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THE IMPACT OF MODIFIED FRUIT JUICE ON ENAMEL MICROHARDNESS: AN IN-VITRO ANALYSIS

Shailee Shelke[1],Shaila Masih[1],Namita Singh[1],Abi M Thomas[1]
1.Department of Pediatric and Preventive Dentistry, Christian dental College, Ludhiana

ABSTRACT:

Aim: To evaluate the pH, titratable acidity of packaged mixed fruit juice modified with tri-calcium phosphate and its erosive potential on enamel.

Study Design: In vitro experimental trial

Methods: From specimen of packaged mixed fruit juice, 2% w/v tricalcium phosphate modified juice was prepared. The pH and titratable acidity for the test drinks was measured before and after addition of tricalcium phosphate.

Samples were prepared from 40 sound deciduous anterior teeth. Teeth were mounted in self cure acrylic and stored in deionized water at room temperature. Four specimen groups of ten samples each were made. Baseline surface microhardness measurements were taken by Vickers indenter- 200gms force for 15seconds. For microhardness testing, three indentations on different locations on same surface were made. Each specimen was then immersed in juice solution for three minutes followed by immersion in artificial saliva for three minutes for three cycles. **Statistics:** The data of enamel microhardness were statistically analyzed with Kruskal-Wallis and Mann-Whitney U test.

Results: There was a significant rise in mean microhardness values of deciduous enamel samples in modified fruit juice group (375.37 VHN) as compared to samples in the mixed fruit juice group (291.05 VHN).

Conclusion: 2% w/v tricalcium modified mixed fruit juice has a higher pH and a lower titratable acidity compared to commercially available packaged mixed fruit juice. The fortification with tri-calcium phosphate minimized the erosive potential of acidic mixed fruit juice on the enamel surface of deciduous teeth.

Keywords: Enamel Microhardness, Packaged Fruit Juice, Dental Erosion, Deciduous Teeth

INTRODUCTION:

Dental erosion can be defined as the dissolution of tooth by acids when the surrounding aqueous phase is under-saturated with respect to tooth mineral [1]. Deciduous teeth are smaller, with thinner enamel than permanent teeth. Hence, erosive processes progress rapidly, leading to advanced lesions after shorter exposures to acids, as compared to permanent teeth [2].

As lifestyles have changed through the decades, the total amount and frequency of consumption of acidic juices or beverages have also increased; and when a dietary acidic challenge acts for long enough, a clinically visible erosive defect occurs [3]. Children constitute over 42% of

*Corresponding Author Address: Dr. Shailee Shelke,Department of Pediatric and Preventive Dentistry,Christian Dental College,Ludhiana- 141008,Punjab, India Email: drshaileeshelke@gmail.com

the total fruit juice consumers [4] and concurrently, a significantly higher prevalence of erosion has been observed in children who frequently consume fruit juices [5].

The pH value, buffering capacity, type of acid, calcium and phosphate content of a beverage determines its driving force for tooth mineral dissolution. Benefits of adding calcium and phosphate have been previously observed in both sports drinks and carbonated beverages with positive results [6]. It has been cited that, after modification with calcium and phosphate the erosive effect of the beverage on permanent enamel was reduced [7]. Currently, there are no studies that evaluate the erosive effects of a packaged fruit juice on primary enamel.

Though timely diagnosis and treatment are currently the modes of managing dental erosion, adequate preventive measures should be undertaken to minimize this tooth-wear process. Thus, the aim of this study was to evaluate and compare the changes in pH, titratable acidity and microhardness of deciduous tooth enamel, before and after the modification of a packaged fruit juice with tricalcium phosphate.

MATERIAL AND METHODS:

Sample preparation: Forty sound, caries-free, extracted or exfoliated deciduous human incisors with relatively planar labial surfaces were taken and their roots were removed with diamond disc bur. Enamel windows measuring 3mm X 3mm were created on each tooth by painting the surface with two coats of acid resistant colored nail varnish. Each tooth crown was then embedded in self-cure acrylic resin using a square mould of 1inch sides. Labial surface of the tooth sample was arranged flat and parallel to the horizontal plane of the mounting.

