

Assessment of the Effect of Fruit (Apple) and Plain Yoghurt Consumption on Plaque pH

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Abstract

Introduction: Nowadays, thanks to improvements in fruit yoghurt tastes, more tendencies are seen in their consumption especially among children. Therefore, their cariogenicity evaluation as healthy snacks is important. The goal of this study was the assessment of the consumption effect of two kinds of Iranian fruit (apple) and plain yoghurts on dental plaque PH.

Methods: In this experimental study, 10 healthy dentistry students were selected upon inclusion criteria. Plaque pH in the certain areas of the mouth was measured by microelectrode and digital pH meter. PH was measured at the baseline and intervals of 2, 5, 7, 10, 15, 20, 30, 40, 50 and 60 minutes after eating test products: fruit yoghurt (apple) and plain Yoghurt. For positive control group, just the baseline PH and at intervals of 2 and 5 min after swishing with 10% sucrose solutions were recorded. The results were analyzed using repeated measures ANOVA. **Results:** Lowest pH was obtained after fruit yoghurt consumption followed by plain yoghurt and %10 sucrose solution and the plaque PH difference was significant ($P=0.05$). Furthermore, time duration which remained below the critical pH was longer after consuming fruit yoghurt. **Conclusion:** Both kinds of yoghurts were considered cariogenic since plaque pH drop below critical points. Average of plaque pH after consuming fruit yoghurt was significantly lower in almost all the time intervals.

Keywords: Acidogenicity, Plaque PH, Plain yoghurt, Fruit yoghurt, Cariogenicity.

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Introduction

Nowadays, diet preferences have been changed enormously and more tendencies are seen in eating healthy foods and snacks. Yogurt and other dairy products are perceived to have important role in human general and dental health(1). Yogurt is highly nutritious and is an excellent source of protein, calcium, potassium, vitamins B2, B6 and B12(2). Moreover, some dairy products are containing probiotics, which may have favorable effect on the reduction of streptococcus mutans in dental plaque(3, 4).

The relative cariogenicity of foods and beverages has been evaluated using various methods comprises enamel surface topography, microhardness, saliva and plaque pH (5). Measurements of the dental plaque pH after eating foods have been widely used for estimating cariogenic potential of them(6) since typical changes in dental plaque pH, named as the Stephan response, occur after consuming sugar containing foodstuffs (7).

Recently, flavored fruit yogurts have been manufactured which have become popular especially among children. Some studies showed that fruit yogurts or yogurts with added sugar have acidogenic properties(8, 9). In contrast, in the another study no correlation was reported between the amount of sugar in yogurt and its acidogenicity(10). However, there are few reports on the cariogenicity of flavored yogurt products and since experimental procedures vary, generalizations must be guarded. Among the testing methods of acidogenicity, Microtouch is considered as reliable one(11, 12).

Although fruit yogurts have been introduced as healthy snacks, there is no data about the cariogenicity of Iranians' yogurt. Hence, this study was conducted with the objectives to compare the consumption effect of plain and fruit yogurts on plaque pH at different time intervals.

Materials and Methods

The study was performed in the Department of Pediatric dentistry of Islamic Azad University, Dental Branch of Tehran. It was a single-blind randomized crossover trial study. Ethical approval was granted by the Ethics Committee of the University. Ten dental students by the mean age of 27.1 ± 3.58 (range from 25 to 32 years) voluntarily participated in the study and informed consent was received from them. The sample size was determined according to Şaroğlu et al. (8). And assuming $\beta=0.2$ with 2-level factorial in Minitab software.

All the participants were healthy and had no systemic disease in their medical history. They had good oral health, normal salivary flow rate, complete

natural dentition, no orthodontic and prosthodontics appliance and no history of drug consumption. Moreover, they were examined not to have any pathologic lesions, faulty dental restorations, current dental caries activity or fluoride releasing restorations. Individuals with milk protein allergies or the history of antibiotic intake two weeks prior the study were excluded(13). Volunteers were examined not to have any filling at distal surface of second premolar and mesial surface of first molar. For verifying normal salivary flow rate, whole stimulated salivary flow rates were analyzed in all participants, not to be less than 1ml/min. Furthermore, salivary streptococcus mutans and lactobacillus count were determined by using a caries risk test kit (CRT-bacteria; Ivoclar-Vivadent, Liechtenstein) and it should be equal or higher than 10^5 CFU/ml(14). The buffering capacity of saliva also investigated to be normal by using CRT buffer test strip (CRT Buffer, Ivoclar-Vivadent, Liechtenstein).

