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Evaluation of Salivary Changes Following Intake of Different Beverages in Children at Different Time Intervals: A Randomized Controlled Trial

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Abstract

Aim: The purpose of this study was to evaluate changes in the salivary pH after the consumption of different beverages in children at different time intervals.

Material and Methods: In this cross-over, single blinded, eighty children between the ages of 7 to 12 years having a strict vegetarian diet were included in the study for consumption of four different fruit juices (i.e. Guava, Orange, Apple, Pomegranate) by dividing them into 4 groups based on caries experience selected. The endogenous pH of the salivary samples was measured at the baseline and after consumption of the fruit juices at 5, 15 and 30 minutes. Data was collected separately for simple fruits, fresh fruit juices and processed juices. The intrinsic pH of fruit juices was noted by the digital electronic pH meter. The collected Data were statistically analyzed by using ANOVA.

Results: The processed fruit juices caused acidity in saliva than fresh juices and fruits after 5 mins. After 15 mins of intake of juices and fruits, the pH of fresh fruit juices moved towards neutral pH while that of processed fruit juices was found to be still acidic. After 30 mins, pH tends to be alkaline for both fresh and processed juices. There was the least change in salivary pH with the use of pomegranate fruits and juices during the whole study.

Conclusion: Pomegranate has less changing effect on saliva pH than all other fruits used in the study and processed fruit juices have a significant influence on the change of salivary pH. The use of fresh fruit juices can be suggested in future.

Keywords: Fresh Juice; Processed Juice; Salivary pH; Salivary Buffer.

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INTRODUCTION

Oral health has often been viewed in isolation from general health.

Diet plays an important role in balancing demineralization and remineralization in the oral cavity.[1]

The normal pH of saliva is 6.7 to 7.4 but as bacteria break down the carbohydrates, they release Lactic

acid, Butyric acid, and Aspartic acid which bring down the pH of saliva.[2]

When the pH level in the mouth goes below 5.5 (i.e., the critical pH value), the acids begin to break down the enamel on the teeth. The longer the teeth are exposed to a low salivary pH, the more likely the development of dental caries.[3]

The composition of saliva is potentially of great importance for the prevention of caries. Saliva contains buffers that tend to reduce the fall in pH that is associated with acid formation from carbohydrates by the dental plaque.[4]

The presence of dental caries makes the oral cavity more vulnerable to the effects of acidogenicity than its absence.[5]

The concept of health has prevailed for centuries, and "Healthy Eating" is perceived to be important dietary habits are changing with modernization and with evolution; drastic transformations in the dietary patterns from a high roughage diet to a refined diet have been observed.[6]

Fruits are always touted as a healthy food choice and are recommended in large quantities. Fruits contain a variety of vitamins and minerals that contribute to the daily functions of the body. Although fruits have many health benefits, there can be possible side effects associated with their consumption.[7-8]

In this modern age, the use of junk food items and snacking between meals is commonly seen in the younger age group and among their peer groups.[9]

Hence, the following study was undertaken to assess the effect of selected locally available fruits and their beverages before and after the consumption on salivary pH, and flow rate changes at various time intervals.

RESEARCH METHODOLOGY

Research Hypotheses:

It was hypothesized that; there is a dynamic two-way relationship between diet and salivary pH.

Research Design:[10]

This study was a randomized (lottery method), cross-over, double-blind, multiple arms, parallel clinical trial, and quasi-experimental pre and post-test study.

Subjects were selected irrespective of their caste, religion, dietary habits, and other lifestyle status.

Blinding, Randomisation and Allocation Concealment:

Enrolled participants in all four arms and study coordinators were blinded to the study group.

All four fruits were provided as identically labelled white packing precoded by the study coordinators intermixed in the box.

Informed Consent and Confidentiality:[11]

All the subjects were invited to participate in the survey.

The nature and underlying principle of the study were explained to the subjects followed by obtaining written informed consent along with their parents, in vernacular language.

None of the subjects participating in the study was coerced in any way or rewarded for their involvement. Their right to withdraw- if so desired at any stage of the study was also stated clearly to them.

To ensure the confidentiality of the data, the crude data as well as responses were destroyed upon completion of this research.

Study setting and Assessors:[12]

The purposive sample was composed of (n=80) of participants without dental knowledge or experience and were of Caucasian origin.

The principal investigator **(Assessors)** was available to clarify their doubts about any point while completing the survey.