Specimens were divided equally into four groups: Group I - baseline, Group II - mixed fruit juice, Group III - 2% tricalcium modified mixed fruit juice and Group IV - control group of deionized water. All the specimens were initially stored in deionized water at room temperature and later subjected to the erosive cycles.

Preparation of test and control solutions: Commercially available FPO-11207(Food Products Order) certified brand of packaged mixed fruit juice was used. From the specimen juice, 2% weight/volume (w/v) tricalcium phosphate modified mixed fruit juice was prepared by adding 20mg of tricalcium phosphate to 1000ml of the juice. Triple deionized water served as the negative control. Beverages were placed in coded containers to blind the examiner to their identity.

Baseline measurements: Baseline microhardness measurements on the exposed surface of group I samples were taken by the means of a Vickers indenter with 200gms of force for 15seconds. Three indentations on different location on the same surface were made and the readings noted.

Physiochemical properties: The pH of each beverage was noted with the use of a digital pH meter at room temperature. Titratable acidity was measured by titrating 20ml of the sample juice or freshly prepared modified fruit juice against 0.1N sodium hydroxide (NaOH) solution till a neutral pH 7 was achieved. The volume of NaOH was recorded. Three measurements were made for each juice and the average noted.

Erosion process: Each specimen from groups II, III and IV was immersed in the assigned solution for three minutes followed by an immersion for three minutes in artificial saliva (Aqwet, Cipla). Three such cycles were followed at room temperature. After which the samples were washed with deionized water and blotted dry.

Analysis of enamel softening: The hardness value of samples was measured after the erosion process. Three indentations on different location on the same surface were made and the readings noted.

Statistical analysis: Data collected was entered into SPSS software version 15.0 for statistical analysis. pH and titratable acidity of the solutions was compared using t- test. The data of enamel microhardness were statistically analyzed with Kruskal-Wallis and Mann-Whitney U test. Significance value in all the tests was set at $p < 0.001$.

RESULTS:

The pH value of the beverages and titratable acidity is represented in Table 1. Mean pH of the fruit juice was 3.2 before the modification with 2%w/v tricalcium phosphate and it increased to 4 after fortification; which was statistically significant.

Mean titratable acidity decreased from 9.8ml for the original unfortified juice to 1.2ml after tricalcium fortification, being statistically significant. (Table 1)

The mean enamel microhardness of the specimens as assessed using the Vickers hardness tester is presented in Table 2 and Table 3. Mean microhardness of samples in mixed fruit juice and modified juice were 291.05 VHN and 375.37 VHN respectively, with the difference being statistically significant. Microhardness had increased from baseline readings (illustrated in graph 1). Supplementation of the juice with 2% w/v tri-calcium phosphate led to a significantly lesser drop in enamel microhardness of primary teeth.

DISCUSSION:

Juice consumption in India has increased exponentially over the past few years. The Ministry of Agriculture, Government of India; valued packaged juice consumption in the country at 550 million liter/year in 2009 [8]; of these, children made up the bulk of consumers. The purpose of the present study was to evaluate the change in enamel microhardness of primary teeth on exposure to such a commercially available packaged mixed fruit juice, and, if the addition of 2% w/v of tricalcium phosphate to the same beverage would influence the microhardness.

The solutions tested were different in terms of pH, titratable acidity, calcium and phosphate content. The pH measures the hydrogen ion (H^+) concentration, whereas titratable acidity measures the concentration of all H^+ ions, both free and bound to un-dissociated acids and anions. Some studies have shown that the pH was a better predictor of erosive potential [9], whereas other studies reported that titratable acidity was more important [10]. Hemingway et al, 2006 [9] noted that the pH is a good predictor of the erosive potential of drinks for the first few minutes of erosion challenge, whereas the titratable acidity characterizes the erosive potential during longer exposure times. The method used in this study to determine the titratable acidity has been employed previously in other studies [11] and is known to give a realistic measure of buffering capacity of drinks by quantifying the amount of alkali required to bring the pH to 7.