To enhance the constancy of conditions, the subjects were carefully instructed to use the same oral hygiene procedure. Therefore, all the participants brushed their teeth with similar toothbrush and dentifrice 3 weeks prior the study until the end of the experiment. Furthermore, for equalization of salivary fluoride level, the use of any other fluoridated product was prohibited(15).

To provide conditions for dental plaque to reach adequate acid production, as well as preventing any conflicts with volunteers dental and periodontal health, they were asked not to perform oral hygiene procedures, including brushing, flossing and using antimicrobial mouthwashes, for 48 hours and not to eat or drink(except water) at least 2 hours prior the experiment session(16). In the first week, each subject was assigned randomly in three groups, consisted of 2 groups of 4 and 1 group of 2 volunteers using convenient sampling. In this study, two test materials (yogurt) and 10% sucrose solution (positive control) were coded from A to C. In every session a recently opened pack of yogurt or fresh 10% sucrose solution were prepared for each participant. Therefore, a total amount of 10cc of the yogurt or 10% sucrose solution was given to each volunteer in a disposable glass. The intervention for each group was carried out in 3 sessions during 3 consecutive weeks. One week interval was considered as the wash out period. After dividing the subjects in the first week randomly, the following sequence was used for other sessions: A-B-C, B-A-C and C-A-B. The content and ingredients of products are presented in Table 1 according to their labeling. Test materials included: A: fruit yogurt (apple), B: plain yogurt and C: 10% Sucrose solution (positive control). In every session, first, a baseline dental plaque pH in all groups was measured by

Metrohm microelectrode (Metrohm, Switzerland, LL micro glass electrode). So the reference electrode connected to a pH meter (Metrohm, Switzerland), was placed at the contact point between the first molar and second premolar in each of four quadrants of the mouth. At this part, the patients were asked to keep 10% sucrose syrup in their mouth for 2 minutes and then spit out (6). Then the plaque pH values were measured at 2 and 5 min after swishing with 10% sucrose solution. Afterwards, subjects were asked to keep 10cc of the test material (A or B) in their mouth for 2 minutes and then swallow it. Plaque pH was measured at intervals of 2, 5, 7, 10, 15, 20, 30, 40, 50 and 60 minutes at the abovementioned sites. For positive control group, just the baseline pH and at intervals of 2 and 5 min after swishing with 10% sucrose solutions were recorded. It should be

mentioned that the operator who recorded the plaque pH, was blind to the type of the consumed products.

Furthermore, it should be noted that the microelectrode was calibrated by 3 mol/L KCL solution with pH of 7. Moreover, the 2% glutaraldehyde solution for 20 min was used for electrodes disinfection(8).

The mean pH in each session was determined in all quadrants according to three test materials. The results were analyzed using repeated measures ANOVA and $P < 0.05$ was considered statistically significant. The time spent below $\text{PH} = 6$ was compared between the three groups using Kruskal-Wallis and Dunn multiple comparison test. The SPSS version 16 was used for statistical analysis.

Table 1. Fruit (apple), plain yogurt and sucrose solution ingredients

	Fruit (apple) yogurt	Plain yogurt	Sucrose Solution
Fat	2.5%	2.5%	-
Dry matter	16.5%	10.8%	-
Protein	3%	3.8%	-
Mineral elements	0.7%	0.7-1%	-
Sucrose	6%	-	10%
Water	-	-	90%
PH	4	5.6	7

Ingredients per serving size (100 gr)

Results

Ten dentistry students by the mean age of 27.1 ± 4.9 participated in this single-blind randomized crossover trial study. The results of mean plaque pH changes analysis before and at certain intervals after the consumption of test materials using Repeated Measure ANOVA are presented in Table 2 and figure 1.

Carry over and the time effect was evaluated and they had not significant effect on the results.

The results of current study revealed that consumption of fruit yogurt had the greatest effect on decreasing the plaque pH in the majority of tested time points. In contrary, plaque pH following consumption of 10% sucrose solution was highest in most of the time points and the effect of plain yogurt was in between the mentioned test materials. There was a statistically significant difference in mean plaque pH values according to test materials at different time intervals ($P < 0.05$).

According to table 2, plaque pH started to drop after consumption of fruit and plain yogurt and reached

its minimum within ten minutes, then it increased gradually but it had not recovered to the pre-experimental level. Consuming sucrose solution decreased pH level very quickly and caused to reach its lowest amount in 7 minutes, then it increased gradually and recovered to the basic level in the experimental period.

