

Analysis of dental erosion induced by different beverages and validity of equipment for identifying early dental erosion, in vitro study

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Abstract

Objective: To understand early dental erosion induced by different beverages and the equipment for its detection.

Methods: The study was conducted at the University Malaya Medical Centre, Kuala Lumpur, Malaysia, from June to September 2014, and comprised single-rooted, unpolished teeth divided into six groups. Electron micrographs and other baseline readings for further analyses were taken before and after the exposure to different beverages. The teeth were exposed to the beverages using a modified Nordini's artificial mouth model. The positioning of the teeth on the motorised stage of the equipment was standardised.

Results: Of the several beverages used, CocaCola had the lowest pH value of 2.53, while tap water had the highest pH of 5.4. Deionised distilled water, which was used as a reference, had a pH near to neutral /alkaline of 7.3. The fluoride content ranged between 9.38ppm in tea and 0.005ppm in orange juice. Teeth exposed to beverages with low pH and considerably high fluoride underwent slight remineralisation (roughness increase 8% from tea), while beverages with no fluoride content and low pH roughened the enamel surface (Coca Cola roughened upto 37%). Quantitative analyses of tooth erosion, micro-hardness, surface-roughness, and surface-height showed that all beverages exhibited positive erosive effect on the tooth enamel surface ($p < 0.005$).

Conclusion: CocaCola was found to be the most erosive agent among both hot and cold beverages (37%), while coffee was more erosive among the hot beverages (29%).

Keywords: Dental erosion, Profilometry, SEM, VHN, Beverages. (JPMA 66: 843; 2016)

Introduction

In the last few decades, a huge increase in the turnover of the acidic beverages has been witnessed, especially in developed countries, leading to a raised tooth erosion index.¹

Dental erosion (DE) is a chronic, localised and painless loss of dental hard tissues which have been chemically etched away from the tooth surface by acid and/or chelation without bacterial involvement.²

Seven different agents with erosive potential were studied in an oral simulation chamber. The results obtained from different methods of detecting early DE were analysed and compared,³⁻⁷ and parameters like Vickers micro-hardness (VHN), surface average roughness (Ra), elemental ratio and scanning electron microscopy (SEM) images of enamel surface were assessed. These studies employed equipment like SEM, profilometer, focus variation microscopy, VHN (with Vickers and Knoop indents) detection and energy-dispersive X-ray spectrophotometry (EDS).

SEM has been used to check the topographical variation

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in the tooth surface before and after the acid attack,⁸ whereas the profilometer/surfometer has been employed to check the Ra.^{4-7,9}

The current study was planned to understand early DE induced by different beverages and the best equipment for its detection.

Materials and Methods

The study was conducted at the University Malaya Medical Centre, Kuala Lumpur, Malaysia, from June to September 2014, and comprised unpolished, single-rooted, non-carious teeth without stains, restorations and cracks. The teeth were mainly extracted in lieu of orthodontic or periodontal treatment. After obtaining ethical approval from the institutional ethical committee, the extracted teeth were disinfected using 0.5% chloramine T trihydrate (Permata Scientific SDN BHD) solution, as per International Organisation for Standardisation/Technical Specification (ISO/TS) 11405:2003 requirements.

The teeth were then scaled using dental scalar (Peizon Master 400, Switzerland), to remove any calculus and debris. The cleaned teeth were then stored in distilled water at 4°C for subsequent experiment as outlined in literature.⁹

SEM (Quanta 250FEG, Japan) was used to record the

qualitative aspect of this research in the form of images of the surface enamel at three different magnifications (1000x, 3000x and 5000x). To evaluate Ra and average height (Sa) of selected surface area, three-dimensional (3D) scanning microscope (Infinite Focus G4 Microscopy; IFM, Alicona Imaging, Grambach/Graz, Austria) was used and profile analysis was done by the IFM G4 software (Alicona imaging/ ISO 4287/4288) provided by the manufacturer.

Vickers diamond micro-indent fitted into the VHN testing machine (HMV 2 Series Shimadzu Micro-hardness Tester, Japan) was used to measure the surface hardness. Modified Nordini's artificial mouth (NAM) model¹⁰ was used for the simulation of oral cavity (Figure-1).