Study Period:

The present study was conducted in two different settings, a private hospital, and a private dental clinic in and around Vellore city. The study period was from Dec 2023 to Jan 2024.

Sample size Calculation and sampling technique:[13]

Before experimenting, a power analysis[13] (n=10) was performed to identify the sample size required to detect significant effects accurately using the ANOVA procedure. They were excluded from the present study.

The results of the analysis indicated that all the measurements were reproducible with significant intra and inter-observer discrepancies.

The Sample Sizes for Two Independent Samples and the dichotomous outcome of the study were determined by fixing the probability of type I error at 5% and that of type II error at 20%.

Thus, Sample size was predicted using 80% power at the 5% level of significance by standard statistical protocol.

$$n \text{ (sample size)} = \{P_1(1-P_1) + P_2(1-P_2)\} (Z/E)^2$$

Were,

- P1 and P_2 are the proportions of successes in each comparison group. The values of P_1 and P_2 that maximize the sample size are P_1-P_2=0.5.
- n- Sample size
- Z = 1.96 (for 80% power).
- E is the desired margin of error (4%).
- Σ- Standard deviation = 2.82.
- Hence, n = 67

This study involved 87 (Age range 7-12 years) consented to healthy children as subjects in the present study.

However, of the total participants, 92% (n=80, Male 44, Female 36 respectively) accepted to sign the consent form and were admitted to this study. The main reason to refuse contribution (n = 7) was due to being observed by another person during his or her task and lack of time.

Sampling Technique: This is a retrospective cross-sectional, stratified randomization method study.

Aim of the Investigation:

The purpose of this study was to evaluate changes in the salivary pH after intake of different beverages in children at different time intervals.

Research Objectives:[14]

The objectives are as follows:

a) To assess the acidogenic potential of fruit and its beverages.

b) To evaluate the buffer capacity and its relationship with the acidogenicity of these products after consumption at various time intervals.

Inclusion Criteria:[14]

- Subjects who were 7-12 years of age.
- Subjects who were caries-free, that is, with ICDAS score = 0.
- Cooperative patient according to Frankel behaviour rating scale. (positive and definitely positive)

Exclusion Criteria:[15]

- Subjects who were using any medication at the time of study or in the period of the last 15 days before the study.
- Children using any Prosthetic/Orthodontic appliance.
- Children with a history of systemic disease or illness and, allergies.
- Parent or child not willing to give consent.

Research Strategy:

Stadiometer calibrated in centimetres (Hospiguard, MG-056) Weighing scale (Hoffen Digital HO-18), Examination Gloves (Surgicare), Eppendorf Tubes, distilled water (Water Care Technologies) GC Saliva-Check BUFFER kit, diagnostic aids, Permanent markers and proforma containing the participants' demographic data.

Research Tool:[16]

Before the study, the investigator was trained and standardized through a series of training exercises. This procedure included a series of theoretical overviews, discussing issues that might be encountered during the study period. The mean Kappa value used to test the reliability of the intra-examiner was > 0.8.

Assessment of pH and titratable acidity[20]

The initial pH was determined using a digital pH meter (Hanna, USA). The pH meter's bulb was immersed in a sample containing saliva to record the salivary pH, after which the pH values were displayed digitally on the pH meter's body.

In between readings, cleaning of the electrode with distilled water was carried out which was then dipped in a standard solution having pH 7·0, to ensure a stable reading.

The digital pH meter, with an accuracy of 0.1 was first calibrated according to the manufacturer's instructions employing buffer standards (pH 4.0, 7.0 and 10.0 respectively) at room temperature. The titratable acidity was then measured by adding 1M sodium hydroxide (NaOH) in increments of 0.2 ml to 100 ml of the freshly prepared/opened fruit-based beverages until the pH reached 5.5 and 7. The pH reading was measured after each increment (0.2 ml) of NaOH until a stable pH was achieved.

All the measurements were repeated in triplicates. The collected data was tabulated, and comparisons were made among the three study groups.

This ensured stable readings and provided a constant check on any drift.

Procurement of raw Material: [17-18]

Fresh Fruit Juices: 100% juice **(**Guava, Orange, Apple, and Pomegranate) was freshly extracted without added sugar or water. Table 1

Fruits were peeled off and pulp was chopped into the desirable size. The seeds were removed from seed fruits.

Processed juices: **Packed Fruit Juice and Packed Fruit Drinks** of Guava, Orange, Apple, and Pomegranate were obtained from the local market of Vellore on the same day of the study. **Table 2**

Three tools were developed for collecting data.