Table 3 shows maximum drop of PH level (max ΔPH) and the minimum amount of PH following the consumption of test materials. Furthermore, the time spent below the critical pH level following consuming test materials was reported in table 4. In fruit yogurt group, this time period was statistically higher than plain one ($P < 0.001$) and also it was significantly higher in plain yogurt than sucrose solution ($P < 0.001$).

Table 2. Comparison of mean plaque PH (\pm SD) between three groups at different time intervals

Time(min)	Fruit yogurt (apple) Group A	Plain yogurt Group B	10% Sucrose solution Group C	P Value	Comparison of groups data
0	6.51 \pm 0.84	6.42 \pm 0.39	6.61 \pm 0.57	0.039*	A=B<C
2	6.19 \pm 0.79	6.21 \pm 0.68	6.32 \pm 0.70	0.185	A=B=C
5	5.87 \pm 0.76	6.01 \pm 0.74	6.03 \pm 0.70	0.037*	A<B=C
7	5.71 \pm 0.86	5.80 \pm 0.87	5.96 \pm 0.45	0.012*	A=B<C
10	5.50 \pm 0.79	5.75 \pm 0.88	6.04 \pm 0.63	<0.001*	A<B<C
15	5.61 \pm 0.79	5.84 \pm 0.80	6.07 \pm 0.67	<0.001*	A<B<C
20	5.79 \pm 0.63	6.10 \pm 0.67	6.29 \pm 0.65	<0.001*	A<B<C
30	6.12 \pm 0.69	6.29 \pm 0.63	6.47 \pm 0.64	0.012*	A<B<C
40	6.21 \pm 0.98	6.41 \pm 0.59	6.36 \pm 0.60	0.013*	A<B=C
50	6.34 \pm 0.75	6.43 \pm 0.60	6.39 \pm 0.64	0.261	A=B=C
60	6.44 \pm 0.73	6.54 \pm 0.64	6.52 \pm 0.79	0.281	A=B=C

*P Value<0.05, significant
P Value>0.05, not significant

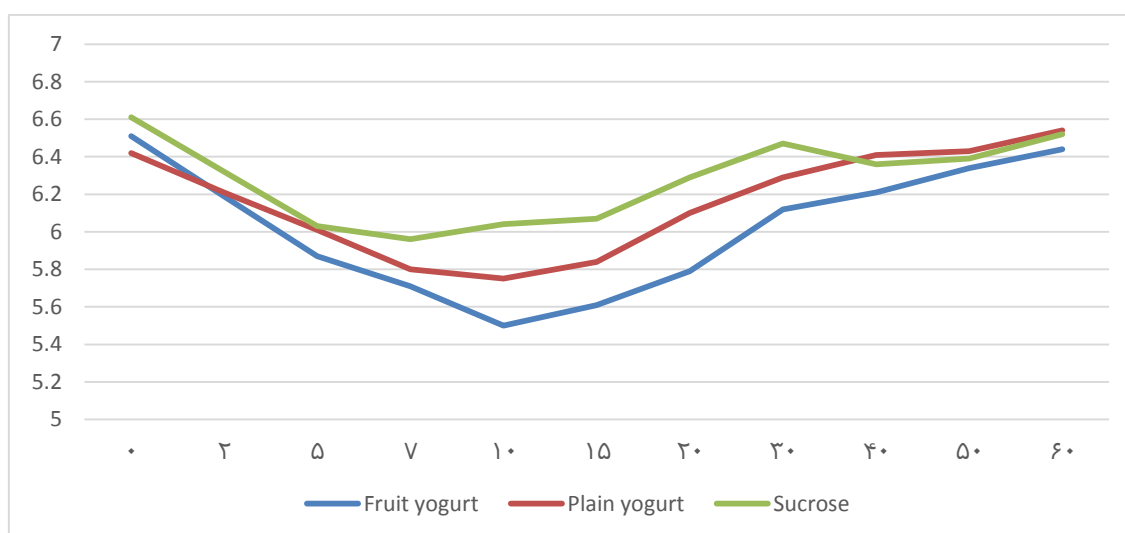


Figure 1. Mean plaque PH following consumption of Sucrose solution, Fruit and Plain yogurt at different time points

Table 3. Maximum PH level drop (max Δ PH) and the minimum amount of PH following the consumption of test materials

	maximum Δ PH	Minimum PH
Fruit yogurt(apple)	1.01 \pm 1.74*	5.50 \pm 0.79*
Plain yogurt	0.67 \pm 1.7	5.75 \pm 0.88*
10% Sucrose solution	0.65 \pm 1.61	5.96 \pm 0.45*
P Value	0.05	0.05

