Original Article

Evaluation of effect of various drinks on tooth surface morphology under scanning electron microscope

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Abstract

Aim and Objectives: The study aimed to evaluate and compare the effect of various beverages carbonated drinks, i.e., thumps up, fresh fruit juice (apple and pomegranate) and packaged fruit juice (apple and pomegranate) on tooth surface morphology under scanning electron microscope.

Materials and Methods: Thirty recently extracted intact caries-free human permanent teeth were disinfected with 5.25% of sodium hypochlorite solution and autoclaved at 240°F, 20 psi pressure for 40 min. Each tooth was sectioned into 4 enamel sections yielding 120 sections which were embedded in resin using a prefabricated mold. Prepared samples were immersed as Group I - thumps up, Group II - fresh apple juice, Group III - packaged apple juice, Group IV - fresh pomegranate juice, Group V - packaged pomegranate juice, and Group VI - normal saline. Each immersion was done daily for 30 min over 14 days after which samples were scanned under scanning electron microscope (SEM). The data obtained were subjected to statistical analysis using Statistical Package for the Social Sciences (SPSS Version 23; Chicago Inc., IL, USA).

Results: The highest surface roughness was noted in the fresh pomegranate and packaged pomegranate groups with a mean of 2.9000 ± 0.30779 followed by thumbs up group. The greatest mean of cracks was noted in thumbs up group with a mean of 2.500 ± 0.51299 which was significant at $P = 0.000$. The greatest mean of score was noted in thumbs up group with a mean of 2.3000 ± 0.65695 which was significant at $P = 0.000$.

Conclusion: The present study demonstrated that all drinks were erosive in nature with thumps and pomegranate juice showing significantly higher erosiveness than apple juice ($P < 0.01$).

Keywords: Carbonated drinks; enamel; erosion; fruit juice; scanning electron microscope

INTRODUCTION

The human enamel is the stiffest and most mineralized tissue in the human body containing minerals such as calcium and phosphate. The oral cavity suffers from cycles of low pH causing demineralization and high pH causing remineralization.[1]

A pH of 5.5 is the "critical pH" for enamel dissolution, loss of mineral tissue begins as the pH falls below this critical pH.

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Many studies have reported the pH of various commercially available beverages to be below 5.5.[2]

Lifestyle changes related to intake of acidic drinks and foods calls for attention to the oral health of children and young youth most importantly.[3]

Dental erosion or "erosion dentium" is used to characterize the physical result of a pathologic, chronic, localized, painless loss of dental hard tissue chemically etched away from the tooth surface by acid and/or chelation without bacterial involvement.[4]

Various drinks have been shown to cause erosion of enamel *in vitro* and *in situ*, that includes carbonated drinks, fruit

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juices, sports drinks, alcoholic drinks, and many other commercially available beverages.[5]

Holding, swirling and/or retaining acidic drinks and foods in the mouth increases the acid exposure on the teeth, increasing the risk of erosion.[6] Wine tasters also have an increased risk of erosion which can be attributed to the acidic contents of red and white wine.[7]

Lifestyle and behavioral factors may also be responsible to increase the direct contact time of acidic beverages with the teeth thereby increasing the risk of dental erosion. The literature has also suggested that the manner of consumption is more important than the overall quantity.[8]

Tooth erosion can be classified as extrinsic, intrinsic, or idiopathic. Extrinsic erosion is due to the result of exposure to exogenous acids. Gastric acid reaching the oral cavity is identified as the major cause of intrinsic erosion. This is possible when person vomits or the result of gastroesophageal reflux. Idiopathic erosion occurs due to exposure to acids of unknown origin.[9]

The degree of erosion has been evaluated using various techniques, such as profilometry, atomic force microscopy, Scanning electron microscope (SEM), microradiography, iodide permeability, confocal laser scanning, and the microhardness test for measuring the loss of dental hard structures and softening of tooth tissues by erosive processes.[10]

Various studies have established the effect of carbonated beverages, fruit juices, and teeth erosion, but studies on the effects of fresh fruit juices are very rare and have not been researched much.[11]

Therefore, this present study aimed to evaluate and compare the effect of various beverages such as carbonated drinks (thumbs up), fresh (apple and pomegranate), and packaged fruit juice (apple and pomegranate) on tooth surface morphology studied under SEM.

