DOI: 10.22037/jds.v40i4.40990
Original Article
ISSN 2645-4351

Comparison of Salivary pH Changes in Children Aged 6-12 Years Following the Consumption of Plain, Probiotic, and Fruit Yoghurt: A Randomized Clinical Trial

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(*Submitted: 29 January 2023 – Revised version received: 25 June 2023 – Accepted: 10 July 2023– Published online: Summer 2023*)

Objectives Tooth decay is a common disease of childhood. If the pH level in the mouth repeatedly falls below 5.5 for an extended period of time, dental demineralization can eventually result in tooth decay or caries. Yoghurt is a fermented milk product that is consumed all over the world. In this study, we aimed to compare salivary pH changes in children aged 6-12 years following the consumption of plain, probiotic, and fruit yoghurt.
Methods A total of 51 children were randomly allocated into three groups of 17 people. On the day of the experiment, a sample of primary non-stimulating saliva was obtained from each participant one hour after breakfast. Next, the participants were given 10 cc of selected yoghurt, including peach, probiotic, and plain yoghurt, all of which were produced by the same company. After 10 minutes, another salivary sample was obtained from each participant. The pH of both samples was calculated using a measuring device, and the difference between the two measurements was considered as the amount of pH change. Analysis of variance (ANOVA) and paired t-test were used for between- and within-group comparisons, respectively.
Results There was a significant difference in the salivary pH level, based on the within-group comparison of peach and probiotic yoghurt groups (P=0.001), while no significant difference was found in the plain yoghurt group (P=0.366). Also, according to the between-group comparisons, significant differences were found between the peach and probiotic yoghurt groups (P=0.001) and also between the peach and plain yoghurt groups (P=0.001) in both comparison, peach group showed more decrease in pH level.
Conclusion Both fruit yoghurt and probiotic yoghurt can temporarily reduce the salivary pH levels. However, due to the lower critical point of demineralization, there are no concerns about the development of dental caries.
Keywords Probiotics; Salivary microbial; Community microbiota; Dental caries

Introduction

Dental caries is considered the most common infectious disease in humans worldwide. Cariogenesis is the outcome of a complex interaction between the host's oral flora and diet. [1] So far, various studies have assessed the effectiveness of different diets in reducing the incidence of dental caries. Probiotics are considered beneficial to human health, as they help to improve the balance of microorganisms in the intestines. When taken orally, probiotics can also have a positive impact on the oral microbiota, which is an important part of the human microbial community. [2] Plain yoghurt is produced by introducing lactic acid bacteria to milk, promoting lactic fermentation. This food product has a high nutritional value and positive bioactive effect. [3]

Probiotics, such as Lactobacillus and Bifidobacterium, are beneficial to the gut microbiota. However, they are closely related to the development of dental caries. [4] A review of the literature suggests that the regular consumption of probiotic products significantly reduces the risk of caries by inhibiting cariogenic bacteria and enriching commensal microbes in the oral cavity. The beneficial effects of probiotics on oral health may be attributed to several possible mechanisms, including buffering the salivary pH, producing bacteriocins and enzymes (e.g., dextranase, mutanase, and urease), and competing for adhesion and colonization on tooth surfaces. [5] Even short-term probiotic yoghurt interventions for infants in the early stages of life may have oral health benefits. [6]

In this regard, Saha et al. reported that probiotics can lower the salivary pH levels and cause essential elements, such as calcium and phosphorus, to leach from the enamel. This can alter the surface topography of the enamel and increase the risk of enamel defects. [7] Moreover, Ayako et al. reported that the decrease in pH under a biofilm produced by Lactobacillus was unusually slow and that the pH level was maintained above 5.5 for 16 hours. Based on their findings, the amount of biofilm and the reduction in enamel hardness were minimal. [8]

Fruit yoghurt typically contains 5% or more of added sugar. A study showed that yoghurt with 5% sucrose has a low potential for causing tooth decay. [9] Additionally, an in vivo study found that the consumption of flavored yoghurt (with strawberries) increased the demineralization of enamel and dentin compared to milk. [10] Also, a study conducted in Tehran, Iran, found that the pH level in the mouth was the lowest after consuming fruit yoghurt. The duration for which the pH level remained below the critical point was longer after consuming fruit yoghurt compared to other types of yoghurt. [11]

Fruit yoghurt is often marketed as a healthy snack option. So far, many studies have evaluated the effects of probiotic yoghurt on Streptococcus mutans and plaque pH in children and adults. However, there is not enough information about the effects of fruit yoghurt consumption

compared to plain and probiotic yoghurt on salivary pH in children. Also, there are concerns about the low pH levels of probiotics and their effects on dental caries. Therefore, in the present study, we aimed to compare the alterations of salivary pH between fruit yoghurt and plain and probiotic yoghurt in children, referred to Tabriz Dental School in Tabriz, Iran.

Methods and Materials

Ethical considerations

The study information was presented to the children's parents, and they were asked to sign an informed consent form before participating in the study. This study was registered in the Iranian Registry of Clinical Trials (IRCT code: IRCT20200411047018N2).

