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Comparative evaluation of salivary pH and buffering capacity of commonly available flavoured drinks in India

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ABSTRACT

Background & objectives: *There is a general perception amongst consumers that fruit flavoured drinks are essentially water with subtle flavoring. As a consequence, they are perceived as being dentally safe. However, these drinks come with a drawback as far as oral health is concerned. An increased consumption of flavoured drinks such as soft drinks, sports drinks and fruit juices is becoming noteworthy because of concern for dental erosion. The objective of this study was to measure the buffering capacity of the drinks used, and evaluate pH of saliva after intake of various flavored drinks at different time intervals.*

Materials and methods: *The present study included 30 subjects, 15 male and 15 female, with the mean age group of 7±1.2 years. Five flavoured drinks were used in this study, to measure the salivary pH after consumption of the drinks, and to assess the buffering capacity of each drink. The drinks used were; apple juice, Appyfizz, milk based drink, Pepsi and Gatorade. Buffering capacity of each drink was estimated by titration with sodium hydroxide. The results were tabulated and analysed statistically.*

Results*: Significant fall in the salivary pH was recorded for Pepsi and Gatorade groups, while the milk based drink maintained the pH of the saliva to the resting value. Therefore, milk based drinks are a safer option as compared to soft drinks, and sports drinks.*

Keywords: *saliva; pH; buffering capacity; dental erosion; fruit juices; soft drinks; sports drinks*

I. Introduction

Over the last decade, prevalence of dental erosion seems to have increased presumably due to an increase in the consumption of soft drinks and fruit juices. [1]

In his article titled “risk factors in dental erosion” V.K Jarvinen, I.I Rytomaa and O.P Heinonen stated that

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Pindborg in 1970 defined dental erosion as "irreversible loss of dental hard tissue due to a chemical process without the involvement of microorganisms." [2] Children and adolescents consume significant amounts of these mostly erosive beverages and therefore their risk of developing dental erosion is high, and have a matter of concern [3]

II. Materials and methods

This study was conducted in the department of pedodontics and preventive dentistry, Institute of dental studies and technologies, Modinagar in collaboration with Saraswati junior high school, to evaluate the pH of saliva following consumption of flavoured drinks in children, and measure the pH and buffering capacity of the drinks.

Selection criteria of children

Sample size and Sample selection:

30 healthy subjects (15 male, 15 female) with a mean age of 7 ± 1.2 years were randomly selected from the standard III-V of the Saraswati junior high school

Inclusion criteria:

- Age group of 8-10 years
- DMFT score not more than 3.
- Written informed parental consent for participation.
- Ability to cooperate and follow the instructions given regarding the beverage intake

Exclusion Criteria:

- Mentally / physically handicapped children
- Children with salivary gland disorders.
- Children on medications that influence the flow of saliva.

Materials used for study (Figure 1, Table 1):

1) Group 1:

Real apple juice (Dabur co.) [pH- 5.2]

2) Group 2:

Appy fizz (Parle Agro pvt. ltd.) [pH- 6.6]

3) Group 3:

Amul cool (Amul pvt. ltd) [pH- 7.3]

4) Group 4:

Pepsi (Pepsi co) [pH- 2.75]

5) Group 5:

Gatorade (Quaker oats) [pH- 4.2]

6) Digital pH meter (ELINCO PHX- 1400 pH meter)

7) Distilled water

8) Buffer tablets (pH 4.00 and pH 7.00) (ELINCO)

9) Beaker

10) Sterilized gloves

11) 0.1 M sodium hydroxide

Saliva collection and methodology

Subjects were instructed not to eat or drink anything except water two hours prior to the study period. In order to account for circadian rhythm, all the beverages were always given at same time of the day on subsequent days at 10.00 A.M at least 60 minutes after the last meal (breakfast). [4, 5]

Subjects were made to sit and relax. After 10 mins, 100ml of the respective drinks were given to the subjects in glasses, to ensure uniform intake (Figure 3).Whole saliva was collected for estimating pH changes.1ml of saliva was collected by asking the patient to bend the head slightly down and accumulating saliva in his mouth, and then he/she was asked to spit the accumulated saliva in a beaker with markings (Figure 4, 5).