MATERIALS AND METHODS

A total of 30 recently extracted intact caries-free human permanent teeth were selected. Extraneous soft tissue, superficial debris, and calculus were removed from the roots with an ultrasonic scaler, and teeth were disinfected with 5.25% of sodium hypochlorite solution. All the 30 teeth were then autoclaved at 240°F, 20 psi pressure for 40 min.

Once the tooth was disinfected, each tooth was sectioned into four enamel sections: buccal, lingual, mesial, and distal section using a high-speed alloy grinder. Each section was 3 mm in thickness measured using Iwanson Metal Caliper (CLP-1).

Hence, we obtained 120 specimens from 30 teeth.

The polymer and monomer of cold cure resin were mixed in a silicon bowl with the help of agate spatula and each specimen was then embedded in acrylic resin using a prefabricated metal mold with the oral surface kept uppermost [Figure 1].

Prepared samples were then cleaned with nonfluoridated pumice, rinsed with water, and dried with compressed air.

A total of 20 specimens each were placed in Group I - Thumps Up (The Coca Cola Company, India), Group II - fresh apple juice, Group III - packaged apple juice (Dabur Nepal Pvt Ltd, Nepal), Group IV - fresh pomegranate juice, Group V - packaged pomegranate juice (Dabur Nepal Pvt Ltd, Nepal), and Group VI - normal saline.

Each group of specimens was exposed to 250 mL of each beverage as per the group division. Immersion was done for 30 min at room temperature with occasional shaking [Figure 2a-f].

After immersion, the samples were cleaned in distilled water and then stored in normal saline in an incubator at 37°C until the next immersion.

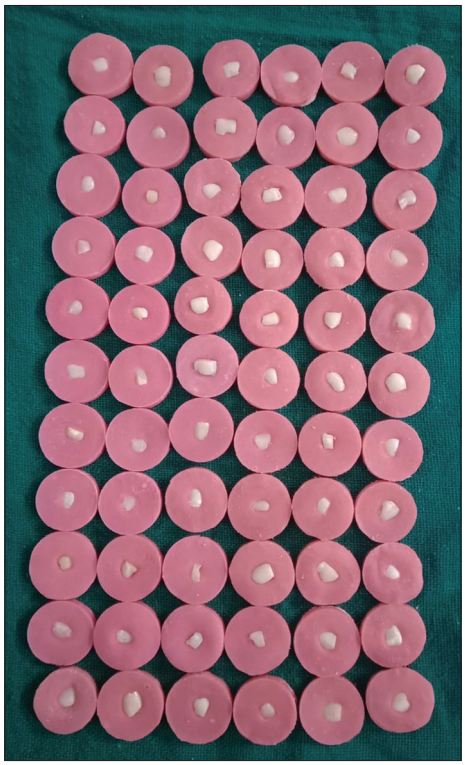

Figure 1: Specimen embedded in acrylic resin

The same cycle of immersion was repeated for 14 days and then the specimens were studied under Scanning Electron Microscope.

Testing of samples

For scanning, the specimens were fixed onto a stub using conductive tape. After the specimens were sputtered with gold, they were scanned under a scanning electron microscope (Apreo S) and studied for changes in surface morphology. Photomicrographs of specimens were produced using SEM (×2500) and studied for surface roughness, exposure of fistula and cracks [Figure 3a-f].

Scoring criteria

The samples were scored for surface roughness, cracks, and exposure of fistula as mentioned in Table 1:

Statistical analysis

The data obtained were subjected to statistical analysis using Statistical Package for the Social Sciences (SPSS Version 23; Chicago Inc., IL, USA). Kolmogorov–Smirnov and Shapiro–Wilk tests were performed to determine the normality of the data. The mean for different readings for surface roughness, cracks and fistula between groups was tested using one-way analysis of variance. Tukey's *post hoc* analysis was applied to find significant differences within groups for surface roughness. $P < 0.05$ was statistically significant.