SPSS 12 was used for data analysis. For the analysis of data between the baseline and final reading measurements, a non-parametric statistical analysis, Wilcoxon Sign Rank Test, was used and $p < 0.05$ was considered significant.

Results

The 42 teeth in the study were randomly divided into seven different groups of erosive agents with 6(14.3%)

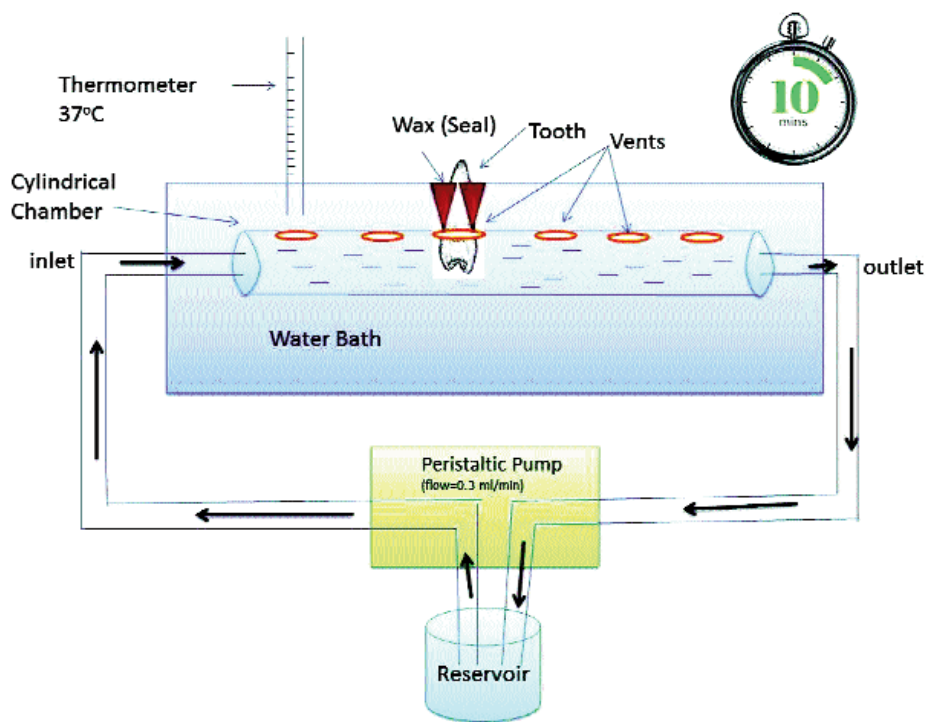


Figure-1: A schematic diagram of modified Nordini's artificial mouth (NAM) model¹⁵ showing how a tooth was positioned in one of the vents of the cylindrical chamber (mimicking the oral cavity) and held firmly by using modelling wax. Beverage was allowed to flow into the cylindrical glass chamber via the inlet from the reservoir and out via the outlet into the reservoir.

Table-1: List of Beverages and their Preparation/Collection for the Experiment.

Beverages Used in Current Study.			
S.NO	Name	Product Information	Purchase and Preparation
1	Deionised Distilled Water	Deionised Distilled Water	Collected from laboratory, University Malaya, faculty of Dentistry.
2	Tap Water	Water from tap	Obtained from water taps of the laboratory at BUA.
3	Coffee	Nescafe Classic, Switzerland	Bought from superstore in Kuala Lumpur, Malaysia. The coffee was freshly brewed (200gm in 100 ml of boiling water) and cooled to 60°C
4	Tea	Lipton Yellow Label, Unilever Scotland UK	Bought from superstore in Kuala Lumpur, Malaysia. Two tea bags were placed in a cup containing 200 ml of boiling water without any additives cooled to 60°C.
5	Coca Cola	Coca Cola Company, USA	Chilled Coca Cola (4°C) were bought from the superstore in Kuala Lumpur, Malaysia. It was used neat without dilution
6	Orange Juice	Tropicana Orange Juice, PepsiCo USA	Chilled Orange Juice (4°C) was bought from superstore in Kuala Lumpur, Malaysia. It was used as it was without dilution