Study design

This single-blind randomized clinical trial was performed on 6 to 12 year-old children, referred to the pediatric ward of Tabriz Dental School (Tabriz, Iran) in 2021. Children were selected based on the inclusion and exclusion criteria. A sample size of 51 children was determined for this study based on previous research. [12, 13] This study was designed with a significance level of α=0.05 and a power of β=0.80, assuming a clinically significant difference of 0.5. A 10% attrition rate was also considered to account for non-respondents and to improve the power of the study. The inclusion criteria were as follows: (1) children in the age group of 6-12 years; (2) cooperative children; (3) children with no systemic diseases; (4) children not using any medications; (5) children not wearing any orthodontic or prosthetic appliances; and (6) children without any symptoms of illness, such as toothache.

An experienced dentist meticulously recorded DMFT using dental instruments and radiographs before commencing the study.

The participants of this study were randomly allocated into three groups of 17 children (A, B, and C), based on a balanced block randomization method. We used 17 blocks, each containing three participants. The participants were assigned into three groups based on the sequence of each block. Initially, a sample of non-stimulating saliva was obtained from each participant one hour after breakfast, and then, each child was given 10 cc of selected yoghurt. Group A received peach yoghurt, group B received plain yoghurt, and group C received probiotic yoghurt. All three types of yoghurt were produced by the same manufacturer. The children were instructed to swirl 10 cc of yoghurt in their mouth for two minutes. After this time, they could either swallow the yoghurt or spit it out, depending on their preference. Ten minutes after consuming the yoghurt, another salivary sample was obtained from each child.

After collecting the samples, they were immediately placed in a refrigerator at a temperature of 0-4°C. At the end of sample collection, they were transferred to the laboratory of Tabriz School of Pharmacy, in a container full of dry ice. At the end of the experiment, to reduce the effects of demineralization caused by yoghurt, fluoride therapy was performed for each patient according to a routine protocol. The pH level of both samples was calculated for all individuals using a pH measuring device, and the difference between the two values was considered as pH. [11, 14, 15] A data analyst, who was blinded to the study groups and the type of yoghurt used, performed the statistical analyses.

Statistical analysis

In this study, SPSS Version 19.0 (IBM, Chicago, IL, USA) was used for data analysis. Kolmogorov-Smirnov test was used for assessing the normal distribution of data. Chi-square ($\chi 2$) test was also performed for categorical variables. Since the data had a parametric distribution, paired t-test and analysis of variance (ANOVA) were used for within- and between-group comparisons of pH in the study groups, respectively. Tukey's post-hoc test was also used for pairwise comparisons of the groups. A P-value less than 0.05 was considered statistically significant on all tests.

Results

Table 1 presents the baseline characteristics of the participants. There were no significant differences between the groups regarding age (P=0.860), sex (P=0.731), or decay-missing-filled teeth (DMFT) index (P=0.935).

Table 1- Baseline (before) characteristics of the participants

Variables		Study Groups			P-value
		Peach (Group A) n= 17	Plain (Group B) n= 17	Probiotic (Group C) n= 17	
Age	Mean ± SD	8.82 ± 1.91	9.0 ± 2.06	8.64 ± 1.61	0.860*
Sex	Female	10	8	8	0.731**
	Male	7	9	9	
DMFT		5.70 ± 3.17	5.35 ± 2.76	5.64 ± 3.06	0.935*

* ANOVA
** $\chi 2$

Table 2 presents the results of within- and between-group comparisons of salivary pH levels. The mean and standard deviation (SD) of pH and the results of Tukey's test revealed significant differences in the mean pH of group A and the mean pH of groups B and C. Based on the results, the mean pH was lower in group A compared to the other two groups (P<0.001). However, no significant difference was found in the mean pH of groups B and C (P=0.990). In groups A and C, there were a significant difference in the mean pH before and after yoghurt consumption, and the mean pH level was found to be higher before consuming yoghurt as compared to after consuming it (P<0.001). In group B, there was no significant difference in the mean pH before and after yoghurt consumption (P=0.366).

Table 2- Comparison of within and between groups of pH level in the study participants based on before and after evaluations

Study groups	PH level (n= 51)		Within-group comparison*	Between-group comparison**	Post-hoc comparisons	
	Before (mean ± SD)	After (mean ± SD)			Two by two comparison	P-value†
A	6.89 ± 0.28	5.74 ± 0.56	0.001		A-B	0.001
B	6.90 ± 0.34	6.93 ± 0.42	0.366	0.001	B-C	0.990
C	7.02 ± 0.40	6.73 ± 0.51	0.001		C-A	0.001

A= peach, B= plain, C =probiotic
* Paired T-test
** ANOVA
† Tukey

Discussion

In this study, we aimed to evaluate salivary pH changes in 6 to 12 year-old children following the consumption of fruit, plain, and probiotic yoghurt. The salivary samples of 51 children were examined before and after yoghurt consumption in three groups of 17 people. The number of boys and girls was almost the same in our study. In a certain age range with a mean age of 8.82 years, the mean DMFT score was 5.56±8.82. The results of this study showed that among the examined yoghurt products, fruit yoghurt showed the greatest effectiveness in reducing salivary pH in the short term, followed by probiotic yoghurt (P<0.001). However, plain yoghurt had no significant effect on reducing or increasing salivary pH levels (P=0.366).