Method for measuring the pH (Flavoured drinks)

The pH meter was calibrated before,in between and at the end of the study using standard solutions of pH 4.00 and pH 7.00.Salivary pH at baseline was determined. Subjects were given 100ml of respective drinks served at room temperature and asked to drink directly from the glass.

Salivary samples were collected at 1min, 5min, 10 min, 15min, and 30 min intervals.

Salivary pH was measured using a portable digital pH meter (ELINCO PHX- 1400 pH METER) (Figure 2).The bulb of the pH electrode was dipped into saliva and switched on. The readings displayed on the screen were recorded when they stabilized to one point (Figure 6)

In between the readings at various time intervals, the bulb was immersed in distilled water to bring the pH reading to neutral.

Measuring the buffering capacity of the beverages [6]

Buffering capacity was evaluated by measuring the volume of 1.0M sodium hydroxide required to; bring the pH of 50 ml of the drinks to pH 5.5 and pH 7.0.

1.0M of sodium hydroxide base was prepared by dissolving 1g of sodium hydroxide pellets in 250 ml of distilled water. This was then titrated against 50 ml of each drink. The values were then put to statistical analysis using SPSS (Statistical Package for the Social Sciences) 15 software and presented with mean & standard deviations. Within group difference in mean were analysed by using repeated measure ANOVA and between the groups difference in the mean at each time point were analysed by using ANOVA and TUKEY POST HOC TEST

$P < .05$ was taken as significant.

III. Results

The present study was carried out to evaluate the pH of saliva and change in pH of saliva after consumption of various flavored drinks. Also the pH of various drinks and their buffering capacities were evaluated.

The study group consisted of 30 subjects; 15 male and 15 females (Table 2). A crossover study design was used and five commercially available flavored drinks were evaluated. Salivary pH was recorded at 1, 5, 10, 15 and 30 minutes

Salivary pH:

Intergroup comparison (Table 3, 4 & 5) (GRAPH 1)

- After consumption of various drinks the pH at 1 min was in the following decreasing order – Group 1 (Apple juice) > Group 2 (Appy fizz) > Group 3 (Milk based) > Group 5 (Sports drink) > Group 4 (Pepsi).
- After consumption of various drinks the pH at 5 min was in the following decreasing order – Group 2 (Appy fizz) > Group 3 (Milk based) > Group 1 (Apple juice) > Group 5 (Sports drink) > Group 4 (Pepsi).
- After consumption of various drinks the pH at 10 min was in the following decreasing order – Group 2 (Appy fizz) > Group 3 (Milk based) > Group 1 (Apple juice) > Group 5 (Sports drink) > Group 4 (Pepsi).
- After consumption of various drinks the pH at 15 min was in the following decreasing order – Group 2 (Appy fizz) > Group 3 (Milk based) > Group 1 (Apple juice) > Group 4 (Pepsi) > Group 5 (Sports drink).
- After consumption of various drinks the pH at 30 min was in the following decreasing order – Group 2 (Appy fizz) > Group 3 (Milk based) > Group 1 (Apple Juice) > Group 5 (Sports drink) > Group 4 (Pepsi).

Groupwise comparison (Table 3, 4 & 5) (GRAPH 1)

- Apple juice group:

The initial pH was 7.34±1.5. After the consumption of drink at 1 min the pH was 6.94 ± 0.11 which after 30 min rose to 7.27 ± .08.

- Appyfizz group:

The initial pH was 7.33±1.5. After the consumption of drink at 1 min the pH fell to 6.74 ± 0.11 which after 30 min rose to 7.25 ± .05.

- Milk based group:

The initial pH was 7.35±0.27. After the consumption of drink at 1 min the pH fell to 6.67 ±0.32 which after 30 min rose to 7.27 ± .18.

- Pepsi group:

The initial pH was 7.42±0.25. After the consumption of drink at 1 min the pH fell to 4.18±0.26 which after 30 min reached a value of 7.27 ± .18. In this group it was noticed that the pH remained below the critical level even after 5min, i.e. 4.84±0.41.

- Gatorade group:

The initial pH was 7.28±0.17. After the consumption of drink at 1 min the pH fell to 4.43±0.25 which after 30 min reached a value of 7.4 ± .14, which was lowest amongst all the groups. In this group it was noticed that the pH remained below the critical level even after 5min, i.e. 5.43±0.26.

