Bangabandhu Sheikh Mujib Medical University Journal 2024;17(1):e70826 bsmmuj.org
ISSN 2074-2908 | eISSN 2224-7750 | © CC-BY-NC-4.0 | Open access doi: 10.3329/bsmmuj.v17i1.70826

BRIEF ARTICLE

Demineralization level of human tooth enamel after exposure to alcoholic and non-alcoholic beverages: A scanning electron microscopic study

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ABSTRACT

Background: In any beverage, human tooth enamel dissociates into its ionic products at a threshold pH of beverage <5.5. This study was done to illustrate and compare the demineralization level of enamel after exposure to alcoholic (beer, wine, distilled spirit) and non-alcoholic (carbonated beverage, apple cider vinegar, commercial fruit juice) beverages.

Methods: Prior to starting this quasi-experimental study of beverage exposure, buccal surface of enamel was scanned under scanning electron microscope to examine the existing score of enamel samples. Twenty-four non-demineralized samples were randomly flushed with attributed beverage and saliva simultaneously for three minutes by a digital automatic flusher. Samples were further scanned under scanning electron microscope after 30 exposures.

Results: pH levels of all beverages were <5.5. After exposure to beverages, all samples were demineralized and 66.6% of samples had the demineralization score of 3. Demineralization score of 2 was observed in 33.3% of samples. The demineralization mean score in non-alcoholic beverages was 3.0 compared to alcoholic beverages 2.3 (P=0.02).

Conclusion: All tested beverages had potential to demineralize the enamel structure. The enamel demineralization capacity of non-alcoholic beverages was higher compared to alcoholic beverages. The demineralization capacity of beverages is inversely proportional to their pH.

Keywords: *demineralization, human enamel, alcoholic and non-alcoholic beverage, scanning electron microscope.*

INTRODUCTION

Human enamel (hydroxyapatite) may dissociates into its ionic products (Ip)[1, 2] if exposed to beverage. By disrupting the concentration of products of hydroxyapatite, the stability of the concentration of beverage is also disrupted and demineralization takes place.[3, 4] Hydrogen ions from any acidic solution directly react with the mineral components of tough enamel structure; it dissolves them, reacts with carbonate ions and phosphates.[5, 6]

The non-ionized form of acid may pass through the interprismatic area and dissolves the minerals under the enamel surface layer.[7] In outer-most enamel surface layer, the presence of aprismatic enamel usually observed.[7, 8]

Alcoholic beverages are intoxicating containing alcohol are produced by fermentation of grapes, grains, barley, fruits, sugarcane, and rice etc.[9, 10] Alcohol's functional hydroxyl group can donate proton act as acidic in nature.[11,12] Non-alcoholic beverages are free

Received: 3 Jan 2024 | **Revised version received:** 13 Mar 2024 | **Accepted:** 13 Mar 2024 | **Published online:** 30 Mar 2024
Responsible Editor: M Mostafa Zaman | **Reviewer A:** Anonymous
This article encompasses PhD thesis of Dr. Rozina Akter

HIGHLIGHTS

1. Human enamel can be demineralized more with non-alcoholic beverages than the alcoholic beverages.
2. The demineralization capacity of beverages was inversely proportional to pH level of beverages.
3. Prevention programme can be designed based on the demineralization/ erosion effect of tested beverages.

from alcohol and most common acids in non-alcoholic beverages are citric, acetic, carbonic and phosphoric acids.[13, 14, 15]

There is paucity of experimental assessment regarding deminerization level of enamel surface after exposure to alcoholic and non-alcoholic beverage. It can be assumed that demineralization level of human tooth enamel might increase after exposure to alcoholic and non-alcoholic beverages. Further research is necessary to clarify the changes of enamel surface following exposure to alcoholic and non-alcoholic beverages by the scanning electron microscope.

This study was done to illustrate and compare the demineralization level of enamel after exposure to alcoholic (beer, wine, distilled spirit) and non-alcoholic (carbonated beverage, apple cider vinegar, commercial fruit juice) beverages.

METHODS

An in-vitro quasi-experimental trial was conducted to assess the demineralization level of enamel after six beverage exposures. Commonly consumed three non-alcoholic beverages (carbonated beverage, apple cider vinegar and commercial fruit juice) were selected as per informal survey among authorized groceries in Dhaka city. Three alcoholic beverages (beer, wine and distilled spirit) were selected according to anecdotal evidence.

Carbonated beverage, fruit juice and beer were not diluted but wine (1:1 ml), distilled spirit (1:1 ml) and apple cider vinegar (3:25 ml) were diluted with distilled water. pH level of beverages was evaluated using pH Meter (HANNA) at the Department of Chemical Engineering, Bangladesh University of Engineering and Technology, Dhaka.

Twenty four extracted human permanent premolar teeth were collected from the Department of Orthodontics, BSMMU. Purposive sampling technique was used to select the tooth samples based on inclusion and exclusion criteria. Teeth with integrity of buccal surface of enamel were included. Exclusion criteria includes absence of integrity of the enamel surfaces, tooth with enamel erosion, dental caries, metallic restoration, fractured tooth, tooth with anomalous shape and structure tooth with external resorption, etc.

