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Comparison of the Effect of Pomegranate Juice and Orange Juice on the Level of pH of Dental Plaque

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ABSTRACT

Background and Aim: Reduction of dental plaque pH is an effective factor in the incidence of dental caries. One of the common methods for assessment of the cariogenic potential of food products is the study of plaque pH changes in the oral environment. The present study was performed due to the importance of dental plaque and its known complications and also the increase in consumption of industrial fruit juices which are encouraged nowadays as healthy drinks and also the positive effect of pomegranate juice on the amount of dental plaque which has been mentioned in the reports.

Methods and Materials: This clinical trial was performed with crossover design. Complete prophylaxis was performed during the first session. Then, the participants were asked to refrain from oral hygiene methods for 48 hours and not to eat or drink for at least 2 hours before the experiment. The baseline plaque pH was measured, and afterwards 10 cc of fruit juice was kept in mouth for 2 minutes and then swallowed. Afterwards, plaque pH was measured at time intervals of 2, 5, 7, 10 and 30 minutes. After one week of wash out period, the participants were again evaluated by the same method and with the other type of fruit juice. The measurement of plaque pH was performed with microtouch method by use of Metrohm electrode. The data were analyzed by repeated measures ANOVA.

Results: PH in pomegranate juice group before fruit juice intake equaled 6.73± 0.24 and reached 5.57±0.34 at the fifth minute and finally reached 6.19±0.32 at the 30th minute ($p<0.01$). Also, in orange juice group, pH before intake equaled 6.16±6.8 and reached 5.62±0.17 at the seventh minute and 6.15±0.2 at the 30th minute ($p<0.01$). The maximum fall in pH for both fruit juices occurred at the fifth and seventh minutes. pH after consumption of both fruit juices began to increase from the tenth minute. These two fruit juices were not significantly different regarding plaque pH at the zero minute and at the time of maximum pH fall and at the 30th minute. ($p<0.08$)

Conclusion: The results showed that plaque pH after consumption of both fruit juices falls below the critical level for seven minutes and this decline is similar for both fruit juices.

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Introduction:

Dental caries is one of the most common and costly infectious diseases.[1] Reduction of salivary and dental plaque pH is one of the effective factors in the incidence of dental caries.[2] Upon encountering dental caries, a dentist needs to be familiar with its etiologic factors and preventive measures in addition to symptomatic treatments.[1]

Estimation of the relative cariogenic potential of foods is of special importance due to the multiplicity of nutritional factors, in a way that great effort has been done for many years to assess the relative cariogenic potential of different food products.[3] Multiple factors play role in the assessment of cariogenic potential including the amount of fermentable carbohydrates, adherence, physical form of carbohydrates and their degree of oral clearance, the effect of mixed consumption of food products, order of consumption, frequency of consumption, etc. Lack of awareness regarding the cariogenic potential of food products leads to inappropriate food intake, dental caries, tooth loss and malnutrition, etc.[4]

Acid production in oral cavity during bacterial fermentation of a food product is a prognostic factor in the assessment of the role of that food product in cariogenicity. One of the common methods for assessment of the cariogenic potential of food products is the assessment of dental plaque pH changes in oral environment.[5]

Saliva has a key role in maintaining the healthiness of oral cavity and teeth and protecting teeth against caries, cleaning the mouth and buffering are among its duties.[6, 7]

Recently, considering the use of healthy food products and changes in nutritional pattern, there is a tendency towards the use of industrial fruit juices especially in children, in a way that their use has been often encouraged as healthy drinks. This claim regarding the safety of these fruit juices for teeth is doubted considering the findings in the literature.[8] In a study by Toumba et al reduction in pH similar or worse than that after consumption of sucrose solution has been reported after consumption of four types of Black currant juice.[9] But in a study by Witjaksono et al in 2013 more severe reduction in pH was reported after consumption of edible sucrose in comparison with edibles containing maltitol.[10] Pomegranate juice has recently attracted a lot of attention as a product with antioxidant properties and has been assessed in a research by Zarban in 2007. In the mentioned study, pomegranate juice concentrate without additives was compared with other commercial Sun Ich fruit juices (pomegranate juice, red grape juice, cherry juice, orange juice, pineapple juice, apple juice and mango juice) and they reported that pomegranate juice had the highest absolute antioxidant capacity. [11] But this product has not been evaluated regarding its cariogenic potential. Therefore, the aim of the present study was to compare the effect of sugar free pomegranate juice and orange juice on the level of dental plaque pH.

Materials and Methods:

This was a randomized crossover clinical trial. The individuals involved in this study were dental students that volunteered to participate in this study after being fully informed of the study protocol. The individuals were examined and healthy subjects without any systemic diseases based on their medical history, that had not consumed any medications for the last two weeks and were not following any special diet, without xerostomia, orthodontic appliances or dental prostheses, non-smokers without periodontal diseases or active dental caries were included in the study.[8] The inspection site was between the distal surface of upper right second premolar and mesial surface of first molar. If this site had any restorations, the surfaces between first and second premolars were selected and if these surfaces were also restored, the individual was excluded from the study.