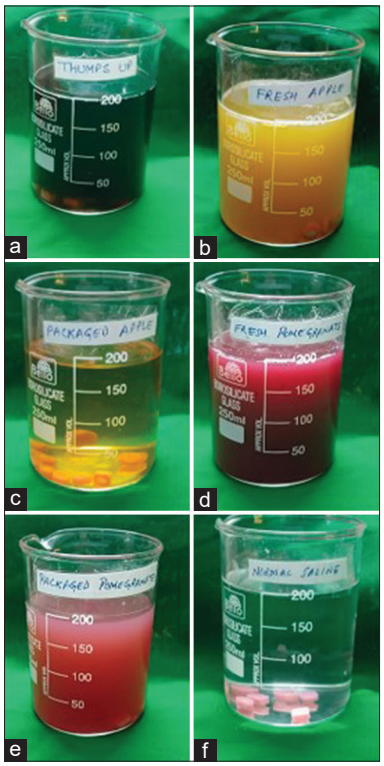

Figure 2: Sample immersed in beverages. (a) Thumps up. (b) Fresh apple juice. (c) Packaged apple juice. (d) Fresh pomegranate juice. (e) Packaged pomegranate juice. (f) Normal saline

RESULTS

Surface roughness

The highest surface roughness was noted in the fresh pomegranate and packaged pomegranate groups with a mean of 2.9000 ± 0.30779 followed by thumbs up group. The least change in surface roughness was noted in the normal saline group with a mean of 0.500 ± 0.22361, which was statistically significant at $P = 0.001$. The surface roughness can be summarized in the following order – Fresh Pomegranate juice = Packaged Pomegranate juice > Thumps Up > Packaged Apple juice > Fresh Apple juice > Normal Saline [Figure 4].

Cracks

Normal saline did not show any cracks followed by the least score in the fresh apple group. The greatest mean of cracks was noted in thumbs up group with a mean of 2.500 ± 0.51299 which was significant at $P = 0.000$.

For the appearance of cracks, the order can be summed up as - Thumps Up > Packaged Pomegranate juice > Fresh

Table 1: Scoring criteria

Notation	Criteria	Grade
−	Not observed	0
+	Little or 1–5 observation	1
++	Intermittently sparse observation	2
+++	Whole surface evenly observed	3

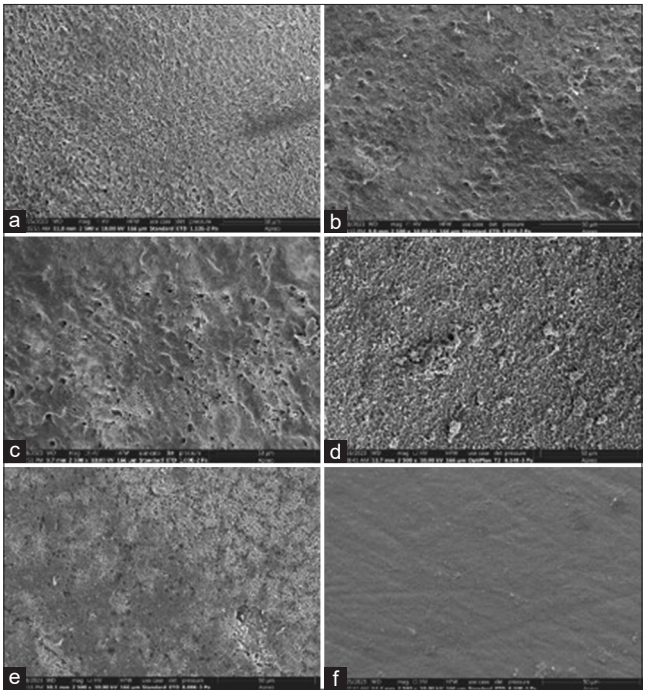

Figure 3: Scanning electron microscopic image. (a) Thumps up. (b) Fresh apple juice. (c) Packaged apple juice. (d) Fresh pomegranate juice. (e) Packaged pomegranate juice. (f) Normal saline