They were divided into 3 tools.

a) Structured Interview Schedule.

b) The intrinsic pH of all four fruit beverages.

c) Estimation of saliva changes.

Tool I: *Structured Interview Schedule:* It was developed by the research team after reviewing the related literature and collecting data related to the parents and children.[10, 19]

This tool included two parts:

- **Part A:** Social-demographic Variables of Respondents such as Age (years), and Gender.[10] **Table 3**
- **Part B:** Estimation of BMI[19]
- The age of participants was recorded from their academic records. Height (Kg) and weight (mts) were measured sequentially for each child before the oral examination. Body mass was measured to the nearest 0.1 kg by a digital balance (Digital best India weighing scale).
- The Body mass index (BMI) was calculated using the formula:

$$BMI\ (Kg/m^2) = Wt\ (Kg)/Ht^2\ (m).$$

Tool II: *The intrinsic pH of all four fruit beverages:* Estimation of the endogenous pH of test beverages was done before the saliva collection.[7-10]

Tool III: *Estimation of saliva changes:*[1,20]

According to the 5th edition of the Basic Methods of Oral Health Survey of WHO (World Health Organization (WHO-2013)[1], 80 children were examined by two experienced Pediatric dentists under natural light using a disposable dental mirror (NMD Dental) and a community periodontal index (Waldent CPI) probe, and the DMFT scores were recorded to assess the caries status of the mixed dentition. Carie's experience level was assessed based on age and DMFT scores. No x-ray was taken.[30]

This tool included Two parts:

Collection of Salivary Samples[20]

The selected children after exclusion and inclusion criteria were assessed on the first day of the study.

Volunteers were asked to refrain from oral hygiene procedures 24 hours before and were kept NPO at least two hours before the study.

To control the circadian variations, samples were collected in the morning. Children were asked to rinse their mouths with water thoroughly 10 minutes before collection of saliva to avoid the contamination of food debris.

For collection of unstimulated saliva, all subjects were asked to sit comfortably on a normal chair with their head bent forward.

The children were instructed to let saliva collect in the floor of the mouth without swallowing it for at least 1 minute, and then to expectorate into the sterile calibrated Eppendorf tub with the help of a sterile funnel. This procedure was continued for 5 minutes until 2ml unstimulated saliva samples were collected.

The sterile calibrated Eppendorf tube was coded with a specific identity number given to each subject for the collection of saliva samples.

All salivary tests were performed by only one person on whole saliva. The saliva collection of children was performed from 9: 00 to 11 am. All samples were transported to the laboratory immediately and stored in a freezer at -80°C.

Stimulated saliva sample post eatable and beverage consumption:[6] The participants were, thereafter, divided into the following three groups: Table 4

Group A (n=20): Consumption of Guava (Table 5)

Group B (n=20): Consumption of Orange. (Table 8)

Group C (n=20): Consumption of Apple. (Table 9)

Group D (n=20): Consumption of Pomegranate. (Table 10)

The children were asked to consume 4 different

types of fruits and beverages according to their allocated group each respectively for subsequent days. After the consumption of thebeverage, a saliva sample was collected at 5, 15, and 30 minutes intervals[7] to determine the salivary pH.

An amount of 100 mL of beverages were consumed by the participants. They were asked to sip each beverage as they usually do at home.

***Estimation of Salivary pH*[20,] Table 1**

The baseline pH of saliva was measured after collecting 5 mL of unstimulated saliva from each subject in a sterile tube.

After the baseline score was documented at each time interval, beverages were tested on all subjects.

The pH of saliva was measured as soon as possible and not later than 10 minutes after the collection of the sample.

***Quantification of Salivary Buffer Capacity for Stimulated Saliva*[21,] Table 2**

Saliva Check Buffer test strips: Itwas carried out buffer capacity was measured by using the colourimetric method by using the GC Saliva Check Buffer kit (GC India Dental Pvt. Ltd.) as per the manufacturer's instructions.

For measurement, the foil package was opened, and a buffer test strip was purged with the test side up and placed onto an absorbent paper. Saliva in sufficient amount was drawn from the collection tube using a pipette provided with the kit and one drop of saliva was released over each of the three well-marked test pads on the strip. To soak up excess saliva on the absorbent paper immediately, the strip was turned 90° to the surface of the paper. By doing this, excess saliva was prevented from swelling on the test pad which could affect the test result accuracy. The test pads began changing colour immediately.

