Original Article

Evaluation of the Effect of Three Different Iranian Industrial Fruit Juices on Plaque PH

P. Moeiny [1], N. Shafizadeh [1], SH. Biabani [2], F. Raofie [3], MJ. Kharazifard [4].

[1]Assistant Professor, Department of Pediatric Dentistry, School of Dentistry, Dental Branch, Islamic Azad University. Tehran, Iran
[2]Dentist
[3]Assistant Professor, Department of Chemistry, Shahid Beheshti University of Sciences, Tehran, Iran
[4]Research Member of Dental Research Center Tehran University of Medical Sciences, Tehran, Iran

Abstract

Background and Aim: Evaluation of cariogenicity of fruit juices as healthy snacks has special importance. The aim of this study was to evaluate the effect of 3 different Iranian industrial fruit juices on plaque pH.

Materials and Methods: In this randomized clinical trial, 10 healthy dental students were selected based on the inclusion criteria namely the amount of streptococcus mutans and lactobacillus in the saliva, salivary secretion rate, buffering capacity of saliva, absence of active dental caries, absence of systemic disease and etc. Dental plaque pH in certain areas of the 4 mouth quadrants was measured by Ω Metrohm microelectrode and digital pH meter before and 2 to 60 minutes after the consumption of Children orange juice, orange nectar with pulp, pineapple nectar and 10% sucrose solution. The pH curve at different time points was then drawn for each product. Data were analyzed statistically by repeated measures ANOVA ($p<0.05$).

Results: The maximum drop in plaque pH occurred 2 minutes after consumption. The time spent below the critical pH was the longest for orange nectar with pulp (10.75 ± 2.24) and shortest for pineapple nectar (3.46±1.14) .At final minutes of study, plaque pH returned to its baseline value after consumption of all fruit juices except for orange nectar with pulp).

Conclusion: After consumption of orange nectar with pulp, the mean plaque pH was significantly lower in comparison to other juices and the time spent under the critical plaque pH was the longest.

Key Words: Dental plaque, Fruit juice, Hydrogen ion concentration, Microelectrodes

Corresponding author:
P. Moeiny, Assistant Professor, Department of Pediatric Dentistry, School of Dentistry, Dental Branch, Islamic Azad University. Tehran, Iran
pmoeiny@yahoo.com

Received: 8 March 2012
Accepted: 28 Oct 2012

Journal of Islamic Dental Association of IRAN (JIDAI) Summer 2013 ;25, (3)

Introduction

At present, significant developments have occurred in nutritional patterns and consumption of healthy foods and drinks has been more emphasized. Industrial fruit juices are now extensively advertised as healthy beverages and are increasingly used around the world. However, their safety in terms of dental health is still a matter of concern [1-3]. Nutritional dietsrichin fermentable carbohydrates are considered strongly cariogenic in populations with poor oral hygiene [4]. Sucrose and fructose are commonly used in industrial fruit juices [5].

Relative assessment ofthecariogenicity of foods and drinks has been conducted by the use of various methods such as enamel surface topography, evaluation of enamel microhardness and measurement of plaque and saliva pH [3]. Stephan in 1940for the first time discussed the alterations in plaque pH following the consumption of foods. Since then, assessment of the acidity of plaque pH has been carried out to estimate the cariogenic potential of foods and drinks. Provided that theacidogenic theory is accepted as an etiologic factor for development of dental caries, assessment of dental pla-

que pH before, during and after food consumption can be a guideline for determining the cariogenic potential of foods [2]. Microtouch method is one of the most reliabletechniques for assessment of dental plaque pH andestimation of theacidogenic potential of different foods [5]. Several researches have been conducted to measure plaque pH changes following the consumption of different snacks such as fruit juices [1,2,6-8]. Considering the increased consumption of industrial fruit juices in Iran and the fact that their consumption rate has increased by three folds in the recent years [9], we may state that industrial fruit juices are a popular snack in Iran. But, to date, no study has evaluated the effect of Iranian fruit juices on tooth structure and dental plaque pH. Enhancing the public knowledge about making the choice to consume less cariogenic foods and drinks is a valuable strategy to prevent dental caries. The present study was conducted to evaluate the effect of three types of Iranian popular industrial fruit juices on dental plaque pH.

Materials and Methods

The study design was approved by the Ethics Committee of Islamic Azad University, School of Dentistry (No#D/378/P) .In this single blind randomized crossover clinical trial, 10 healthy dental students aged 20-27 years were evaluated. The study design was completely explained to subjects and written informed consent was obtained from them.