Buffering capacity of each drink

Table 1 shows that Pepsi had phosphoric acid as acidulant; apple juice and Appy fizz had malic acid, while Gatorade had only citric acid, and the milk based drink had linoleic acid as ingredient. Baseline pH on opening the drinks showed that Pepsi had the lowest pH 2.75; and milk based drink had the highest pH of 7.3.

The volume of sodium hydroxide base needed to raise the pH of the drinks to 5.5 ranged from 0.1 to 1.5ml; and 0.4 to 9.5ml of the base was needed to raise the pH of the drinks to 7.0.

Gatorade needed the most base to raise its pH to 5.5 and 7.0, while milk based drink needed the lowest volume of base to raise its pH to 5.5 and 7.00. Pepsi having the pH value lower than Gatorade on opening needed comparatively lesser base to raise the pH to both 5.5 and 7.0.

The buffering capacities of beverages can be ranked as follows: milk based drink > Appy fizz > apple juice > Pepsi > Gatorade.

Table 1 :Age and sex wise distribution of subjects

AGE IN YEAR	MALE	FEMALE	TOTAL NUMBER OF SUBJECTS EXAMINED
7	1	0	1
8	8	10	18
9	5	4	9
10	1	1	2
TOTAL	15	15	30

Table 2 : Mean ± sd values for all groups at all time intervals

GROUPS	INITIAL	1 MIN	5 MIN	10MIN	15MIN	30MIN
1 (Apple Juice)	7.34 ± .15	6.94 ± .11	7.04 ± .08	7.14 ± .07	7.22 ± .08	7.27 ± .08
2 (Appy Fizz)	7.33 ± .15	6.74 ± .33	6.86 ± .30	7.01 ± .23	7.16 ± .15	7.25 ± .20
3 (Milk based)	7.35 ± .27	6.67 ± .32	6.87 ± .28	7.1 ± .20	7.2 ± .19	7.27 ± .18
4 (Pepsi)	7.42 ± .25	4.18 ± .26	4.84 ± .41	6.09 ± .44	6.9 ± .33	7.21 ± .21
5 (Sports Drink)	7.28 ± .17	4.43 ± .25	5.43 ± .26	6.4 ± .39	6.98 ± .19	7.4 ± .14

Table 3 : Comparative analysis of salivary pH at different time intervals using anova

		Sum of Squares	df	Mean Square	F	Sig (p<0.05)
Initial	Between Groups	.336	4	.084	1.928	.109
	Within Groups	6.323	145	.044		
	Total	6.660	149			
0 min	Between Groups	222.585	4	55.646	762.245	.000
	Within Groups	10.585	145	.073		
	Total	233.171	149			
5 min	Between Groups	120.432	4	30.108	354.533	.000
	Within Groups	12.314	145	.085		
	Total	132.745	149			
10 min	Between Groups	26.599	4	6.650	72.566	.000
	Within Groups	13.288	145	.092		
	Total	39.887	149			
15 min	Between Groups	2.479	4	.620	14.105	.000
	Within Groups	6.370	145	.044		
	Total	8.849	149			
30min	Between Groups	.714	4	.178	6.027	.000
	Within Groups	4.292	145	.030		
	Total	5.006	149			

Table 4 :Post hoc analysis at different time intervals

GROUPS	1 MIN	5 MIN	10MIN	15MIN	30MIN
1 (Apple Juice)	6.94 ± .11	7.04 ± .08	7.14 ± .07	7.22 ± .08	7.27 ± .08
2 (Appy Fizz)	6.74 ± .33	6.86 ± .30	7.01 ± .23	7.16 ± .15	7.25 ± .20
3 (Milk based)	6.67 ± .32	6.87 ± .28	7.1 ± .20	7.2 ± .19	7.27 ± .18
4 (Pepsi)	4.18 ± .26	4.84 ± .41	6.09 ± .44	6.9 ± .33	7.21 ± .21
5 (Sports Drink)	4.43 ± .25	5.43 ± .26	6.4 ± .39	6.98 ± .19	7.4 ± .14
P VALUE	.001	.001	.001	.001	.001
POST HOC	1>4, 5 2>4,5 3>4,5 5>4	1>4, 5 2>4,5 3>4,5 5>4	1>4, 5 2>4,5 3>4,5 5>4	1>4, 5 2>4,5 3>4,5 5>4	1>4, 5 2>4,5 3>4,5 5>4