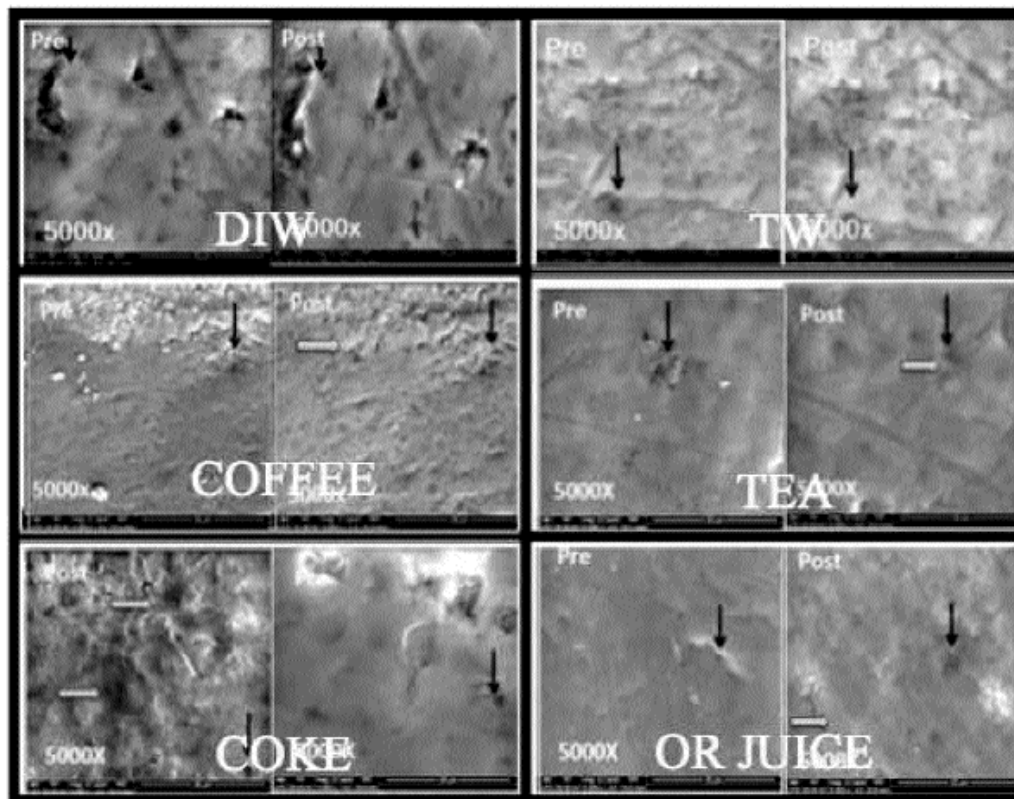
Table-2: Mean and Standard Deviation of the pH and Fluoride Content Noted. (Fluoride content is measured in ppm)

The pH and the fluoride content.						
Variables	Deionised distilled Water	Tap Water	Coffee	Tea	Coca Cola	Orange Juice
Fluoride (Mean/ SD)	0.00±0.001	0.73±0.11	0.05±0.002	9.38±0.111	0.01±0.004	0.01±0.005
pH (AVG/ SD)	7.30±0.032	5.36±0.021	4.46±0.015	4.90±0.015	2.53±0.035	3.41±0.015

Table-3: Statistical Analysis of the parameters treated by different beverages (Negative Z score shows that the raw score in all groups is less than the mean value.).