The deionized water group showed minimal decrease in the microhardness owing to the higher pH. Citric acid, iso-citric acid, maleic acid etc are present in packaged juices and they contribute to the erosive potential of drinks by decreasing the pH. Also, the presence of acid would effectively increase the titratable acidity, thus increasing erosive potential. This is attributed to the fact that citric acid acts as a chelator capable of binding to calcium ions of the tooth structure, effectively causing demineralization [12].

The erosive potential of acidic drinks can be reduced by adding calcium, phosphate or milk products. The optimum Ca:P ratio is achieved by adding tricalcium phosphate to an acidic beverage at 2%-2.5% concentration [13]. Previous studies by Attin et al, 2005 [11], Larsen and Nyvad, 1999 [6] have shown that a modified beverage will reduce erosion if immersed in solution over long periods of time (few hours to few days). But the present study demonstrates the immediate effect on microhardness with tricalcium phosphate enriched juice. The supplementation with tricalcium phosphate increases the pH, reduces the titratable acidity and saturates the solution with tooth structure minerals, contributing collectively to the reduced erosive potential of the modified mixed fruit juice.

Reduced hardness of enamel and loss of mineral, which accompanies the dental erosion of the enamel surface by the acidic beverages, can be measured using various physical techniques such as microhardness tests and chemical analysis. In the present study, the assessment of erosion of the enamel surface was verified by measurement of the surface hardness. The present results thus show that the microhardness of primary tooth enamel is significantly reduced after treatment of the enamel surface by packaged mixed fruit juice and deionized water.

In previous studies, the surface enamel was removed so that the reaction surface was the softer subsurface enamel which is probably more easily eroded. In contrast, in the present study, the acidic treatment was rendered on the original, untouched enamel surface of the tooth. These unchanged enamel surfaces are more likely to reflect the clinical situation of microhardness in the erosive process.

The oral environment while drinking juices was simulated by a salivary cycling model at room temperature. The artificial saliva used cannot completely reflect the oral environment as other factors such as the salivary flow, buffering capacity, salivary pellicle, frequency and mode of consumption of juices (straws, glass, ice-lollies) influence the erosive effect of juices. Hence, the findings do not accurately reflect an in-vivo condition.

This in-vitro study confirmed the erosive potential of the mixed fruit juice studied. The results were similar to a study by Attin et al in 2005 [11] on modified carbonated drinks, but in this case the microhardness significantly increased with the modified beverage.

CONCLUSION:

It can be concluded that:

1. The commercial packaged mixed fruit juice can reduce microhardness of primary tooth enamel.
2. With addition of tricalcium phosphate, the erosive potential decreased and microhardness increased significantly. This can be attributed to increased pH, reduced titratable acidity of the modified drink.

