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QUANTITATIVE EVALUATION OF SOLUBILITY AND SURFACE MICRO-HARDNESS OF ENAMEL ON EXPOSURE TO VARIOUS SOFT DRINKS - AN *EX-VIVO* STUDY

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ABSTRACT: Objective: To evaluate and compare pH and titrable acidity (TA) of various commonly consumed commercially available soft drinks. To evaluate and compare the weight of enamel dissolution and surface micro-hardness of the tooth after exposure to commonly consumed commercially available soft drinks. **Methods:** 60 human enamel specimens were randomly divided into 5 groups: Group 1: Kinley water (control), Group 2: Pepsi cola, Group 3: Urzza the liquid charger, Group 4: Tropicana orange delight, Group 5: Patanjali amla amrit. For the five solutions pH and TA was calculated. Each of the specimens were then pre-weighed and exposed to the soft drink for 14 days and weighed again. The mean weight difference was calculated for all of the samples. The same samples were subjected to the micro-hardness test (Vickers hardness number). **Results:** The pH of all the test drinks ranged from 2.55 to 3.74, which is far below critical pH. The pH and TA of carbonated drinks were statistically significantly lower than non-carbonated groups. Statistical analysis revealed that TA at 5.5 has a positive correlation to weight difference and a negative correlation to micro-hardness. **Conclusion:** TA is a more appropriate criteria than pH to analyze the erosive potential of a drink. Non-carbonated beverages have a higher TA than carbonated beverages which are associated with increased enamel solubility and least micro-hardness values. Patanjali amla amrit had a low pH, high TA and was proven to be the most erosive of the soft drinks tested.

INTRODUCTION: In the modern fast-track lifestyle there is a change in the eating and drinking habits of the population which is more focused towards convenience. Even though traditional and homemade drinks will always remain popular, packaged beverages are gaining traction with Indian consumers who are now frequently reaching for their more convenient-to-consume counterparts soft drinks. As per market research, the non-alcoholic beverage industry in India stood at US$2.5 billion in 2015.

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The market is estimated to grow at a CAGR (compound annual growth rate) of over 17% in between the period from 2016 to 2020 to reach US$4.9 billion by 2020 [1]. There is increasing evidence, from *in-vitro* and *in-situ* studies, about the association between consumption of acidic drinks and dental erosion [2, 3, 4]. To overcome these adverse effects of soft drinks people are oriented towards holistic healthier alternatives. In the present scenario, herbal health drinks are overtaking carbonated and fruit drinks due to its therapeutic benefits and boosting of the immune system with minimal or no side-effects. It has the added advantage as they are extracted from natural sources.

Indian gooseberry, commonly known as Amla belongs to the Euphorbiaceae family, is considered

the powerhouse of nutrients and has shown to have hepatic, cardio, nephron, neuroprotective effects, antioxidant, anti-inflammatory, analgesic, antipyretic and restorative properties [5]. To the best of our knowledge, there is no literature available on the effect of health drinks on the tooth. Hence, this study was undertaken to assess the pH and titrable acidity (TA) of common commercially available soft drinks and their effect on enamel dissolution and surface micro-hardness of the tooth.

MATERIALS AND METHODS: This study was carried out in the Department of Conservative Dentistry and Endodontics, J.S.S. Dental College and Hospital, Mysuru in the year 2017 after obtaining ethical clearance from the Institutional Ethical Committee [JSS/DCH/IEC/MD-15/2015-2016(2)].

Specimen Preparation: 60 extracted non-carious human permanent molar teeth which were free of caries, cracks, fractures, non-carious lesions, restorations and visible features of fluorosis were collected. They were decoronated at the cement-enamel junction after obtaining flat occlusal enamel surface using diamond disks in a low-speed hand-piece, and two coats of nail varnish were applied to the decoronated part of the dentinal surface.

Solution Preparation: Commercially available, carbonated drinks, *i.e.* Pepsi cola and Urzza the liquid charger and non-carbonated drinks, *i.e.* Tropicana Orange delight, Patanjali Amla Amrit and Kinley water were selected and subjected for the following assessment immediately after opening container.

i. pH Measurement: The initial pH was measured by using 10 ml of soft drink at room temperature placed in a test-tube and a Benchtop Digital pH meter (Mettler Toledo India Pvt., Ltd., Mumbai, India). The electrode was calibrated at the beginning of each session using standard buffers of pH 5.5 and 7.0, and the mean of 3 readings was taken for each sample.

ii. TA Measurement: TA was estimated by acid-base titration with a standard NaOH solution using phenolphthalein indicator. It was estimated by the amount of 0.1N NaOH required to bring the pH of 5 ml of the test drink to a pH of 5.5 and 7.0. A total of 3 readings were taken for mean measurement of the TA for each sample. The specimens were randomly assigned to five groups **Table 1**.