		DIW		TW		Coffee		Tea		Coke		Or Juice	
		PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST
Micro Hardness	Median	245.50	241.83	252.67	213.33	285.50	180.67	223.67	177.67	255.34	156.50	266.67	158.67
	ST DEV	±12.14	±10.77	±74.29	±64.36	±61.79	±57.12	±22.53	±7.27	±71.05	±31.98	±54.69	±24.96
	Z-Statistics		-0.11		-2.20		-2.20		-2.20		-2.20		-2.20
	P Value		1.00		0.03		0.02		0.03		0.02		0.03
Surface Roughness	Median	0.22	0.22	0.22	22.66	0.26	0.36	0.27	0.28	0.19	0.26	0.21	0.24
	ST DEV	±0.02	±0.05	±0.03	±0.02	±0.21	±0.25	±1.00	±0.09	±0.03	±0.04	±0.56	±0.08
	Z-Statistics		-0.37		-1.36		-2.21		-2.20		-2.21		-2.20
	P Value		0.88		0.22		0.02		0.03		0.02		0.03
Surface Height	Median	4.75	4.48	4.17	3.78	3.48	4.36	4.22	3.86	3.49	4.80	4.02	5.06
	ST DEV	±1.67	±1.71	±1.89	±1.63	±1.34	±1.91	±1.67	±1.34	±1.17	±1.97	±1.14	±1.29
	Z-Statistics		-0.31		-0.31		-0.73		-1.11		-1.99		-1.36
	P Value		0.84		0.84		0.28		1.00		0.03		0.22

DIW: Deionised water.

**Figure-2:** Scanning electron microscopy (SEM) images of enamel of the surface at baseline and final reading after exposure for 10 minutes to different beverages.

(SEM Images at 5000X)

DIW: Deionised water

TW: Tap Water

OR Juice: Orange Juice

teeth in each group (Table-1). All the selected beverages had low pH value, with CocaCola having the lowest at 2.53 and the tap water having the highest at 5.4. Deionised distilled water, which was used as a reference, had a pH near to neutral/alkaline at 7.3.

The fluoride content ranged between 9.38ppm in tea and 0.005ppm in orange juice (Table-2).

Deionised distilled water, tap water and tea-treated teeth did not show any significant changes. In the case of coffee, a slight change to the tooth surface was noticeable when seen under the SEM at the 3000X magnification. The change was even more pronounced at 5000X magnification, demonstrating an early

phase of DE and demineralisation. The small debris seen in the pre-treatment picture at 3000X magnifications may occur during sample preparation.

After 10 minutes of exposure to CocaCola, the tooth surface showed a marked visible effect. Prisms were clearly visible in the images taken post-treatment. The cracks seen were more obvious, and the surface looked quite rough, showing marked signs of DE. Orange juice, with a lower pH and almost negligible fluoride, showed noticeable alteration in the tooth surface. The tooth surface after exposure to orange juice appeared to affect the enamel prisms being exposed and this was visible at all the three magnifications (Figure-2).

Both the deionised distilled water and tap water did not appear to roughen the tooth surface enamel to a significant level. The teeth exposed to CocaCola exhibited surfaces which were coarse in which the Ra increased significantly by 37% ($p < 0.05$). Coffee-treated teeth showed an increase in Ra by up to 29% ($p < 0.05$), followed by orange juice 18% ($p < 0.05$). Tea showed a minimal increase of only 8% ($p < 0.05$) in Ra.

Results obtained with respect to the effect of the beverages, except CocaCola, on Sa were not significant (Table-3). For CocaCola, a decrease of 38% in Sa was significant ($p < 0.05$). For coffee and orange juice, which appeared to cause a decrease in Sa of about 29% and 21% respectively, the difference was not significant ($p > 0.05$).

Discussion

DE induces demineralisation, therefore affecting the hardness of tooth tissue, which is believed to be related to the degree of remineralisation. Demineralisation of enamel was measured as a surface VHN using Vickers diamond indentation method. This method is suitable for the determination of small changes in the surface hardness due to erosive attack.^{5,11} There was a notable difference between the baseline and final measurements in the VHN measurements observed in this study. All of the teeth except those exposed to deionised distilled water showed a statistically significant decrease in the surface VHN ($p \leq 0.05$). Another measuring tool used in our study was IFM, the non touch profilometry, which is a laser profilometer used for the measurement of Sa and Ra. The specialised software provided with the machine was used to detect the change in the pre- and post-treatment Sa. Special reproducibility protocol was taken to assure that the pre- and post-treated sites observed were exactly the same.

The beverages used in this study were based upon the frequency of consumption among the Malaysians. Their

effect on the human tooth enamel surface was analysed qualitatively (using SEM) and quantitatively (using VHN, Sa and Ra).