After cleaning, tooth samples were stored in distilled water. The roots of samples were discarded with micromotor. Polishing the lingual surface of tooth sample was done to flat the surface for placing on the stab of scanning election microscope. Prior to beverage exposure, all crown samples were dried in oven at 37^{o}C for 7 days and mounted with gold sputtering.

The pre-exposure/ baseline investigation of buccal surface of crown sample was carried out under scanning electron microscope at magnification of X over 10,000 to evaluate the existing features or demineralization score of samples according to Galil and Wright's classification[13] by Department of Biomedical Engineering, Bangladesh University of Engineering and Technology, Dhaka. All non-demineralized crown samples were randomly attributed to 6 beverages (4 samples for each beverage group).

All samples were flushed with beverage and saliva bathing simultaneously for 3 min using a customized digital automatic flusher (INVOLUTE Tech Limited, Dhaka). Goal was to create an environment that can simulate the real-life conditions (inside a person's oral cavity/ mouth) for the experiment to achieve the best result. Therefore, all necessary conditions like the simultaneous presence of natural saliva inside the mouth, periodic consumption of beverage at a certain volume in a specified time was considered.

Total amount of beverage for a single exposure/ experimental trial was 250 ml. Total amount of saliva for a single exposure/ experimental trial was 16 ml. Total exposure time for a single exposure/ experimental trial was 3 minutes. Each exposure/ trial consists 10 cycles and each cycle duration was 18 seconds. Each cycle had active phase (9 seconds) and interval phase (9 seconds). Active phase includes flow of 25 ml of beverage in acrylic oral cavity model for 3 seconds, immersion of crown sample within beverage for 3 seconds and flash out of beverage in 3 seconds. Beverage exposure trial to every crown sample was performed for 30 times. After drying and gold sputtering, further scanning election microscope investigation of the buccal surface of crown sample was done at a magnification of X over 10,000. The representative digital photomicrographs were evaluated individually by two evaluators with the attributed evaluation scores according to Galil and Wright's.[13]

Score-1	No demineralization/change of structural morphological features of enamel surface
Score-2	Distinct change of structural morphological features of enamel surface/ loss of integrity of enamel surface
Score-3	Etch pattern showing preferential dissolution of the enamel surface/ prism peripheries giving a 'cobblestone' appearance
Score-4	Preferential dissolution of the enamel surface/ prism cores resulting in a 'honeycomb' appearance
Score-5	A more random etch pattern, corresponded to score-3 and score-4 together
Score-6	Pitted enamel surface

Statistical analysis

Frequency and percentage were presented for categorical variables. The mean and standard deviation of the demineralization score was compared between alcoholic and non-alcoholic groups using *t* test. *P* value <0.05 was considered significant.

RESULTS

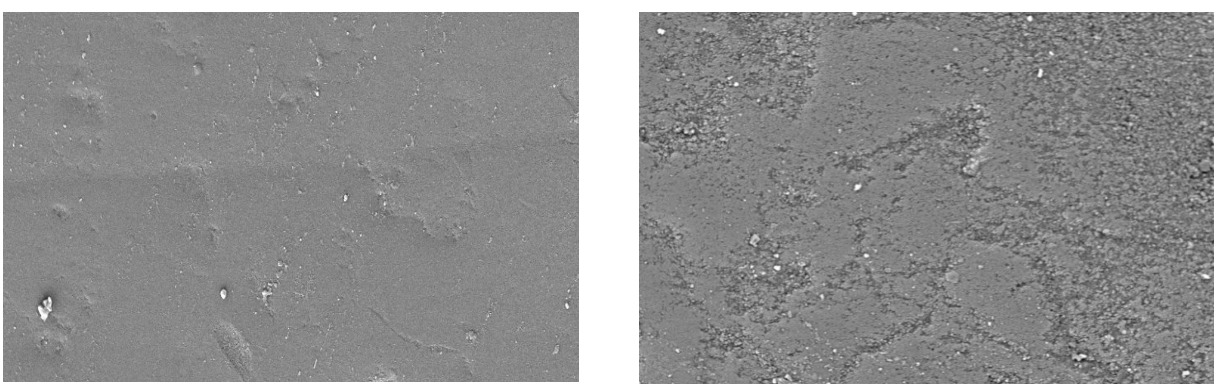
Initially, samples (n=24) were non-demineralized. After exposure to beverage, 100% of samples were demineralized. Total 66.6% of samples showed demineralization score of 3 after exposure non-alcoholic beverages that indicated preferential dissolution of the enamel surface layer. Demineralization score of 2 was observed among 33.3% of samples exposed to distilled spirit and beer beverage that indicated the distinct change of structural morphological features of enamel surface or loss of integrity of enamel surface. The demineralization mean score of samples exposed to non -alcoholic beverages (3.0) was significantly higher compared to alcoholic beverages (2.3) (*P*=0.02) **(TABLE 1)**.