The result was calculated after 2 minutes by adding points based on the observed final colour for each of the pads on the strip. The conversion table for test pad colour was provided in the instruction manual provided by the manufacturer.

The result was interpreted using the scheme in the kit, where each resulting total value corresponds to a degree from "very low" to "normal" salivary buffer capacity, as follows: 0–5: very low; 6–9: low; 10–12: normal.

The flow rate was measured directly from the calibrated test tube after each sample collection.

The recording of the data was done by a well-trained recorder who recorded data on a proforma containing details on the general information and frequency of uptake of the selected test food of each study subject.

To minimise bias or errors in the data, an independent observer, blinded to the study's aim, recorded all the digital pH readings.

The outcome variables are changes in salivary pH on consumption of beverages post acidogenic challenge.

Data Processing and Statistical Analysis[22]

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp. was used to perform statistical analyses.

Descriptive Statistics: The frequency distribution for categorical data is expressed in terms of number and percentage, whereas for continuous data, it is expressed in frequency, mean, and standard deviation for salivary flow, pH, and Buffer.

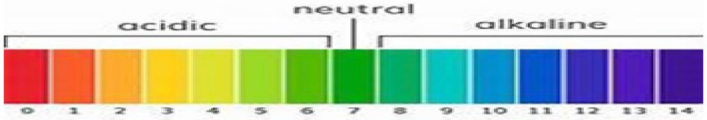
Inferential Statistics: Repeated measures of ANOVA followed by Bonferroni post hoc analysis were used to compare the mean Salivary Flow Rate and hand Buffering Capacity between different time intervals in each study group.

A 95% Confidence Level was used, and a p-value of less than or equal to 0.05 was considered statistically significant.

RESULTS

Table 1: Interpretation of salivary pH

Combined total	Buffering ability of saliva
0-6	Acidic
7	Neutral
14-Aug	Alkaline



Citation: [20] **Franklin** S, Masih S and Thomas AM. Effect on oral pH changes and taste perception in 10–14-year-old children, after calcium fortification of a fruit juice. *Eur Arch Paediatr Dent* 2015; 16: 483–489.

Table 2: Quantification of Salivary Buffer Capacity for Stimulated Saliva

Conversion table: Test pad color after 2 minutes	
Green	4 points
Green/blue	3 points
Blue	2 points
Red/blue	1 points
Red	0 points

When any color combination indicates unclear result, use intermediate scores.

Citation:[21] Maldupa I, Brinkmann A, Mihailova A. Comparative analysis of CRT buffer, GC saliva check buffer tests and laboratory titration to evaluate saliva buffering capacity. *Stomatologija* 2011; 13(2):55-61.

Table 3: Collection of saliva samples at different time intervals

Base line	Before consumption of sample
After 0 minute	Right after last sip or bite of sample
After 5 minutes	After last sip or bite of sample
After 15 minutes	After last sip or bite of sample
After 30 minutes	After last sip or bite of sample

Citation:[6-7]Anum Nazir, UswaAhmad, Nizwa Qamar, Zunaira Abaid, Nishat Zafar, and Sidra Anam. Evaluation of changes in salivary pH after the intake of fruits, fresh fruit juices and processed juices: a randomized control trial. Pure *Appl. Biol*2020; 9(3): 1976-1981.

Table 4: Packed Fruit Juice (Commercially available) DABUR

Fruit Juice	Composition	pH	Manufacturer (Batch Numbers) LOT Number
Guava	Water, 100% pure Guava juice (filtered water and concentrated Guava juice), contains added flavour (natural Guava flavours)	**3.5**	Dabur India Limited NW00137-B
Orange Juice	Water, 100% pure orange juice (filtered water and concentrated orange juice), contains added flavour (natural orange flavours)	**3.7**	Dabur India Limited MU23022B/2
Apple Juice	Water, Concentrated apple juice (17%), Reconstructed 100% apple juice, Contains added flavour (natural Apple flavours)	**3.6**	Dabur India Limited RU0435
Pomegranate	Water, 100% pure Pomegranate juice (filtered water and concentrated Pomegranate juice), contains added flavour (natural Pomegranate flavours)	**3.4**	Dabur India Limited RU0215

Table 5: Packed Fruit Drinks (Commercially available-Tropicana)