*P Value<0.05, significant
P Value>0.05, not significant

Table 4. Time (min) spent below pH 6.0 for all of the test products

	Fruit yogurt(apple) Group A(n=10)	Plain yogurt Group B(n=10)	10% Sucrose solution Group C(n=10)
Time (min) spent below pH 6.0	22.5 \pm 3.21	12.82 \pm 3.64	0.05 \pm 0.28

Discussion

The current study revealed that the fruit yogurt had greater impact on reduction of dental plaque pH in most of the time points. The plain yogurt placed in second rank and 10% sucrose solution was in next level. From the minute 2, the plaque pH decreased following consumption of all studied products. In the minute 5, the reduction of pH due to fruit yogurt was significantly more than other materials. Additionally, from this moment in fruit yogurt group, the dental pH fell to the degree below the critical limits. From the minutes 5 to 50, the reduction of dental plaque pH due to the consumption of fruit yogurt were more than plain yogurt and sucrose solution in all the minutes of study. Near the minutes 10, the plaque pH raised to recovery pH following sucrose consumption but in both yogurt groups it continuously decreased and showed the maximum downfall. Near the minute 20, plaque pH after consumption of both types of yogurts began to recover. So from the minute 20 in the plain yogurt group and from the minute 30 in the fruit one, plaque pH achieved the higher level than critical limits but as demonstrated in the table 2, even at the final minutes after consumption of both yogurts specifically fruit one, significant difference existed with baseline pH.

Another criteria which was evaluated in this study was the period of time that plaque pH remained below the critical limits. This critical pH is not constant, because the levels of calcium and phosphate in plaque fluid vary among individuals and also in different sites of the mouth in each individual(17). According to some researches, the plaque pH below 5-6 was supposed as the danger zone for initiation of enamel demineralization(18, 19). In this study, pH=6 was considered as the critical pH to be more conservative for assessment of the acidogenicity of snacks(5,6,14). Due to our study, the time interval that pH remained below the critical level following consumption of fruit yogurt was 1.7 times more than plain one. Moreover, it should be mentioned that the plaque pH drop below the critical level following consuming 10% sucrose solution was not significant.

Şaroğlu Sönmez et al. investigated the effect of white cheese and sugarless yogurt on plaque acidogenicity(8). According to their results, in consistent with our findings, plain yogurt did not lower the plaque pH below 5.7 and the maximum drop of pH was occurred in the minute 10. But in our research, the time interval that plaque PH descended below the critical level was lower than theirs (12.82 min in comparison to 19.7 min). Furthermore, regarding the plain yogurt consumption the plaque PH was reduced to less than the critical limits in the minute 1 in Şaroğlu Sönmez et al. but in the minute 7 in ours. These

differences could be interpreted by dissimilarity of test materials pH. The yogurt in their study was more acidic (4.3 vs. 5.6) which led to the rapid and longer downfall in dental plaque pH.

However, it should be bear in mind that the demineralization effect of foods and beverages cannot be attributed just to the pH. Other factors should also be considered, including the physical and chemical properties that affect the adhesion of microorganisms to the dental surface, the stimulation of salivary flow, the buffering capacity and the presence of fluoride, calcium and phosphate(20).

Ferrazanno et al. investigated the protective effect of yogurt extract on tooth enamel demineralization(21). The results demonstrated that casein phosphopeptides (CPP) of yogurt had an inhibitory effect on demineralization and promoted the remineralization of dental enamel. This in vitro analysis was relatively in contrary with current study, since dental plaque pH was considerably decreased below the critical level due to yogurts consumption which would lead to enamel demineralization. This contradiction could be interpreted with differences in study design (in vitro) and test material (soluble fraction containing the CPPs was separated by centrifugation). However, from this study, it was postulated that besides pH, protective mechanisms could affect buffering, salivary stimulation, reduction of bacterial adhesion, reduction of enamel demineralization, and promotion of remineralization by casein and ionizable calcium and phosphorus.

Caglar et al. evaluated dental erosion in an Istanbul public school. Of subjects who consumed fruit yogurt, 36% showed dental erosion. This study revealed that although milk products were rich in calcium and phosphate content and this would inhibit demineralization, product's pH would be important(22).

Due to another in vitro study results, fluoride levels were the same for all tested fruit yogurts, and the saturation degrees of hydroxyapatite and fluorapatite was positive, indicating supersaturation. Furthermore, they stated that fruit yogurt had no erosive potential, but more clinical trial studies would be required for generalization(23).