Tea and coffee were taken hot (60°C) while Coca-Cola and orange juice were taken cold (4°C). Tap water and deionised water was taken at room temperature as test group. Tap water was included in the study as it has a major role in our daily life and is frequently in contact with our teeth. It is ostensibly in contact with teeth during tooth brushing as well as mouth rinsing especially during ablution amongst the Muslim community.

The pH measured for the beverages ranged from very acidic as in Coca-Cola (2.53) to alkaline as in deionised distilled water (7.3). Lussi et al.¹² has reported that any pH below 4.5 is critical.¹² They also have reported that any pH above 4.5 will cause precipitation of fluoroapatite and hence DE becomes less likely. The process of DE in relation to pH, phosphate and fluoride is still not clear.

Based on the pH levels, a progressive enamel loss was expected in accordance with the previous studies.^{6,8,13} The presence of fluoride was also noted as it is believed to play a negative role against DE because it promotes remineralisation and inhibits demineralisation by deposition in the form of CaF₂.¹⁴ Hydroxyapatite (HAP) gives rise to the fluorapatite Ca₁₀(PO₄)F₂, when the hydroxyl ions are substituted by the fluoride ions. The presence of fluoride ions in the lattice allows it to resist the acid challenge.¹⁵

Any beverage or food with intrinsically lower pH, including carbonated drinks, fruit juices, diluted juices, salad additives and flavoured mineral water, may cause DE.⁴⁻⁶ The severity of the erosive potential is thought to be related to pH, titratable acidity, buffering capacity and calcium chelating property of the beverages.¹⁶ The hydrogen ion concentration (H⁺) of the acidic substances which are available to interact with the tooth surface, also called the titratable acidity, is considered to be more important than the actual pH.⁶ Almost no morphological differences in the SEM images could be detected in the case of teeth exposed to hot tea and hot coffee. The SEM image analyses are a qualitative assessment that did not appear to give a clear correlation with the ingredients (fluoride content) and property (acidic) of the beverages.

The sample size and duration of the test varies from researcher to researcher. Hunter et al. used six enamel specimens from deciduous and permanent teeth for each of their five groups of beverages and four hours exposure time in their protocol.¹⁷ Kitchen and Owens immersed four teeth in the respective seven different groups of

beverages (28 teeth in total) for 14 days,⁶ Larsen and Richards used two human molars per group of beverage for seven different group beverages and 48-hour exposure protocol.¹⁸ Ren et al. used 30 enamel samples from third molars which were dipped in orange juice for 20 minutes with and without applying toothpaste.⁹ The usual contact time between a beverage consumed and teeth in the oral cavity depends on the type and viscosity of the beverage. In this study, a standardised volume of 250 ml for the respective beverages was allowed to flow through the glass chamber (simulated mouth) for 10 minutes.

Keeping in mind all the information gathered from literature on the average daily consumption of soft drinks and time in mouth that is around five seconds per sip, we decided to keep the exposure time of teeth to the beverages at 10 minutes.^{6,9,17}

According to the present study, exposure of the teeth to hot black tea showed slight decrease in surface VHN. This difference was significant but among the least when compared to the other beverages used. When seen qualitatively, under SEM, tea failed to show much of the difference of the surface structure between the baseline and final images of the teeth after exposure. This result could be seen in accordance with Zahara et al.³ who categorised tea as a non-erosive drink and stated that its intake to about 150ml/day may be associated with the reduction of the DE in vivo. Nasition et al.¹⁹ have stated that coffee is more erosive than tea. This appears to be in agreement with results obtained in our study. SEM images of the tooth surface exposed to hot coffee showed visible difference with signs of demineralisation. The opposite effect was observed with hot black tea. The scratches that were initially present on the tooth surface as shown in the SEM image before being exposed to hot black tea appeared to fade away after being exposed to hot black tea. This might be attributed to a glimpse of remineralisation promoted by the high fluoride content in tea. In addition, the surface hardness of the tooth surface seemed to decrease almost two times in coffee than in teeth exposed to hot black tea. Coffee also caused the Ra to increase by almost 28%, while the Sa and calcium and phosphate content decrease.