Before the immersion, the samples were preweighed and recorded in grams using a microbalance with a sensitivity of 0.0001 (Schimadzu Analytical India Pvt., Ltd., Delhi). All specimens were exposed to 5 ml of soft drinks at room temperature in closed transparent plastic boxes which was changed every 24 h. After 14 days the specimens were dried and weighed again. The mean weight loss was calculated. Samples were then subjected to Vickers micro-hardness after embedding in acrylic resin in micro-hardness tester (HWMMT- XT; Highwood). Three indentations were made on the flattened occlusal surfaces with a load of 100 g, 15 s dwell time, and the mean was calculated for each specimen in Vickers hardness number (VHN).

TABLE 1: BASIC INFORMATION OF THE VARIOUS SOFT DRINKS EVALUATED

Groups	Material	Manufacturer	Ingredients
1	Kinley water	Coca-cola India Pvt., Ltd., (CCIPL)	Treated water, salts of sodium and magnesium
2	Pepsi Cola	Pepsi Co.	Carbonated water, high fructose corn syrup, caramel color, sugar, phosphoric acid, caffeine, citric acid, and natural flavors
3	Urzza the liquid charger	Bisleri International Private Limited	Carbonated water, sugar, citric acid, sodium benzoate, Vitamin C, sodium citrate, tartaric acid, B group Vitamins, and natural flavors
4	Tropicana orange delight	Pepsi Co.	Water, concentrated orange juice, sugar, acidity regulator, stabilizer, salt, natural color and added flavor
5	Patanjali amla amrit	Patanjali	Sodium benzoate, amla swaras (*Phyllanthus emblica*)

Statistical Analysis: Descriptive statistics were done to obtain mean and standard deviation for all the parameters in the groups. Comparison between the groups was analyzed using one-way analysis of variance (ANOVA) and Scheffe's post-hoc test to determine the presence or absence of statistically

significant difference wherever necessary. The p-value of <0.001 was set as the significance level. Pearsons, correlation coefficient test, was done to check if there was a correlation between any parameters. SPSS Version 22 was used for statistical analysis.

RESULTS: The comparison of the mean pH, titrable acidity at 5.5 and 7, weight loss in grams and Vickers micro-hardness in VHN of all the five groups and carbonated and non-carbonated groups are given in **Table 2** and **Table 3** respectively.

TABLE 2: COMPARISON OF THE VARIOUS PARAMETERS OF ALL THE GROUPS

Groups	pH	TA		Weight loss in grams	Vickers micro-hardness in VHN
		5.5	7		
1	6.72[a]	-	-	.0004[a]	283.65[a]
2	2.553[b]	3[a]	8[a]	.0226[b]	253.9042[b]
3	2.693[c]	1.8[b]	2.7[b]	.0783[c]	217.0075[c]
4	3.741[d]	4.4[c]	6[c]	.0655[d]	213.4075[c]
5	2.642[e]	4[d]	5.5[d]	.1133[e]	190.5475[d]

*****values denoted with the same superscript alphabet in a column have no statistically significant difference

TABLE 3: COMPARISON OF THE VARIOUS PARAMETERS OF CARBONATED AND NON-CARBONATED GROUPS

Groups	pH	TA		Weight loss in grams	Vickers micro-hardness in VHN
		5.5	7		
1 (Control)	6.72[a]	-	-	.0004[a]	283.65[a]
2 and 3 (Carbonated drinks)	2.623[b]	2.4[a]	5.35[a]	.0505[b]	235.4558[b]
4 and 5 (Non-carbonated drinks)	3.1915[c]	4.2[b]	5.75[a]	.0894[c]	201.9775[c]

*values denoted with the same superscript alphabet in a column have no statistically significant difference

Pearson's correlation indicated that there is a negative correlation between pH with weight difference (.650) and a positive correlation with micro-hardness (.719) which is statistically significant. Titrable acidity at 5.5 has a positive correlation to weight difference (.640) and a negative correlation to micro-hardness (-.774) that is statistically significant. Titrable acidity at 7 has a negative correlation with micro-hardness (-.428) that is statistically significant but has a positive correlation with weight difference (.286) which isn't significant.