All recruits were in good health condition, had not taken any antibiotics since two weeks before the study, were not on aspecific diet, did not have diagnosed xerostomia, hadno orthodontic appliance or prosthesis, were nonsmokers, were not pregnant and did not have periodontal disease. Subjects had noactivecarious lesions or restorations at the testing site (between distal surface of second premolar and mesial surface of first molar). They also had no permanent or temporary glass ionomer restorations in their mouth. Their stimulated salivary secretion rate was equal or more than 1 ml/min, indicating normal salivary secretion.Furthermore, number of salivary Streptococcus mutans and lactobacilli in subjects was measured using the CRT-Bacteria (Ivoclar Vivadent) kit andwas equal or more than 10^5 CFU/ml.

The buffering capacity of saliva in our understudy subjects was determined bythe CRT buffer test strip (Ivoclar-Vivadent) andonly those with normal buffering capacity of saliva were entered the study. After selection of understudy participants, similar toothpastes were given to subjects and they were asked to brush their teeth as usualwith the given toothpaste starting three weeks before the initiation of studyuntil the completion of the experiment. Subjects were also asked not to use any other fluoride-containing compounds in order to match thefluoride content of their saliva as much as possible.

Volunteers were requested not to use oral hygiene measures such as tooth brushing, flossing or using antibacterial mouthwash for 48 hours before the test session and to avoid eating or drinking (except for water) for 2 hours prior to testingin order to allow the accumulation of dental plaque to reachits acidogenic potential but at the same time not compromising dental or periodontal health. Three understudy fruit juices and 10% sucrose solution (positive control) were coded from A to D. Arecently opened pack of juice or fresh10%sucrose solution were prepared and offered to each participant. A total amount of 10cc of the juice (after shaking) or10%sucrose solution was given to participants in disposable glasses in each session. At first, participants were divided into2 groups of 4 and 1 group of 2using convenient sampling. The intervention for each group was carried out in 4 sessions during 4consecutiveweeks. One week interval was considered as the wash out period. Each group in each week randomly consumed one of the understudy products in a cross over method. The content and ingredients of products are presented in Table 1 according to their labeling.

Understudy solutions included:

A: Orange juice for children

B: Natural orange nectar with pulp

C: Natural pineapple nectar

D: 10% Sucrose solution (positive control)

In each session before the experiment, the baseline pH of plaque wasmeasuredwithMetrohm microelectrode (Metrohm Switzerland LL micro glass electrode) connected to a digital pH-meter (Metrohm-Switzerland) at the embrasure between the distal surface of the second premolar and mesial surface of the first molar in all 4 quadrants ofthe mouth. Afterwards,the participants were asked to hold 10cc ofthe understudyfruit juice in theirmouth for2 minutes and then swallow it.Plaque pH was measuredat 2, 5, 7, 10, 15, 20, 30, 40, 50 and 60 minutes after drinkingof the solution at the above-mentioned siteas described above. It should be mentioned that the operator who measured thepH of dental plaque was blindedto the type of consumed solution.

It should also be noted thatthe microelectrode was calibrated by 3 mol KCl solution with a pH=7 and washed

Table 1. Fruit juice ingredients

Fruit juice type * Ingredients	Orange nectar with pulp (50% fruit juice)	Children orange juice (25% fruit juice)	Pineapple nectar (45% fruit juice)
Sodium (mg)	21	22/8	18
Potassium (mg)	430	360	223
Total carbohydrate (gr)	30	30	29
Sugars (gr)	25	24	26
Protein (gr)	1/07	1/5	2/3
Calcium %	2%	2%	2%
Iron %	< 2%	< 2%	< 2%
Vitamin C %**	94%	80%	60%
Vitamin A %	< 2%	< 2%	< 2%
PH***	3/23	3/03	3/45

*Ingredientsper each serving. Serving size:240 ml
** % indicates percent daily value
***Was measured by Dr. Farhad Raofie (R –F)

with distilled water before startingthe tests and also in between two tests. Themicroelectrode was immersed in 2%glutaraldehydesolution for 20 minutes for disinfection before testing each participant.

The mean pH was determined in all quadrants at different measurement time points and separately for the4solutions. The results were analyzed using repeated measures ANOVA and P<0.05 was considered statistically significant.

Results

A total of 10 dental students with a mean age of 27.1±4.9 years participated in this experimental single blindcrossover study. The results of analysis of mean-plaque pH changes before and at certain time points after the consumption of understudy solutions using repeated measures ANOVA (p<0.05) are presented in Table 2 and Diagram 1.