The results in previous studies,^{3,6} cannot support the results for coffee in the present study. This is because they have put this beverage under the non-erosive category, where the results in the present study indicated coffee as a beverage with higher erosive potential. The temperature difference may be counted as a reason to explain, as we had used hot coffee, while in the previous

literature cited, temperature was not indicated. Results for both Coca-Cola and orange juice showed positive erosive response that is well supported by the literatures.^{5-7,11,13}

The SEM analysis of the teeth that were exposed to Coca-Cola, and orange juice for 10 minutes presented early signs of DE. Surface irregularities were observed, characterised by dissolution of the enamel rods and inter-rod area with exposed dentinal tubules especially for teeth exposed to Coca-Cola. The SEM images of the pre- and post-treated teeth exposed to the orange juice also showed distinct changes as observed for teeth exposed to Coca-Cola.

Tap water also did not show any change in the topography under the SEM after 10 minutes of exposure. The fluoride content of 0.7 ppm also seemed not to show much of the difference.

The ingredients in the beverages may play an important role in their erosive capacity. Citric acid (also found in orange juice) is an important constituent of various acidic beverages with an erosive potential inducing enamel demineralisation.²⁰ It has also been documented that the citric acid has a higher potential for the dissolution of the hydroxyapatite crystals due to the formation of the calcium citrate and the chelating (calcium binding) action of the citric acid that withdraws Ca²⁺ from the tooth surface enamel resulting in an increased dissolution tendency due to the loss of common ion effect. Citric acid irreversibly chelates with the calcium with net effect of an accelerated loss of calcium from tooth structure.²¹ The sugars added to some drinks may also increase the erosive potential of the drinks.²²

When talking about the cold beverages, chilled Coca-Cola showed to be the most destructive to the tooth surface, as evident from other studies.^{4,5,7} On the SEM, images of enamel surface showed an intense scalloping of the entire surface, with debris visible and tubules exposed on the surface. The hardness tends to be decreased by almost 45% with a 24% percentage difference in calcium loss. There was an increase in Ra by 37%, with around 38% decrease in the Sa. Chilled orange juice also appeared to show a marked destruction of human tooth enamel as can be observed in SEM images. This was supported by the elemental analysis where calcium loss was 17% and surface hardness was decreased by 35% while the Ra increased by 18%.

From this study, it shows that SEM alone failed to give us a satisfactory result. However, it can be considered as an evaluation tool for DE to support, in addition to the other

measuring parameters employed.

The pH of beverages that are acidic may have a role in DE and the fluoride in the beverages can also interfere with the progress of DE. As for the measuring parameter for assessing DE, hardness appears to be a reliable parameter compared to other quantitative measuring parameters.

In the current study, the change in the Sa between the pre- and post-treated tooth surfaces exposed to beverages, except in Coca-Cola, was not significant. This finding is also reported by Fuji et al.⁷

The change in the Ra between the pre- and post-treated tooth surface to the beverages, with the exception of the tap water and distilled water, was significant. Hence, the Ra measurement is supporting the results of the VHN measurements and also the SEM images.

Acknowledgement

We are grateful to University of Malaya for financial assistance, and to Professor Noorlede Abu Kassim for statistical help.

Conflict of Interest: There is no conflict of interest.

Financial Assistance: The financial support was provided by IPPP, University Malaya(Account No. PU 103_2011A).

Disclosure: This research was conducted under financial assistance of University of Malaya, and has not been submitted or presented at any other journal/ conference.