Fruit Juice	Composition	Ph	Manufacturer (Batch Numbers LOT Number)
Guava	Water, Guava Pulp 20%, Sugar, Acidity Regulator (330), Antioxidant (300). Contains Permitted Natural Color {160a (I)} and Added Flavor.	**3.8**	PepsiCo,Inc 7467D02L23
Orange Juice	Orange juice 45% (reconstituted), Water, Concentrated orange juice 8.2%, Sugar, Acidity regulator 330, Salt, Stabilizer 440	**3.9**	PepsiCo,Inc 7467D05A24
Apple Juice	Apple juice 44% (reconstituted), water, concentrated apple juice 7%, Sugar, Acidity regulator 296	**3.8**	PepsiCo, Inc 7467 D024O23
Pomegranate	Water, sugar,Concentrated fruit juices (concentrated Pomegranate juice (3.4%), Concentrated red grape juice (0.7%), concentrated apple juice (0.5%), concentrated Aroniaberry juice), Acidity regulator 330, Salt, Stabilizer (466,440), Sweetener (960)	**3.4**	PepsiCo, Inc 7467E09K23

Table 6: Social-demographic variables of respondents

Individual scenario-Descriptive statistics					
Variables		**Respondents (Percentage)**	**ANOVA analysis (Inference)**		**Inferential Statistics**
			Mean ± SD. Comparisons	**Z score Comparisons**	
Total number of respondents			**100%(n=80)**		
Age (years)	7 years	13 (16.2)	7.95 ±1.71	33.85	p< 0.0001 HS*
	8 years	15 (18.7)			
	9 years	20 (25)			
	10 years	17 (21.2)			
	11 years	10 (12.5)			
	12 years	5 (6.2)			
Gender	Male	44 (55)	-	10	p< 0.0001 HS*
	Female	36 (45)			
BMI		80 (100)	15.34±2.79	23.17	p< 0.0001 HS*

Legends/Capitations:

- **SD:** Standard Deviation.
- **ANOVA:** Analysis of Variance.
- **Citation:** Hajifattahi F, Hosseini Jeddi S and Khatibi M. Comparison of the Effect of Pomegranate Juice and Orange Juice on the Level of pH of Dental Plaque. *J Res Dent MaxillofacSci 2016*; 1(3): 23-27.
- **Data Source:** Field work, 2023-2024
- **Inferential Statistics:** Significance level p< 0.0001, p value-Two-tailed.
  : *Significant- HS: Highly significant.
  : p=Probability

Table 7: Mean Salivary changes at different intervals of time after consumption of GUAVA.

Group–A (n=20) Caries active group

Individual scenario-Descriptive statistics						
Variables		**Time interval**	**ANOVA analysis (Inference)**			**Inferential Statistics**
			Mean ± SD Comparisons	**Z score Comparisons**	x^2	
Salivary flow rate (mL/min)	Unstimulated Saliva Range (0.1 mL/min)	Base line	3.54±0.28	58.78	19.06 df=2	p< 0.0001 HS*
	Stimulated Saliva Range (0.2 mL/min)	30 min	2.6±0.20	86.7		p< 0.0001 HS*
Buffering capacity Range (4–12)	Unstimulated Saliva	Base line	7.30±0.11	11.45		p< 0.0001 HS*

Variables	**ANOVA analysis(Inference)**							
	Time interval							
	Unstimulated Saliva	**Stimulated Saliva**				**Z score Comparisons**	**x^2**	**Inferential Statistics**
	Mean ± SD Comparisons (before)	**Mean ± SD Comparisons (0 min)**	**Mean ± SD Comparisons (5 min)**	**Mean ± SD Comparisons (15 min)**	**Mean ± SD Comparisons (30 min)**			
Simple fruit	7.11±0.096	5.36±0.59	6.51±0.66	6.86±0.68	7.06±0.37	20.9	14.61 df=2	p< 0.0001 HS*
Fresh fruit juice	7.14±0.86	4.54±0.35	5.18±0.95	5.73±0.70	6.91± 0.50	14.24		p< 0.0001 HS*
Processed fruit juice	7.105±0.23	4.49±0.42	4.56±0.37	6.51±0.57	7.064±0.19	12.31		p< 0.0001 HS*

table cont....

Legends/Capitations:

- **SD:** Standard Deviation
- **ANOVA:** Analysis of Variance
- **Citation[12]:** Shetgar S, Kemparaj U, Chavan S and Patel R. Effect of Fresh Fruit Juices on Salivary pH: A Randomized Controlled Trial. *Int J Oral Health Med Res* 2017;3(5): 28-32.
- **Data Source:** Field work, 2023-2024
- **Inferential Statistics:** Significance level p< 0.0001, p value-Two-tailed.
  : *Significant- HS: Highly significant.
  : p=Probability

Table 8: Mean Salivary changes at different intervals of time after consumption of ORANGE.