Table 2. Mean ± SDof plaquepHat different time points afterthe consumption of understudydrinks

Studied material / Time (min)	Children orange (a)	Juice Orange (b)	Nectar Pineapple (c)	10% sucrose (d)	P.V	Order of pH pH drop
0	6/71±0/14	6/70± .12	6/64 ± .12	6/67 ± .17	0/217	a=b=c=d
2	5/33± .15	5/38± .13	5/62± .13	6/31 ± .18	<0/001	d>c>b=a
5	5/84± .12	5/60± .14	6/04± .14	6/09 ± .16	0/004	d=c>a>b
7	5/86± .10	5/84± .11	6/23± .11	6/04 ± .15	<0/001	c>d>a=b
10	6/08± .09	5/96± .08	6/43± .08	6/10 ± .12	<0/001	C>d=a>b
15	6/20± .11	6/07± .14	6/44± .14	6/03 ± .12	<0/001	C>a>b=d
20	6/44± .09	6/06± .12	6/54± .12	6/26 ± .10	<0/001	C>a>d>b
30	6/46± .09	6/08± .14	6/44 ± .13	6/44 ± .13	<0/001	C=d=a>b
40	6/58± .11	6/16± .13	6/56± .13	6/54 ± .12	0/003	C=d=a>b
50	6/63± .12	6/19± .14	6/67± .14	6/54 ± .17	<0/001	C=d=a>b
60	6/54± .13	6/14± .15	6/67± .15	6/64 ± .16	<0/001	C=d=a>b

The results of this study showed that consumption of orange nectar with pulp had the highest effect on decreasing the plaque pH in the majority of tested time points compared to other solutions. Children orange juice ranked second in this regard. However, the difference between the mentioned two juices was not statistically significant. Pineapple nectar and 10% sucrose solution ranked next, respectively. Regular yoghurt is

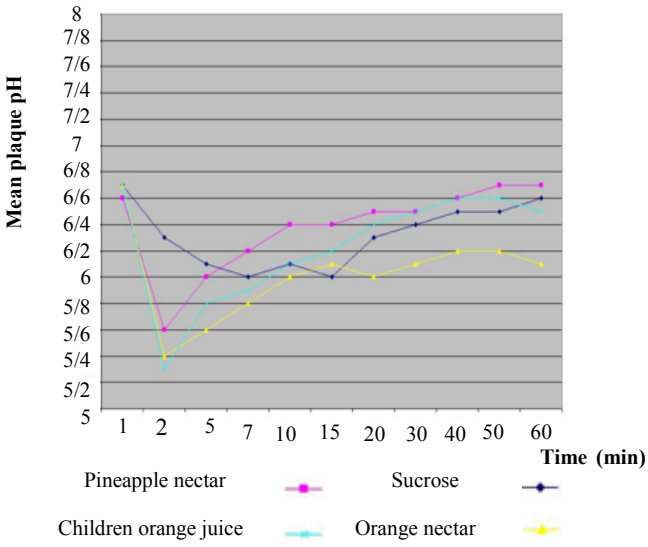

Diagram 1. Mean plaque pH measured at different time pointsfollowing theconsumption of understudydrinks

placed somewhere between the mentioned two materials.

Also, as observed inTable 2, the maximumdrop inplaque pH after the consumption of understudy fruit juicesoccurred at 2 minutes after juiceconsumption. Plaque pH recoveryoccurred faster after the consumption ofpineapple nectarand children orange juice, as the plaque pH returned to its baseline valueat final minutes (Diagram1).Thesituation was different for orange nectar with pulp asthe plaque pH recovery was slower and did not return to the baseline value even at the final minutes.

Also, in our study weassessed the time spent below the critical plaquepH levelafter the consumption of the 4 understudy drinks (Table 3). The differences in this respectbetween the studied drinks were statistically significant ($p<0.05$). The longest time spent below the critical pH leveloccurred following the consumption oforange nectar with pulp (pH=6).Children orange juice and pineapple juiceranked next,respectively;the least time spent belowthecritical pH was due to the consumption of 10% sucrose solution.

Table 3shows the time spent below the critical pH level following the consumption of the understudy products. The respective durationforfruit yoghurt has shown to be 1.7 times more thantheregular yoghurt. It should be mentioned that the drop in plaque pH below the critical levelafterthe consumption of 10% sucrose solutionwas not significant (Table 3).