References

1. Arnadottir IB, Holbrook WP, Eggertsson H, Gudmundsdottir H, Jonsson SH, Gudlaugsson JO, et al. Prevalence of dental erosion in children: a national survey. *Community Dent Oral Epidemiol* 2010; 38: 521-6.
2. Ganss C. Definition of erosion and links to tooth wear. *Monogr Oral Sci* 2006; 20: 9-16.
3. Manaf ZA, Lee MT, Ali NH, Samynathan S, Jie YP, Ismail NH, et al. Relationship between Food Habits and Tooth Erosion Occurrence in Malaysian University Students. *Malays J Med Sci* 2012; 19: 56-66.
4. Passos VF, Melo MA, Vasconcellos AA, Rodrigues LK, Santiago SL. Comparison of methods for quantifying dental wear caused by erosion and abrasion. *Microsc Res Tech* 2013; 76: 178-83.
5. Caneppele TM, Jeronymo RD, Di Nicoló R, de Araújo MA, Soares LE. In Vitro assessment of dentin erosion after immersion in acidic beverages: surface profile analysis and energy-dispersive X-ray fluorescence spectrometry study. *Braz Dent J* 2012; 23: 373-8.
6. Kitchens M, Owens BM. Effect of carbonated beverages, coffee, sports and high energy drinks, and bottled water on the in vitro erosion characteristics of dental enamel. *J Clin Pediatr Dent* 2007; 31: 153-9.
7. Fujii M, Kitasako Y, Sadr A, Tagami J. Roughness and pH changes of enamel surface induced by soft drinks in vitro-applications of stylus profilometry, focus variation 3D scanning microscopy and micro pH sensor. *Dent Mater J* 2011; 30: 404-10.
8. Lussi A, Bossen A, Höschle C, Beyeler B, Megert B, Meier C, et al. Effects of enamel abrasion, salivary pellicle, and measurement angle on the optical assessment of dental erosion. *J Biomed Opt* 2012; 17: 97009-1.
9. Ren YF, Zhao Q, Malmstrom H, Barnes V, Xu T. Assessing fluoride treatment and resistance of dental enamel to soft drink erosion in vitro: applications of focus variation 3D scanning microscopy and stylus profilometry. *J Dent* 2009; 37: 167-76.
10. Rahim ZHA, Fathilah AR, Irwan S, Wan Nordini Hasnor WI. An artificial mouth system (NAM Model) for oral biofilm research. *Res J Microbiol* 2008; 3: 466-73.
11. Seow W, Thong K. Erosive effects of common beverages on extracted premolar teeth. *Aust Dent J* 2005; 50: 173-8.
12. Lussi A, Jaeggi T, Jaeggi-Schärer S. Prediction of the erosive potential of some beverages. *Caries Res* 1995; 29: 349-54.
13. Willershausen B, Schulz-Dobrick B. In vitro study on dental erosion provoked by various beverages using electron probe microanalysis. *Eur J Med Res* 2004; 9: 432-8.
14. Cate JT. Review on fluoride, with special emphasis on calcium fluoride mechanisms in caries prevention. *Eur J Oral Sci* 1997; 105: 461-5.
15. De Carvalho Filho ACB, Sanches RP, Martin AA, do Espirito Santo AM, Soares LES. Energy dispersive X-ray spectrometry study of the protective effects of fluoride varnish and gel on enamel erosion. *Microsc Res Tech* 2011; 74: 839-44.
16. Jensdottir T, Arnadottir I, Thorsdottir I, Bardow A, Gudmundsson K, Theodors A, et al. Relationship between dental erosion, soft drink consumption, and gastroesophageal reflux among Icelanders. *Clin Oral Investig* 2004; 8: 91-6.
17. Hunter ML, Hughes J, Parker D, West N, Newcombe RG, Addy M. Development of low erosive carbonated fruit drinks. 1. Evaluation of two experimental orange drinks in vitro and in situ. *J Dent* 2003; 31: 253-60.
18. Larsen M, Richards A. Fluoride is unable to reduce dental erosion from soft drinks. *Caries Res* 2002; 36: 75-80.
19. Nasution AI, Gani BA, Pospos RM. Relationship between Fluor Concentration and Structure Pattern of Enamel Prism in Enamel Surface after Coffee and Black Tea Exposure. *World* 2012; 3: 284-9.
20. Sauro S, Mannocci F, Piemontese M, Mongiorgi R. In situ enamel morphology evaluation after acidic soft drink consumption: protection factor of contemporary toothpaste. *Int J Dent Hyg* 2008; 6: 188-92.
21. Williams N, Covington 3rd J. Methamphetamine and meth mouth: an overview. *J Tennessee Dental Assoc* 2005; 86: 32-5.
22. von Fraunhofer JA, Rogers MM. Dissolution of dental enamel in soft drinks. *Gen Dent* 2004; 52: 308-12.