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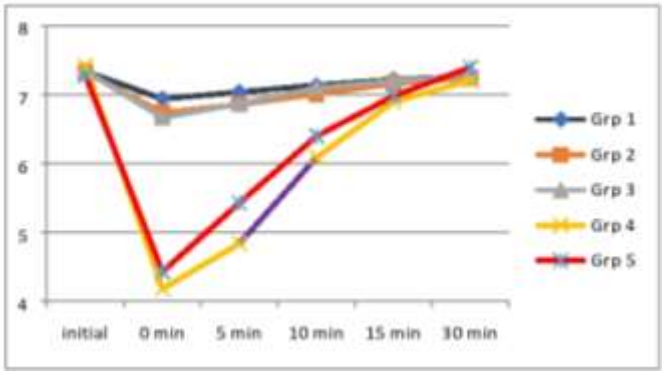
Figure 1:Respective drinks were given to the subjects in glass

Figure 2: Saliva collection from the subject

Figure 3: 1 ml. of salivary sample

Figure 4: Measurement of salivary pH

Figure 5: Mean values of salivary pH of all drinks at all time intervals



IV. Discussion

Dental erosion is defined as an irreversible loss of dental hard tissue by a chemical process without the involvement of microorganisms and is due to either extrinsic or intrinsic sources.[6,7,8] Dietary erosion may result from food or drinks containing a variety of acidic ingredients.[7] Frequent consumption of these easily and widely available beverages has been shown to cause erosion of the enamel in both in vitro and in vivo studies. Children and adolescents consume significant amounts of these mostly erosive beverages and therefore their risk of developing dental erosion is high. Soft drink intake in children is generally greater than adults, but has a huge individual variation.Deciduous teeth are smaller than permanent teeth, the enamel is thinner, and there are morphological differences compared to permanent teeth. Therefore, the erosive process reaches the dentine earlier and leads to an advanced lesion after a shorter exposure to acids, compared with permanent teeth.[9] It can be stated that dietary factors represent the most important external risk factor for children to develop dental erosion. [7, 9, 10] Majority of studies dealing with pH and food intake are carried out in relation to bacterial plaque and caries development. There has been a tectonic shift in the last few years in contemporary lifestyle which paired with easy availability of

different kinds of drinks for children makes it imperative that results from studies such as this be made known. Most carbonated beverages and sport drinks have a pH below 3.5 and experiments have shown that enamel dissolution occurs below pH 4.

The erosive potential of the beverages is thought to involve several factors, including low pH, and high buffering capacity of the drink. [11, 12] Soft drinks may contain several different types of acid that contain carbonic acid formed by carbon dioxide solution.

In our study, thirty subjects were randomly selected and were made to drink one of the drinks of the five groups on subsequent days. Unstimulated saliva samples of 1ml were collected from the subjects by asking them to slightly bend down their heads and expectorate in a plastic beaker with markings. Then salivary pH values were measured at 1min, 5min, 10 min, 15min and 30 minutes.

In order to account for the circadian rhythm, all the beverages were always given at the same time of the day on subsequent days. [4, 5] For, pH measurements, all the subjects sipped the beverages from the same type of glasses and saliva was collected.

The beverages reported on here are representatives of most frequently consumed acids, viz, citric acid, malic acid and phosphoric acid.

Theoretically, the erosive potential of a soft drink must be dependent upon the immediate effect of the drink on the tooth surface, the time it takes to clear from the mouth, the drinking method, the protective effect of saliva, the amount of residual drink after swallowing, the actual amount of beverage consumed and frequency of consumption. [12, 4] In a review by Lussi and Jaeggi, [13] pH values of a drink or foodstuff among other factors was important in explaining the erosive attack.

The lowest pH value recorded in our study was Pepsi, i.e. 2.75, and it proved to be the most erosive. The salivary pH values recorded for each drink 30 min after consumption was similar for Apple juice and milk based group, i.e. 7.27; whereas the lowest values were recorded for Pepsi (7.21) and Gatorade (7.4).