Jenson and coworkers reported that dairy products with the exception of sweetened yogurt reduced the amount of dentin demineralization and also actual remineralization of enamel caries-like lesions were detected(24). This was, to some extent, in consistent with our results since apple yogurt group remained 1.7-fold period of time below critical PH. Interestingly, Jenson et al. stated that the lesion body showed a net increase in mineral content even in fruit yogurt group.

In the other study by Saeed et al. the effect of some beverages on plaque pH was evaluated. According to their report, cola and orange juice are acidogenic and frequent intake should be discouraged but a reasonable intake of unsweetened milk may be advised safely(25).

Blacker et al. investigated the erosive potential of smoothies in an in vitro study. They stated that the majority of the drinks had a baseline pH below the critical pH of enamel (5.5) except the yogurt, vanilla bean and honey. The immersion of the tooth samples in the drinks led to reductions in their surface hardness but these were only significant for the cranberry, blueberry and cherry fruit smoothie and homemade strawberry and banana fruit smoothie. The most depth loss was recorded in kiwi, apple and lime smoothie groups(26).

An interesting observation in the study by Bamise et al. was the greater amount of titratable acid in the yogurt drinks than acidic cola drink. Due to their findings yogurt drinks have greater erosive potential. Cola are usually acidulated with phosphoric acid while yogurts contains lactic acid, which has been found to be one the most erosive acidizing agents and cause linear release of calcium and phosphorus, while phosphoric acid lead to only calcium release(27).

Based on the data which was delivered by the factory, the fruit yogurt of current study contained 6% sucrose. But higher reduction in dental plaque PH were recorded in different time points in fruit yogurt group compared with 10% sucrose solution group. The logical justification would be the yogurt acidic degree, or the factory claim inaccuracy about the yogurt's sucrose content.

The acidic capacity of juice and other beverages in enamel demineralization depends on the PH, acid type, and titratable acidity(TA)(27). Some researchers suggest that TA would be a better guide than the inherent pH of the product for potential dental erosiveness. TA or buffering capacity is defined as the total number of acid molecules and actual availability of hydrogen ion for interaction with the tooth surface and thus it is an indicator of erosive potential. So the greater the TA, the longer time it will take for saliva to restore the pH value (salivary clearance)(28, 29).

In our study, TA of yogurts did not measure but according to table 1, fruit yogurt PH (PH=4) was lower than plain yogurt (PH= 5.6). Therefore, this could be the logical reason for its rapid and longer reduction of plaque pH. The other factor which can influence the plaque PH is the amount of added sugar. Sucrose is considered to be much more cariogenic than other sugars. The fruit Yogurt tested in current study had 6% additional sucrose while the plain yogurt was sugar-free and it could be the other explanation for plaque PH variations.

However, several mechanisms are existed by which dairy products may reduce enamel demineralization. Firstly, adsorption of milk proteins to the enamel surface may impede demineralization; secondly, milk fat could be adsorbed onto the enamel and have a protective role; and thirdly, milk enzymes may reduce the growth of acidogenic bacteria(21, 30). Other parameters which may exert a direct effect on the tooth surface include calcium, phosphorus and CPPs. Since milk and yogurt have similar elements, it can be supposed that both products may have similar protective effect against dental caries. Furthermore, the protein content of yogurt is higher than milk because of the addition of non-fat dry milk(21).

Yogurt is a dairy product produced by bacterial fermentation of milk. Fermentation of the milk sugar (lactose) under controlled temperatures and environmental conditions produces lactic acid, which cause milk protein to give yogurt its texture and its special taste(31). Fermentation has little effect on the mineral ingredients of milk, thus the total mineral content remains unchanged in the yogurt. Moreover, because of the lower pH of yogurt compared with milk, its calcium content would be in ionic form(21).

Regarding the table 1, the mineral content of both yogurts were similar and the main difference was the protein level which in plain yogurt was 0.8 percent more than fruit one. Furthermore, the percentage of dry matter was approximately 6% more in fruit yogurt. Perhaps adding the fermentable carbohydrates to fruit one might cause the acidic action, till the protective mechanism of existed protein could not be able to inhibit more and longer reduction in plaque pH.

Generally, it can be concluded that although yogurt is considered as the healthy snack, improper consumption of this product may lead to erosive and permanent damage to teeth especially in children and adolescent with immature teeth.

Although it is not logical to set limits on usage of this nutrient snack, children should be advised and encouraged to keep yogurt to mealtimes if possible and to swallow it down in one rather than sipping it over a long period. Furthermore, eating of plain yogurt should be more encouraged.

In conclusion, although the average of plaque pH following fruit yogurt consumption was lower than plain one, both types of yogurt could have adverse effect on dental enamel especially in high risk children.

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