Group–A (n=20) Caries active group

Individual scenario-Descriptive statistics						
Variables		**Time interval**	**ANOVA analysis (Inference)**			**Inferential Statistics**
			Mean ± SD Comparisons	**Z score Comparisons**	**x^2**	
Salivary flow rate (mL/min)	Unstimulated Saliva	Base line	2.57±0.19	91.73	22.4 df=2	p< 0.0001 HS*
	Stimulated Saliva	30 min	3.43±0.21	78.9		p< 0.0001 HS*
Buffering capacity Range (4–12)	Unstimulated Saliva	Base line	7.31±0.10	126.9		p< 0.0001 HS*

	ANOVA analysis (Inference)							
	Time interval							
	Unstimulated Saliva	**Stimulated Saliva**				**Z score Comparisons**	**x^2**	**Inferential Statistics**
	Mean ± SD Comparisons (before)	**Mean ± SD Comparisons (0 min)**	**Mean ± SD Comparisons (5 min)**	**Mean ± SD Comparisons (15 min)**	**Mean ± SD Comparisons (30 min)**			
Simple fruit	7.20±0.25	5.71±0.77	6.11±0.52	6.46±0.77	7.16±0.20	23.24		p< 0.0001 HS*
Fresh fruit juice	7.14±0.83	5.53±0.36	6.23±0.39	6.83±0.58	7.087±0.46	22.4	4.68 df=2	p< 0.0001 HS*
Processed fruit juice	7.08± 0.46	3.33±0.307	4.56±0.27	5.96±0.49	6.86± 0.50	10.17		p< 0.0001 HS*

Legends/Capitations:

- **SD:** Standard Deviation
- **ANOVA:** Analysis of Variance
- **Citation[12]:** Shetgar S, Kemparaj U, Chavan S and Patel R. Effect of Fresh Fruit Juices on Salivary pH: A Randomized Controlled Trial. *Int J Oral Health Med Res* 2017 3(5): 28-32.
- **Data Source:** Field work, 2023-2024.
- **Inferential Statistics:** Significance level p< 0.0001, p value-Two-tailed.
  : *Significant- HS: Highly Significant.
  : p=Probability

Table 9: Mean Salivary changes at different intervals of time after consumption of APPLE.

Group–C (n=20) Caries active group

Individual scenario-Descriptive statistics						
Variables		**Time interval**	**ANOVA analysis (Inference)**			**Inferential Statistics**
			Mean ± SD Comparisons	**Z score Comparisons**	**x^2**	
Salivary flow rate (mL/min)	Unstimulated Saliva	Base line	2.66±0.20	86.7	14.10 df=2	p< 0.0001 HS*
	Stimulated Saliva	30 min	3.54±0.28	58.78		p< 0.0001 HS*
Buffering capacity Range (4–12)	Unstimulated Saliva	Base line	7.31±0.10	126.9		p< 0.0001 HS*

ANOVA analysis (Inference)								
	Time interval							
	Unstimulated Saliva	**Stimulated Saliva**				**Z score Comparisons**		**Inferential Statistics**
	Mean ± SD Comparisons (before)	**Mean ± SD Comparisons (0 min)**	**Mean ± SD Comparisons (5 min)**	**Mean ± SD Comparisons (15 min)**	**Mean ± SD Comparisons (30 min)**			
Simple fruit	7.20±0.25	5.31±0.60	5.86±0.75	6.51±0.71	7.11±0.29	18.89		p< 0.0001 HS*
Fresh fruit juice	7.19±0.86	3.52±0.48	4.63±0.94	6.33±0.49	6.96±0.48	10.05	6.0 df=2	p< 0.0001 HS*
Processed fruit juice	7.15±0.29	3.28± 0.17	4.66± 0.41	6.21±0.55	7.014±0.28	9.68		p< 0.0001 HS*

Legends/Capitations:

- **SD:** Standard Deviation
- **ANOVA:**Analysis of Variance
- **Citation[12]:**Shetgar S, Kemparaj U, Chavan S and Patel R. Effect of Fresh Fruit Juices on Salivary pH: A Randomized Controlled Trial. *Int J Oral Health Med Res* 2017; 3(5): 28-32.
- **Data Source:** Field work, 2023-2024.
- **Inferential Statistics:** Significance level p< 0.0001, p value-Two-tailed.