The stages of the study were verbally explained to the participants and then they signed informed consent forms before the experiment.
Determining the pH level of dental plaque The participants were asked not to use any fluoride-containing products or antimicrobial mouthwashes.[8] In order for the dental plaque to reach the appropriate acid production ability and yet

not to create conflict with dental and periodontal health, in the first session, total mouth prophylaxis was performed and then the participants were asked to refrain from oral hygiene procedures such as tooth brush, dental floss or antimicrobial mouthwashes for the next 48 hours and not to eat or drink for at least 2 hours before the experiment (expect water).[12]

Afterwards, the two experimental drinks were coded as A and B. For each participant in each experimental session, a box of juice was opened after shaking and 10 cc of the juice was poured into a disposable cup.

In this stage, the baseline pH level of dental plaque (before intervention) was measured. Afterwards, the participant held 10 cc of the juice in his/her mouth for 2 minutes and then swallowed the juice.[12] Then the pH of dental plaque was measured immediately and at time intervals of 2, 5,7,10 and 30 minutes after drinking the juice. The values were recorded after the number on the pH meter was fixed for 30 seconds. Measurement of the pH of dental plaque was performed In vivo with microtouch method using Metrohm glass microelectrode connected to Metrohm digital pH meter ((Metrohm LTD CH-9101 Herisau,781 PH/Ion Meter, Switzerland).[12] In microtouch method, thin glass or metal probes which penetrate the depth of dental plaque are placed in contact with the dental surface and the connected pH meter shows the value of pH.

Before each experiment and also between readings, the microelectrode was calibrated with 3 mol of KCl solution with pH 7 and the electrode was washed with gentle flow of distilled water. Glutaraldehyde 2% was used for disinfection for 20 minutes.[12]

The minimum pH after consuming each juice and the difference between the rest pH (base pH) and minimum pH (ΔpH) and also the time period when the pH was below critical pH=5.5 were determined for all participants. After one week of wash out, the participants in each group were assessed again using the same method with the other type of juice. (Crossover)

Afterwards, pH curve was drawn for each experimental juice for all the participants in the mentioned time intervals.

The findings were analyzed with repeated measures ANOVA with 95% confidence interval and 80% power.

The specifications of the experimented products

After visiting the supermarkets in Tehran in search of different types of fruit juice, we realized that Sun Ich Company produces the widest variety of fruit juices. Two types of sugar free fruit juices without additives produced by this company were selected for this experiment.

The specifications and ingredients of fruit juices are summarized below.

Ingredients * / Fruit juice	Sugar free natural pomegranate juice (100% pure)	Sugar free natural orange juice (100% pure)
Total fat (gr)	0	0
Cholesterol (mg)	0	0
Sodium (mg)	36	6
Potassium (mg)	480	400
Total carbohydrates (gr)	30	25
Dietary fiber (gr)	0	0
Sugars (gr)	23.5	17
Protein (gr)	1.4	2.5
Vitamin A **	<2%	<2%
Calcium %	2 %	2%
Vitamin C %	80%	200%
Iron **	<2%	<2%

*Ingredients in each serving. Serving size -8 Oz (240 ml)

**Percent daily value

Result:

This study was performed on 20 participants and considering the study design (crossover) on 40 samples.

6 participants (30%) were male and 14 participants (70%) were female with the average age of 26±1.22 years and all of them were dental students which met the inclusion criteria.

Based on the performed analyses in each of the groups, the level of pH at 0 and 30 minutes (end of the experiment) were not significantly different. Moreover, the initial and final pH values in the two groups were not significantly different. ($0.57<p<0.63$)

Based on the findings, the level of pH in pomegranate juice group began to decrease from the zero minute and decreased by 17 % at the fifth minute which was significantly different from the first minute ($p=0.01$), and afterwards the level of pH increased.

In orange juice group, the decrease in pH at the seventh minute equaled 17.3% which was significantly different from the zero and 30th minutes ($p=0.01$).

According to the performed analyses, no significant differences existed between pomegranate juice and orange juice groups during the mentioned time intervals. (0.46<p<0.78)

The level of pH based on the follow-up time and divided by the type of studied fruit juices are summarized in Table 1.

Table 1- Plaque pH based on follow-up time and divided by fruit juice type

Plaque pH / Fruit juice / Time		Value	C.V.
Pomegranate juice	0	6.73± 0.24	4
	2	5.7± 0.37	6
	5	5.57± 0.34	6
	7	5.61± 0.22	4
	10	5.76± 0.25	4
	30	6.19± 0.32	5
Orange juice	0	6.8± 0.16	2
	2	5.86± 0.51	9
	5	5.67± 0.62	11
	7	5.62± 0.17	3
	10	5.78± 0.48	8
	30	6.15± 0.2	3

Coefficient of variation (CV) showed that plaque pH was low during all the mentioned time intervals and the maximum level equaled 11%.