New approaches have been developed and studied to improve oral health by modulating inflammation and reducing the amount of biofilms or microorganisms present in the mouth. One of these new strategies to combat dental biofilm diseases is the use of probiotics, especially strains of the genus Lactobacillus and Bifidobacterium because of their capacity to adhere to different surfaces of the oral cavity. [16] It has been suggested that the consumption of probiotic yoghurt, containing Bifidobacterium lactis Bb12, may modify the oral biofilm formation. [17]

In this regard, a study by Natassa SE et al., evaluating the effects of probiotic and non-probiotic milk consumption on salivary pH and the number of S.mutans, found that salivary pH levels increased and that the amount of S. mutans reduced within 3-7 days after the consumption of the probiotic product. [18] Also, a significant increase was observed in salivary pH levels on days 14 and 28, and a significant decrease in S. mutans was reported on day 28 after receiving multi-dose probiotics. [19] The increase in pH levels after probiotic consumption could be explained by the ability of probiotic bacteria to compete with oral microorganisms and reduce their frequency. Therefore, the reduced frequency of acidogenic microorganisms also decreased acid production and consequently increased the salivary pH levels. This finding is interesting for dental caries control, which is influenced by pH imbalances. [20]

The abovementioned studies reported pH alterations in longer periods. However, some studies have indicated pH reduction in the first steps of probiotic dairy consumption, which is consistent with our results [8, 21]; this can be a cause for concern regarding the effects of probiotics on oral health. Generally, a negative correlation has been observed between salivary pH levels and DMFT; in other words, when salivary pH decreases, the incidence of tooth decay increases. [22] Moreover, based on new findings, exposure to probiotics can affect microhardness and surface roughness and cause leaching of essential elements, such as calcium and phosphorous, from the enamel. [7]

The results of a study by Srivastava et al., examining the effects of normal and probiotic curd on salivary pH, are similar to the findings of our study, as consuming probiotic curd was found to reduce salivary pH in a short period of time (30 minutes). [21] Moreover, a study by Moeiny P et al. in 2017 found the lowest pH level in the mouth after consuming fruit yoghurt. Also, the duration for which the pH level remained below the critical point was longer after consuming fruit yoghurt compared to the other types of yoghurt. [11] The results of this study, which indicated a reduction in plaque pH levels after consuming fruit yoghurt, are consistent with the findings of our study that also found a decrease in salivary pH levels after consuming fruit yoghurt. Although the parameter of comparison in this study differs from our study (plaque pH vs. salivary pH), the similarity in the results is probably due to similar study

designs.

An in vitro study by Kargul et al., which aimed to measure the initial pH of several types of yoghurt and examine their degree of saturation with respect to hydroxyapatite and fluorapatite to determine the buffering capacity and erosive potential of seven types of yoghurt, found that fruit yoghurt had the highest buffering capacity among the yoghurts tested. [23] The results of our study showed that the mean pH was significantly lower in the fruit yoghurt group compared to both normal and probiotic yoghurt groups. The difference between the results of this in vitro study and our research, which was conducted in the oral cavity, may be due to the influence of saliva and its buffering capacity.

In the current study, we could not precisely control for some variables, such as environmental and genetic conditions, oral health status, and oral hygiene, which is one of its limitations. It can be concluded that probiotic products reduce the pH of saliva within the first minutes after consumption. However, if they are consumed for a long period of time (e.g., 3-7 days), they can increase the pH of saliva and exert anti-caries effects in the long term. The probable reason for the immediate decrease in salivary pH levels in our study could be the inherent acidity (3.4-4.4) of yoghurt. It can be also attributed to the fermentation effects of carbohydrates and the production of carboxylic acids by lactic acid bacteria, which leads to a reduction in pH levels.

On the other hand, there are various mechanisms through which dairy products may reduce enamel demineralization in the long term. First, the uptake of milk proteins by the enamel surface may prevent its demineralization. Second, milk fat may be absorbed on the surface of the enamel and play a protective role, and third, milk enzymes may reduce the growth of acidogenic bacteria. [24, 25] Despite this reduction, the pH levels were still above the critical level (5.2-5.5), which usually causes the development or progression of dental caries; therefore, despite being acidic, yoghurt does not increase the risk of developing dental caries. [1]

Conclusion

Both fruit yoghurt and probiotic yoghurt could temporarily reduce the salivary pH levels. Fruit yoghurt was found to cause a greater decrease in pH levels as compared to probiotic yoghurt. However, due to the lower critical point of demineralization, there are no concerns about the development of dental caries.

Conflict of Interest

No Conflict of Interest Declared ■

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How to cite:
Sohrabi A, Teimoory N, Gholizadeh T, Zohdi M. Comparison of Salivary pH Changes in Children Aged 6-12 Years Following the Consumption of Plain, Probiotic, and Fruit Yoghurt: A Randomized Clinical Trial. J Dent Sch 2022;40(4):148-152.