  : *Significant- HS: Highly Significant.

  : p=Probability

Table 10: Mean Salivary changes at different intervals of time after consumption of POMEGRANATE.

Group–D (n=20) Caries active group

Individual scenario-Descriptive statistics						
Variables		**Time interval**	**ANOVA analysis (Inference)**			**Inferential Statistics**
			Mean ± SD Comparisons	**Z score Comparisons**	**x^2**	
Salivary flow rate mL/min	Unstimulated Saliva	Base line	2.49±0.16	109.43	28.8 df=2	p< 0.0001 HS*
	Stimulated Saliva	30 min	3.30±0.15	111.1		p< 0.0001 HS*
Buffering capacity Range (4–12)	Unstimulated Saliva	Base line	6.98±0.10	130.2		p< 0.0001 HS*

Table Cont...

	ANOVA analysis (Inference)							
	Time interval							
	Unstimulated Saliva	Stimulated Saliva				Z score Comparisons	x^2	Inferential Statistics
	Mean ± SD Comparisons (before)	Mean ± SD Comparisons (0 min)	Mean ± SD Comparisons (5 min)	Mean ± SD Comparisons (15 min)	Mean ± SD Comparisons (30 min)			
Simple fruit	7.15 ± 0.15	4.51 ± 0.45	6.21 ± 0.41	7.064 ± 0.45	7.16 ± 0.20	13.45		p< 0.0001 HS*
Fresh fruit juice	7.16 ± 0.205	3.64 ± 0.54	4.53 ± 0.89	6.087 ± 0.23	6.96 ± 0.48	10.45	3.03 df=2	p< 0.0001 HS*
Processed fruit juice	7.105 ± 0.37	3.3 2± 0.16	4.56 ± 0.37	5.71 ± 0.56	6.49 ± 0.26	10.79		p< 0.0001 HS*

Legends/Capitations:

- **SD:** Standard Deviation
- **ANOVA:** Analysis of Variance
- **Citation[12]:** Shetgar S, Kemparaj U, Chavan S and Patel R. Effect of Fresh Fruit Juices on Salivary pH: A Randomized Controlled Trial. *Int J Oral Health Med Res* 2017; 3(5): 28-32.
- **Data Source:** Field work, 2024
- **Inferential Statistics:** Significance level p< 0.0001, p value-Two-tailed.
  : *Significant- HS: Highly Significant.
  : p=Probability

- A total of 80 children aged 6-12 years were enrolled in this study. The numbers of high, medium, and low caries activity children were20 in each group.
- The data presented in the form of a questionnaire was taken from each subject who included biological ages between 6 and 12 years (mean age **7.95 ± 1.71**). Therewas a significant difference between the two genders.

Mean salivary flow rates: (Table 7-10)

- For the entire group**, salivary flow rates** at the baseline and 30 min respectively were different and the difference was found to be statistically significant ($p < 0.01$).
- In the case of Guava, it was observed that the mean salivary flow rate was minimal immediately after consumption which increased to a maximum of 30 minutes.
- In the case of other fruits (Orange, Apple, Pomegranate), it was observed that the mean salivary flow rate was maximum immediately after consumption which reduced eventually at 30 minutes. There was statistically significant in all four groups at 0,5,15 minutes.

Buffering capacity: (**Table 7-10)**

- For the entire group, the Mean Buffering capacity at the baseline was found to be statistically significant ($p < 0.01$).

Salivary pH measurement: (**Table 7-10)**

- Results indicated that before intake of fruits and juices, salivary pH was normal (Table 7-10).
- The results of the measurement of salivary pH for patients after consumption of different fruits and beverages presented the means and standard deviation of each salivary pH measurement.
- There was a slight decrease in the pH of saliva at the 0 min time point in all four experiments, following which the pH reverted to baseline value at 30 min.

DISCUSSIONS

Saliva is essential for the maintenance of oral health.[1] Human salivary glands produce from 0.5 to 1.5 litres of total saliva in a 24-hour period. A complex fluid, containing electrolytes, salivary and serum proteins, and small organic molecules, as well as metabolites and debris from microorganisms that colonize the mouth, saliva functions to moisten and

protect oral tissues, clean the gingiva and teeth, and aids speaking and swallowing. Additional essential roles include buffering of the oral cavity, protective pellicle formation, tooth mineralization, antimicrobial activity, tissue repair, and taste and digestion.[23]

The buffer systems of saliva are liable for retaining suitable acid–base equilibrium. Buffer solutions retain an approximately constant pH even when small amounts of ether acid or base are added, or when saliva is diluted, being resistant to changes in the oral pH. The normal pH range for resting saliva is between 6.2–7.6. There are three possible buffer systems found in saliva, namely protein buffer, phosphate buffer, and carbonic acid/bicarbonate buffer (with the most important role).[24-25]

To date, the purpose of this present study was to evaluate changes in the salivary pH after intake of different fruits and beverages in children at different time intervals.