Table 3. Duration of time spent below the critical plaque pH following the consumption of understudy solutions

Test products	Time (minute) ± SE
Orange nectar	10/75±2/24
Children orange juice	7/88±1/45
Pineapple nectar	3/46±1/14
10% sucrose solution	0/05±0/28

Discussion

In the present study, consumption of orange nectar with pulp caused the greatest drop in plaque pH followed by children orange juice (but with no significant difference). Pineapple nectar ranked next and 10% sucrose solution ranked last in this respect.

In a study by Banan and Hegde, plaque pH drop following the consumption of grape juice was greater compared to orange juice, and pineapple juice caused the least drop in pH compared to other fruit juices [2]. In a laboratory study by Grenby, citrus fruit juices caused hydroxyapatite demineralization more than pineapple juice and other beverages [6]. In a study by Sudhanshu, of the 4 understudy solutions, maximum drop in plaque pH occurred after the consumption of sweet lemon juice followed by apple juice, papaya juice and sucrose solution [1]. Al-tinawi and Saeed in their study evaluated the effect of coke, orange juice and milk on plaque pH and found that orange juice, coke and 10% sucrose solution led to the highest drop in pH, respectively but milk could not decrease the plaquepH below the baseline value [7]. In a study by Toumba and Duggal, drop in plaque pH following the consumption of mixed citrus drink was significantly higher compared to other fruit juices [8].

In our study, maximum drop in plaque pH occurred 2 minutes after the consumption of fruit juices. Plaque pH was then gradually increased for all groups but the pH recovery trend was especially significant for pineapple juice and had a slower pace for the other two fruit juices. The time spent below the critical pH was 3.45 min for the pineapple nectar, 7.88 min for children orange juice and 10.75 min for orange nectar (Table 3). The exact determination of critical pH is not feasible because it is variable in different individuals and at different areas of the oral cavity [4, 10]. However, most references consider the pH below 5-6 as the danger zone for initiation of enamel demineralization [2, 4, 11-13]. In our study, pH=6 was considered as the critical pH that appears to be more conservative for assessment of

theacidogenicity of snacks [3]. It should be mentioned that the plaque pH returned to its baseline value at the final minutes (at 50 to 60 min) following the consumption of pineapple juice and 10% sucrose solution; but this did not happen for children orange juice and orange nectar. Especially for orange nectar with pulp, plaque pH at the final minutes had a significant difference with the baseline value ($P<0.003$).

In a study by Johansson et al, plaque pH had the maximum drop at 2 minutes following the consumption of orange juice and remained below the critical threshold for about 15 min in subjects with normal salivary flow and returned to the baseline value at the final minutes (at 50-60 min)[14].

In a study by Banan and Hegde, maximum drop in plaque pH occurred at 5 minutes after the consumption of understudy fruit juices. Plaque pH recovery happened gradually and returned to its baseline value during 30 minutes. The difference between their study results and ours may be due to the different additives, ingredients, acidity and natural pH of the understudy beverages [2].

In a study by Toumba and Duggal, maximum drop in plaque pH happened at 5 minutes after the consumption of mixed citrus drink and remained below the critical pH for 5 minutes. They stated that taking a primary sample of the plaque at 5 minutes after the consumption may not always show the minimum plaque pH and primary pH measurement at 2 (similar to our study) or 3 minutes may better indicate maximum drop in plaque pH following the consumption of fruit juices. Furthermore, the natural pH of the mixed citrus drink used was higher than that of orange juice used in our study and had lower sugar content as well (almost one-third compared to our understudy fruit juices). Also, pH measurement in their study was carried out using the sampling method [8]. Lingestrom et al. have stated that this method shows the drop in plaque pH significantly less than the microtouch microelectrode [5].

Jensen and Schachtele compared the effect of consumption of several snacks on plaque pH and reported that maximum drop in plaque pH occurred 10-15 min after the consumption of fruit juice (type of the fruit juice or its characteristics have not been mentioned, it has only been stated that the fruit juice contained 10% carbohydrates)and then rapidly recovered to its baseline value. However, for other products such as 10% sucrose solution, recovery in plaque pH did not occur within the first 30 min. The authors stated that the added flavors to fruit juices might act as a sialogogue and quickly raise the plaque pH after its primary drop [15]. This phenomenon may justify the slow recovery of plaque pH following its primary drop after the consumption of sucrose solution in comparison to our understudy fruit juices.

Beighton believes that the quick primary drop in plaque pH (sooner than 5 minutes) after the consumption of fruit juices may be mainly due to their acidic content rather than sugar fermentation by the plaque bacteria [16]. This finding may explain the delayed maximum pH drop (at 7 minutes) and smoother trend of plaque pH variations following the consumption of 10% sucrose solution compared to our understudy fruit juices.