DISCUSSION: Erosion is the abnormal loss of tooth substance caused by a chemico-mechanical action, but not from acids associated with bacteria. The causative factors for tooth erosion are numerous and can either be intrinsic or extrinsic in origin [6]. Diagnosis of the exact causative agent for erosion in an individual is very challenging and can be frequently misdiagnosed. Previously pH (initial H^+ ion concentration) was considered as the indicator of the erosive potential of a food or drink. Review of literature concluded that TA is a more accurate measure of the total H^+ content of a drink, therefore be a more realistic means of predicting erosive potential [7].

The pH of all the test drinks ranged from 2.55 to 3.74 which are far below the critical pH of enamel. At critical pH, the titrable acidity of Tropicana orange delight was the highest followed by Patanjali Amla Amrit which were both fruit juices. The pH and titrable acidity of carbonated drinks were statistically significantly lower than non-carbonated groups which were similar to previous studies [3, 8, 9].

Comparison of the weight loss and VHN among the groups showed that amla Amrit (*Phyllanthus Emblica*) showed the highest value and Pepsi cola showed the least value. Non-carbonated drinks showed higher weight loss and VHN which is by other studies [3, 8, 10]. So, the findings are correlated more so with the titrable acidity rather than the pH. According to a study conducted by Anthony JF and Matthew MR, enamel dissolution by non-carbonated drinks and cola drinks was 90 - 180 and 55 - 65 greater than water respectively [8]. Seow WK and Thong KM reported that the Vickers hardness of enamel was significantly reduced by about 50% in the case of fruit juice and 24% in the case of Coca-cola [10]. Soft drinks contain acidulants such as phosphoric, citric, malic, tartaric, and other organic acids.

These polybasic acids exhibit buffering capacity and can maintain the local pH at the tooth surface below the threshold value. Beverages supplemented with refined carbohydrates or sugars (sucrose, high fructose corn syrup), sticky nature of fruit drink, the chemical composition of the fruit and the additives may be contributing factors to tooth dissolution.

Contradicting results was found in a study by Tadakamadla J *et al*., who observed that carbonated drinks were observed to have more enamel solubility potential than fruit juices as the viscosity of a drink, together with contact angle and surface tension, determines its ability to penetrate a capillary space such as pores in enamel [11] and Wongkhantee S *et al*., in their study found that the softening effect on enamel by cola was significantly higher than the sports drink which was significantly higher than the orange juice which could be due to the relatively short soaking protocol [2].

In this study, both Pepsi cola in the carbonated group and Tropicana orange in the non-carbonated group showed higher titrable acidity, but they showed relatively lower weight loss compared to their counterpart in the same group. This may be attributed to the fact the composition of the drinks may undergo oxidative changes on exposure to the atmosphere over 24 h; this may alter the pH and titrable acidity of the drink which was not evaluated. On comparison of Tropicana orange delight group and the Urza group, there is statistically significant lower enamel solubility in the later than but no significant difference in their micro-hardness value. The probable cause for this may be the surface enamel characteristic in all the samples may vary, which could play an important role in determining the micro-hardness of the tooth. It may be argued that the results of this study may not be directly related to the clinical setting.

In the oral cavity, the quantity and quality of saliva along with numerous other factors like salivary clearance, composition, and viscosity also play a role on the degree of erosion. Remineralizing agents like fluoridated products also alter the rate of erosion. However, in this study, we were mainly concerned with the quantitative erosive potential of the soft drink. These findings play a pivotal role in diet counseling so to decrease the incidence of erosive lesions. Patients with dry mouth, dehydration and debilitating conditions which are advised to consume a lot of non-carbonated beverages must be made aware of the risks and advised to take the appropriate preventive measures.

CONCLUSION: Within the limitations of this study, it can be concluded that:

1. Titrable acidity is a more appropriate criteria than pH to analyze the erosive potential of a drink.
2. Non-carbonated beverages have a higher titrable acidity than a carbonated beverage which is associated with increased enamel solubility and least micro-hardness values.
3. Patanjali amla amrit had a low pH, high titrable acidity and was proven to be the most erosive of the soft drinks tested.
4. Kinley water was the least erosive of all the soft drinks tested

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CONFLICT OF INTEREST: The authors had no conflict of interests.

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