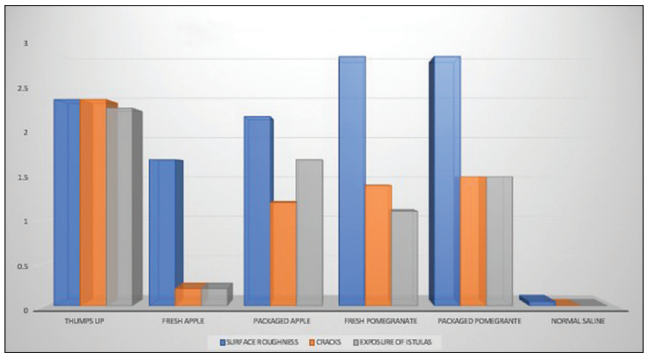

Figure 4: Statistical analysis of various beverages

Pomegranate juice > Packaged Apple juice > Fresh Apple juice > Normal Saline [Figure 4].

Exposure of fistula

The greatest mean of score for exposure of fistula was noted in thumbs up group with a mean of 2.3000 ± 0.65695 which was significant at $P = 0.000$. Fresh apple showed the least mean score for fistula which was significant at $P = 0.000$.

For exposure of the fistula, the order is as follows – Thumps Up > Packaged Apple juice > Packaged Pomegranate juice > Fresh Pomegranate juice > Fresh Apple juice > Normal Saline [Figure 4].

DISCUSSION

Dental erosion (erosive tooth wear) is the situation of chronic loss of dental hard tissue that is chemically etched away from the tooth surface by acid and/or chelation without bacterial involvement.[12]

Literature indicated a strong correlation between erosion and the frequency and amount of beverage intake.[13]

The range of prevalence found in the literature varies widely as much as 4%–100% in adults.[14] The rising prevalence has been linked to the increasing consumption of soft drinks and other beverages.[15]

Early diagnosis of the erosive process can be difficult, but frequent exposure to acidic challenges can eventually lead to permanent and clinically detectable loss of dental tissue. Clinical signs of rapid progression may be dentin hypersensitivity and absence of staining of the lesion. However, most patients do not present with symptoms, even when progression is slow and reparative dentin has time to obliterate tubules. Sometimes, the most severe erosion cases leave the patients asymptomatic, emphasizing once again the importance of dental practitioner's awareness and early detection.[14]

The erosive potential of beverages has been attributed to low pH and the buffering capacity of the drink. Carbonated beverages contain carbonic acid formed by carbon dioxide in solution. Even when the carbon dioxide has been blown off and the drinks have become "flat" the pH remains low. Fruit juices and fruit-flavored drinks are made from a concentrated source of fruit and consist of organic acids derived from the fruit such as citric acid from oranges, tartaric acid from grapes and malic acid from apples. Added Vitamin C (ascorbic acid) may also contribute to the acidity of soft drinks.[15]

Many studies have evaluated effects of wide variety of beverages on the tooth surface. A study by Shroff *et al*. (2018) evaluated the effect of variety of carbonated drinks and packed juices on the percentage loss of enamel.[16]

Another study by Azher *et al*. (2021) evaluated titratable acidity of fresh and packed juices and showed that all juices having pH below 5.5 were erosive.[17]

In the present study, carbonated beverage, fresh, and packaged fruit juice have been explored for their effect on the tooth surface. The effect was observed based on surface roughness, exposure of fistula, and cracking of surface under a Scanning Electron Microscope (SEM). Similar observations were included by Jeong *et al*. in their study which analyzed the effect of energy drinks on tooth enamel erosion. Jeong *et al*. in their study analyzed the samples using a grading system based on the surface involvement. The presence of cracks or roughness or exposure of fistula were noted all over the microphotograph and scored "0" if no observations were made. If observations were 1–5 in number on the photomicrograph it was scored "1" denoted as '+'; intermittently sparse observations were scored "2" denoted as '++' and if whole surface on photomicrograph had cracks or exposure of fistula or roughness a score of "3" was given denoted as '+++'. In the present study, similar grading criteria was followed for the specimen analysis.[18]

All the specimens were immersed in 250 mL of each drink for 30 min for 14 days. After 14 days, the specimens were analyzed under SEM.