TABLE 1 Mean scores tooth samples before and after exposure to beverages (n = 24)

Exposures	pH	Number	Before	After	Mean (SD)[a]	P
Non-alcoholic beverage					3.0 (-)	0.02
Carbonated beverage	2.4	4	1	3		
Apple cider vinegar	2.9	4	1	3		
Commercial fruit	3.5	4	1	3		
Alcoholic beverage					2.3 (0.5)	
Wine	3.5	4	1	3		
Distilled spirit	3.6	4	1	2		
Beer	4.3	4	1	2		

[a]Mean of post-exposure scores; SD indicates standard deviation

pH level of carbonated beverage is lowest (2.9) and pH level of beer (4.3) is highest among all tested beverages. Based on pH level, the non-alcoholic group of beverages is more acidic compared to alcoholic group of beverages.



A B

FIGURE 1 **Scanning electron microscope image of enamel surface before (A) and after (B) beverage exposure**

Scanning electron microscope image of enamel surface before beverage exposure showed no demineralization. However, demineralization of enamel surface was observed after beverage exposure **(FIGURE 1)** with a 'cobblestone' appearance. The demineralization pattern of score-2 showed distinct change of structural morphological features of only outer enamel surface not inner prism structure.

DISCUSSION

We report that both alcoholic and non-alcoholic beverages cause demineralization. However, it is more prominent in case of non-alcoholic beverges. Mulic *et al.* revealed that fruit juices and carbonated beverage are potential to cause tooth demineralization which findings supported the present study findings.[1] Li *et al.* concluded that carbonated beverages showed the highest and statistically significant odds ratio for the development of tooth demineralization which findings are also similar with the present study findings.[2] Similarly, Habib *et al.* found acidic fruit juice consumption as a significant factor for tooth demineralization.[3] Swahn *et al.* and Somayeh *et al.* found a correlation between demineralization of tooth and pH of beverages.[4, 5] Zimmer *et al.* reported that men with demineralized tooth twice who consumed carbonated beverage more than men without tooth demineralization/ erosion.[6] Toole *et al.* reported an association between incisal tooth demineralization and habit of holding of beverages in mouth prior to swallowing in children aged 6 years.[5, 7]

The basic cause of enamel demineralization is the existence of the critical pH value of a solution/ beverage is 5.5.[8, 9] If the pH value of the solution is below 5.5, the solution is not saturated, hence, it causes demineralization.[10] Hydrogen ions from any acidic beverage/ solution may dissolve the mineral components of enamel structure of tooth and reacts with carbonate and phosphate ions.[11]

The demineralization in non-alcoholic beverages was higher than that of alcoholic beverages because higher of their lower pH level.[12] A beverage's acidity level is determined by how much acid it contains from citrus or other additives.[13] The pH scale measures this trait, but any pH level below 7.0 is considered acidic, and the lower the number, the greater the potential to cause demineralization.[13, 14] Therefore, the demineralization capacity of beverages was inversely proportional to pH of beverages.[14, 15]

Scanning election microscope was recommended as a fruitful evaluation technique for the direct qualitative analysis of the enamel structure.[15] The signals that derive from electron-sample interactions reveal information about the external morphology of crystalline structure.[16] Areas ranging from approximately 1-10 microns in width was imaged at magnification 10,000X. The scanning election microscope was also capable of analysis of selected point locations on the sample. This approach is capable in scoring qualitatively the crystalline enamel structure.[16, 17] Further study regarding the changes in Ca and P levels following exposure to beverages with a quantitative analysis would be useful.

It is essential to notify that in previous studies, direct immersion of enamel sample in beverage was done in vitro setting because it was not possible to replicate intra-oral conditions in the laboratory nor it was ethical to conduct such a study in humans.[18, 19] In the present study, it was possible to simulate intra-oral conditions by the help of a digital automatic flusher.

Conclusion

Evidence-based programme can be designed based on the demineralization/ erosion effect of tested beverages on human enamel surface.[20] The demineralization capacity of beverages was inversely proportional to pH of beverages. Although all tested beverages had potential to demineralize the enamel structure, demineralization capacity of non-alcoholic beverages was higher compared to alcoholic beverages.

Acknowledgments

The authors gratefully acknowledge to Prof. Dr. M. Tarik Arafat, Chairman, Department of Biomedical Engineering, BUET for helping to conduct the investigation under scanning electron microscope

Author contributions

Conception and design: RA, AAM, AKMB, MK. *Acquisition, analysis, and interpretation of data:* RA, MMMH. *Manuscript drafting and revising it critically:* RA, AAM, AKMB, MK. *Approval of the final version of the manuscript:* RA, AAM, AKMB, MK. *Guarantor of accuracy and integrity of the work:* RA, AAM, AKMB, MK, MMMH, MHU.

Funding

The study received financial support from Bangabandhu Sheikh Mujib Medical University. Memo number: BSMMU/2023/13314 (19), Date: 31/10/2023.

Conflict of interest

We do not have any conflict of interest.

Ethical approval

This study was conducted after getting ethical clearance from the Institutional Review Board of BSMMU (vide BSMMU/2021/12935, dated 26/12/2021). Prior to performing lab work, permission was taken from Department of Biomedical Engineering, Bangladesh University of Engineering and Technology.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

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