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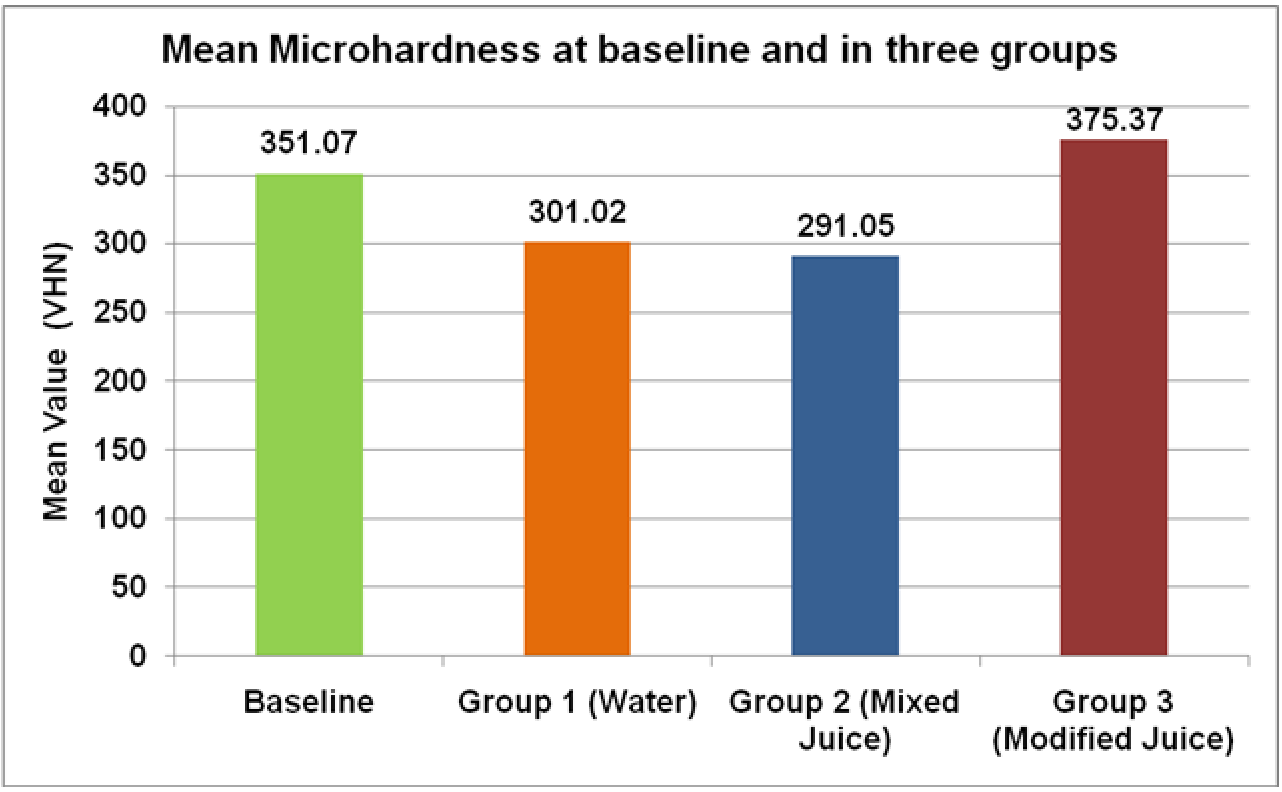
TABLES:

TABLE 1: PHYSIOCHEMICAL PROPERTIES OF THE JUICES		
	pH	Titratable acidity
Mixed fruit juice	3.2	9.2ml of 0.1N NaOH*
Modified mixed fruit juice	4	1.8ml of 0.1N NaOH*
Deionized water	6.7	-
*NaOH : sodium hydroxide		

TABLE 2: ENAMEL MICROHARDNESS DIFFERENCES IN MEAN VALUES			
Baseline reading	Deionized water	Mixed fruit juice	Modified fruit juice
351.07 VHN	301.02 VHN* Not significant	291.05 VHN* Significant†	375.37 VHN* Significant
*VHN: Vicker's Hardness Number † Significance: p value <0.001			

TABLE 3: MICROHARDNESS VALUES		
Group	Microhardness (VHN)	
	Range	Mean ± SD
Baseline	338.90 – 377.30	351.07 ± 9.87
Group 1 (Water)	249.80 – 324.30	301.02 ± 22.24
Group 2 (Mixed Juice)	265.70 – 333.80	291.05 ± 20.31
Group 3 (Modified Juice)	345.20 – 392.40	375.37 ± 14.87

GRAPH:


Mean Microhardness at baseline and in three groups
Mean Value (VHN)
400
350
300
250
200
150
100
50
0
351.07
301.02
291.05
375.37
Baseline
Group 1 (Water)
Group 2 (Mixed Juice)
Group 3 (Modified Juice)