Stephen, Robert M. stated a 60-min immersion simulates 1 month of beverage consumption with a 2-min drinking assumption.[19] Hence, we employed a 30-min immersion time over 14 days to simulate youngsters' pattern of beverage consumption.

The results of the present study demonstrated that all drinks used were erosive in nature. Pomegranate Juice (both packaged and fresh) showed the highest mean values for roughness followed by Thumps Up ($P = 0.01$) that was statistically significant. This was in accordance with a study conducted by Seoul Hee Nam in 2021 observed the effect of commercially available fruit juice on the surface

roughness of enamel. It was concluded that fruit juices are erosive and the roughness increases with increase in the duration of exposure.[3]

The greatest mean of cracks and exposure of fistula was noted with thumps up whereas fresh apple juice showed least mean values ($P = 0.01$).

The study by Shroff *et al*. in 2019 demonstrated similar results wherein most erosive drinks were found to be thumps up and apple juice which can be attributed to their higher titratable acidity.[16]

Normal saline which was used as a control group did not show any alteration in the surface morphology after the experiment. This was in accordance with a study conducted by Korte *et al*. in 2019 where they evaluated effect of soda beverages on primary tooth under three-dimensional Laser Measuring Microscope and used 0.9%NaCl as control group.[4]

The present study also demonstrated that packaged fruit juices were found to be more erosive than fresh juices. This was in accordance with a study conducted by Halageri *et al*. where oral clearance rate between packaged fruit drink and fresh fruit juice among dental undergraduate students were compared. It was concluded in their study that the packaged fruit drink group showed maximum pH drop immediately after consumption.[20]

Enam *et al*. in 2017 carried out a study to evaluate dental erosion potential of soft drinks, energy drinks, fruit juices, and packaged drinking water. This SEM study concluded that soft drinks, energy drinks, and fruit juices have low pH causing the tooth to erode. However, packaged drinking water showed very low degree of saturation indicating that prolonged use can cause leaching of minerals from tooth tissue.[21] The present study was in accordance as carbonated drinks and fruit juices both pomegranate and apple juice were found to be erosive in nature.

Another study by Gumilang *et al*. assessed impact of fresh and packaged orange juice on enamel hardness. Their investigation concluded that decline in enamel hardness was higher with packaged orange juice which has been attributed to the loss of minerals over time.[22]

Behl *et al*. had demonstrated use of bioactive agents for eroded enamel lesions wherein they concluded that self-assembling peptides had better remineralization potential than CPP-ACP and Biomin-F.[23]

Several fruit extracts have been investigated for their remineralizing effects as well. A study by Nandakumar and Nasim investigated grape seed and cranberry extracts for remineralizing potential. It was concluded that grape seed extract had better remineralizing potential.[24]

The intake of drinks with low pH also affects the bonding of restorative material to enamel thereby lowering treatment success.[25]

Thus, it may be inferred that whether it be carbonated drink or fruit juices, all these beverages are highly erosive in nature which causes deleterious effects on teeth.

The method used in the present study presents a limitation that the amount of mineral loss in the erosive process cannot be quantified and hence more than one type of analysis should be performed. Another limitation is that in the present study, we have taken fresh fruit juices whose concentration cannot be determined. Furthermore, *in vitro* studies can only be taken as a prediction of relative erosive potential.

CONCLUSION

Within the limitations of the study, it has been concluded that among all beverages tested, thumps up and pomegranate juice were found to have a more erosive potential than apple juice, and least erosive potential was noted in normal saline.

Among the packaged and fresh juices, packaged juices demonstrated more erosive potential than fresh juices.

Erosion compromises the esthetic appeal and jeopardizes structural integrity, potentially leading to more severe dental problems in the long term. The negative impact of these dietary habits can have psychological and social consequences, affecting an individual's self-esteem and overall quality of life.

To mitigate these effects, individuals must be educated and encouraged to adopt healthier dietary choices.

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Conflicts of interest
There are no conflicts of interest.

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