Discussion:

The aim of the present study was the In vivo assessment of changes in the pH level of dental plaque after consuming sugar free orange juice and pomegranate juice by use of microtouch method with crossover design.

Critical dental plaque pH is a pH range in which the solubility of hydroxyapatite crystals of dentin and enamel is initiated.[9]

In the present study, consumption of orange and pomegranate juices similarly reduced the pH of dental plaque. In a study by Saha et al reduction of pH of dental plaque after consumption of apple juice was further than that after drinking Guava, pomegranate and lemon juice.[13]

In a study by moeiny et al assessment of the effect of orange juice, orange concentrate and pineapple concentrate on plaque pH was performed and consumption of orange concentrate with pulp had the highest effect on pH decline during the majority of the time intervals in comparison with other products.[12]

In a study by Toumba et al black currant juice mixed with sugar free apple juice and black currant juice mixed with citrus juice with higher concentration of carbohydrates than other products, black currant drink containing 7% concentrate and black currant drink containing 10% concentrate with new formula were compared. Among these four types of fruit juice, black currant drinks with new formula which contained low carbohydrate had lower acidogenic potential and prevented the fall of pH to below the critical level.[9] By evaluating four types of fresh and packaged fruit juices, Chaly et al reported that plaque pH didn't fall below the critical level.[14]

Nowadays, significant transition has taken place towards the consumption of healthy food products and drinks in different societies. Fruit juices are one of the food products which have a great market as healthy drinks.[4]

Dental caries is a process during which the mineral tissue of teeth deteriorates gradually due to the effect of acid produced by the effect of microorganisms on fermentable carbohydrates.[15]

In the present study, the maximum fall in plaque pH occurred at the fifth and seventh minutes after intake of fruit juices. Afterwards, plaque pH followed an ascending order for both fruit juices which showed the compensating process of pH.

In the study by Toumba et al the maximum fall in plaque pH occurred following the intake of mixture of citrus juices at the fifth minute and remained below critical level for five minutes. This study suggests that the preliminary sampling of the plaque five minutes after consumption may not always record the minimum plaque pH, and the initial time intervals of 2 minutes or 3 minutes may better represent the maximum fall in plaque pH after intake of fruit juices.[9]

Also, in a study by Azrak et al after drinking orange juice, milk, mineral water and instant Fennel tea, the maximum fall was shown to be during the fifth to 10th minutes except for mineral water

which exhibited high pH at all the time intervals. [16] In the present study, at the 30th minute after the intake of both fruit juices plaque pH almost returned to the initial level. The difference between the pH decline time and returning time was close in these two studies.

Johansson and colleagues stated that significant decline of the baseline pH following the consumption of acidic drinks also occurs after the intake of sugar free types of these drinks which can be an important risk factor for the initiation of dental erosion.[5]

On the other hand, Beighton believes that rapid preliminary decline of plaque pH (less than 5 minutes) after consumption of fruit juices is related to the acidic contents of these drinks rather than to sugar fermentation by plaque bacteria. [18]

In the present study, both fruit juices were sugar free and caused a significant reduction in plaque pH.

It seems that the acidic content of fruit juices (citric, acetic, maleic, and ascorbic acid, based on the type of fruit juice) is washed rapidly by saliva, and after this cleansing, acids produced by fermentation of carbohydrates by bacteria (lactates and succinates) reach the maximum concentration. It has been stated that exposure of bacteria to food stuff with low pH decreases their ability of carbohydrate fermentation and acid production.[18]

Johansson et al have stated that overall, pH curve after consumption of foods such as fruit juices follows a typical pattern (Stephen curve) which is due to three main factors:

1) The ingredients of a product such as acids and sugar

2) Individual factors such as salivary conditions, amount of dental plaque and type of microflora

3) Food and drink consumption pattern

In the present study which was a crossover experiment the last two factors were homogenized as possible. Therefore the results are highly reliable. Moreover, the method for pH measurement used in the present research is an up-to-date and reliable method.[10, 19]

A single test with the ability to unequivocally determine the cariogenicity of foods and drinks in vivo is not yet available, but if caries acidogenic etiologic theory is accepted, measurement of pH before, during and after food consumption can be considered as a guide for determining the cariogenic potential of food products.[17] In addition, glass microelectrode used in the present experiment is extremely more accurate and reliable compared to the types which need separate reference electrode and salt bridge and have been used in previous studies [9, 17]. In more recent studies which have used digital glass electrodes, the speed of readings and the accuracy of pH measurements have been higher. [10, 18]

One of the limitations of our study was the type of experimented fruit juices. The results of this study are only valid for these two types of fruit juices of particular brand and due to the limitations in experimental use of natural juices and impossibility of procurement of similar samples we could not use fresh fruit juices. However, the results are valid for these two commercial products in the market and in the mentioned time intervals.

Conclusion:

The results of the present study showed that plaque pH declined below the critical pH till the seventh minute after the intake of fruit juices and this decline was similar for both fruit juices. Plaque pH returned to baseline value 10 minutes after fruit juice consumption.

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