In the intergroup comparison it was found that salivary pH values dropped to the lowest level within 5min after drinking, which was in accordance with the study done by Jensdottir et al. where it was pointed out that the pH of drinks determines their erosive potential within first minutes of exposure. [14]

Since, this study evaluated and compared pH changes following consumption of most frequently available flavoured drinks in the Indian market, we did not encounter any previous study in literature which had similar drinks for research. Hence, it was not possible to make any comparison with other similar studies. According to Edwards M., Creanor S.L., Foye R.H., and Gilmour W.H it is generally accepted that titratable acidity, which is a measurement of the total acid content, is a more important indicator than actual pH value in determining erosive potential of beverages. [11]

In our study, values of salivary pH were statistically significant between the milk based group (pH-7.27) and Pepsi (pH- 4.12) making milk based drinks a safe alternative to soft drinks.

The pH of all drinks investigated in our study ranged from 2.75-7.30 on opening, amongst which Pepsi (pH-2.7), apple juice (pH-5.2) and Gatorade (pH-4.2) had values below the critical pH at which enamel dissolution occurs. [15]

This was quite similar to the finding of Touyz who concluded that Canadian fruit juices had pH below the critical dissolving pH of enamel [16]. Buffering capacity has been found by several studies to affect the erosive potential of soft drinks and Zero has suggested that it should be considered more important than pH in determining the erosive potential of drinks. [6]

Fruit juices needed the most base to neutralize thereby having greater erosive potential than the cola and non-cola drinks. The cola drinks despite having the lowest pH on opening were easy to neutralize than the fruit juices and non-cola drinks, i.e. they required only 1.5ml and 3.8 ml of NaOH to make the pH 5.5 and 7.0 respectively; whereas, the sports drink required the maximum amount of NaOH, i.e 7ml and 9.5 ml to make the pH, 5.5 and 7.0 respectively.

This was quite similar to the findings of two studies by Jensdottir and co-workers and Bamaise C.T et al. [13, 14]

It was also interesting to note that initial pH value gave no indication of the underlying buffering capacity and, therefore, the erosive potential of the drink. Generally, the pure fruit juices had a higher initial pH than the carbonated drinks but required much more NaOH to raise the pH. This study agrees broadly with those already found in the literature which state that fruit juices have greater erosive potential.

Maximum fall in the salivary pH was recorded by subjects within 1 minute, followed by a gradual recovery to near normal values in 30 minutes. The gradual recovery of pH can be attributed to neutralizing effects of saliva, by virtue of its buffering system (bicarbonates) which gets activated with the increased salivary secretions, as a result of an acidogenic challenge. [17]

The resting salivary pH usually ranges from 6.5-7.4. When a low pH drink is consumed it causes a fall in this resting salivary pH. The length of the time for which this low pH remains at its minimum is important, since it reaches the critical pH, it initiates dissolution of enamel hence lower the pH, faster is the demineralization. [18]

In our study, the salivary pH values were recorded as: Milk based >Appyfizz>Apple juice>Gatorade> Pepsi

Among, the five drinks used in the study, Gatorade showed significantly greater fall in the salivary pH followed by Pepsi.

Milk based drinks, showed the least fall in salivary pH, even at 1 minute. But, within limitations of using these milk based drinks, as they can lead to dental caries, it can be stated that, if pressed for a choice they can be used as an alternative to these carbonated drinks and fruit juices.

V. Conclusion:

Thus, from the present study, the following conclusions can be drawn:

- The drop in salivary pH value in the 30min duration was recorded minimum for the milk based group; whereas the maximum drop in salivary pH values were recorded for the sports drink group.
- The buffering capacity was found to be lowest for the Milk based group. Pepsi having the lowest pH amongst all the drinks used, comparatively needed lesser base to raise the pH to both 5.5 and 7.00, than Gatorade; thus having a lower erosive potential than Gatorade. Gatorade had a higher buffering capacity as compared to the Pepsi, Apple juice and Appyfizz group.
- Taking into the consideration, the salivary pH values at all time intervals, the initial pH and buffering capacity of all the drinks used; it was found that Milk based drink had the lowest erosive potential, whereas Gatorade had the highest erosive potential.

Apple juice and Appyfizz groups were also found to cause a significant fall in salivary pH values, so they cannot be considered to be dentally safe.

Hence caries risk assessment and a customized approach to dietary advice and future care is mandatory for children who frequently consume soft drinks and fruit juices. Milk based drink may be a safer alternative for consumption as compared to cola and sports drinks.

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