The study was carried out on 80 subjects with a mean age of 7-12 years and selected two different settings, a private hospital, and a private dental clinic in and around Vellore city.

In the present study, all groups showed changes in salivary pH after intake of beverages and eatables but after immediate and 5 mins, 15 mins, 30 mins follow-up showed significant changes in fruit and fruit juice groups. When a food is consumed, an admixture of saliva and food is formed.

Neha Awasthi ***et al.,*** conducted a studyon 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, Appy fizz, milk-based drink, Pepsi, and Gatorade. Buffering capacity of each drink was estimated by titration with sodium hydroxide. 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.[6]

Fruits and vegetables contain many nutrients which promote good health and to fight against diseases, but pH of many fruits can be acidic which promotes the tooth decay.[7]

The juice and peel of pomegranate is involved in lowering of pH and the polyphenolic compounds present in pomegranate helps in treatment of cavities.[7]

Azima Hanin S.M ***et al.,*** conducted a studyon the Analysis of Salivary pH Before and After Intake of Sugary Drinks - An In vitro Study. They had there is a significant drop in the mean pH value (pH=5.45) after consumption of fruit drink from the baseline pH value (pH= 7.15). These findings are in concordance with the present study.[26]

Lata K Mehta ***et al.,*** conducted a study on the Analysis of acidogenic potential of the various commercially available fruit juices and to evaluate the salivary and plaque pH changes before and after consumption of the fruit juices that were kept at various temperatures. Among the three juices compared, grape juice was found to be more acidic compared to the orange juice and pineapple juice. The pH fall was maximum after consumption of grape juice followed by orange and pineapple juice, respectively. These findings are in concordance with the present study.[27]

Anum Nazir ***et al.,*** conducted a studyto check saliva pH after intake of fresh and processed fruit juices (orange, apple, pomegranate, and guava). Pomegranate has less changing effect on saliva pH than all other fruits used in study and processed fruit juices have significant influence on change of salivary pH. These findings are in concordance with the present study.[28]

The use of other fruits and juices also depicted that fruit juices were more dangerous and may interfere with the salivary pH than consuming whole fruit. The reason may be that processed juices contain a lot of artificial sugars which cause an increase in their shelf life and protect from any bacterial or viral attack. But also causes a lowering in the pH of saliva leading to dental caries.[23]

Simple fruits contain a lot of nutrients and fibers but during juice making, these fibers are lost.[24]

Hence, based on the observations, it was concluded that the maximum drop of salivary pH was found with processed orange fruit juice in the caries active group.

The neutralization of an acidogenic challenge in the oral cavity is a complex phenomenon.

Limitations:

Further studies with larger sample sizes are required taking into consideration the salivary electrolytes, and the knowledge, attitude, and practices of beverage consumption study.

CONCLUSION

It was found that commercially available processed fruit juice caused a greater drop in

salivary pH than that of fresh fruit juices. The results provide basic information to the general dental practitioner regarding the consumption of fruit juices and their potential role in the development of dental erosion and/or dental caries.

It could also provide the basis for the development of less acidic (erosive) fruit juices.

It is suggested that dietary advice and preventive care are mandatory for individuals who frequently consume fruit juices.

Clinical significance (Recommended):

4. It is recommended that further studies should take into consideration the handedness of the participants.
5. The dentist must provide appropriate diet counselling tailored for a particular individual to maximize compliance.
6. Ideally, fruit juices should be served only at mealtimes, the frequency of consumption of fruit juices should be decreased and straws should be used whenever possible.

Footnotes

1. Conflict of interest (Statement) and source of funding:

a) The authors declare that the research was conducted in the absence of any commercial or financial relationships from the funding agency that could be construed as a potential conflict of interest or bias among the authors.

2. Ethical disclosures:

a) Confidentiality of data: The authors declare that no patient personal data appear in this article.

b) Right to privacy and informed consent: The authors have obtained the written informed consent of the patients or legal guardians/next of kin mentioned in the article. The corresponding author owns this document.

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