It appears that the acidic component of fruit juices (acetic acid, citric acid, malic acid and ascorbic acid based on the type of fruit juice) is quickly washed off the oral cavity by the saliva and after this cleansing acids produced through the fermentation of carbohydrates by the bacteria (lactate and succinate) reach their maximum concentration. It has been stated that exposure of bacteria to foods with low pH reduces their ability to ferment carbohydrates and produce acids [16]. Johansson et al, and Sudhanshu et al, in separate studies confirmed this finding [1, 14].

The pH curve after the consumption of fruit juices follows the typical pattern of the Stephan curve and this is due to three main factors:

1.Product ingredients such as acids, sugars, calcium, phosphorus, etc

2.Individual factors such as salivary content, plaque index, plaque age and type of oral microflora

3.Pattern of food and drink consumption [13, 14]

Of the mentioned factors, the latter two were standardized and matched as much as possible. Thus, in order to explain differences in plaque pH following the consumption of fruit juices, further attention had to be paid to the first factor namely product ingredients (Table 1). Although the three understudy drinks had a pH of 3-3.5, pH of the children orange juice (containing 25% fruit juice) was the lowest followed by the pH of orange nectar (containing 50% fruit juice) and pineapple nectar (containing 45% fruit juice). In a study by Grenby, pure fruit juices had a higher pH compared to orange juice (containing 10% fruit juice). In addition to natural pH, the titratable acid content of drinks was evaluated as well and it was mentioned that the purer the fruit juice, the higher its titratable acid content. This characteristic is found to be responsible for greater dissolution of hydroxyapatite calcium and phosphorus [6]. Furthermore, researchers mention that this is the buffering capacity of

the fruit juice that enables it to neutralize salivary buffers and cause further reduction in plaque pH. Thus, fruit juices with higher titratable acidity can cause longer drop in plaque pH. In the mentioned study, the titratable acidity was defined as the volume of 0.1 N sodium hydroxide required to neutralize the beverage andbring the pH of 10 cc of fruit juice to 5.5 [1]. Furthermore, according to Saeed, the pH of a beverage is not an indicatorof its titratable acidity and the titratable acid contentis better indicative of the potential dental erosivenessof a drink [7].

In our study, the titratable acid was not determined in the understudy fruit juices but according to Odebunmi the acidity of fresh orange juice (31% M) is much higher than that of fresh pineapple juice (31% M)[17]. This finding can explain the lower drop in plaque pH and shorter time spent below the critical plaque pH after the consumption of pineapple juice compared to the two types of orange juice in our study. Comparison of fruit juices only in terms of acidity may not be adequate. Grenby et al. discuss that only the acidity of a solution is not sufficient for the assessment of its acid content and chemical nature and power of acids (H^+ release) are different as well [6]. Citric acid and malic acid are the two main organic acids in orange juice and pineapple juice (18, 19). But, the ratio of citric acid to malic acid in orange juice is greater than that in pineapple juice [20]. Citric acid is more potent and the most destructive one for tooth enamel. Since the purer the fruit juice, the higher its acidity [6], we may justify the longer time spent below the critical plaque pH and its slower recovery towards the baseline plaque pH after the consumption of orange nectar with pulp in comparison to children orange juice. Furthermore, difference in physical characteristics of orange nectar with pulp and children orange juice may also be responsible for the longer time spent below the critical pH. Gustaffson et al.mention that the risk of plaque pH drop following the consumption of sugars becomes greater and longer given that they are consumed in high viscosity form having greater tendency to remain on the tooth surface [19].

As observed in Table 1, the totalcarbohydrate and sugar contentof these 3 fruit juices are almost the same. In general, the main sugars present in fruit juices are sucrose, glucose and fructose. Although according to Grenby the content of these three types of sugars is different in fruit juices [6], it has been mentioned that all the three types of sugars have similar capability in reducing plaque pH [19]. One ingredient of the understudy fruit juices (Table 1) with a significant difference in amount between pineapple and orange juice isthe potassium content. Its amount in the orange nectar and children orange juice was twice and 1.5 times its value in the pineapple juice, respectively. Ho-kwon et al, in their clinical study indicated a direct association between DMFS in high school students and daily potassium intake but mentioned no reason for this correlation [21]. Marsh in an in-vitro study on the effect of sodium and potassium on acid production by Streptococcus mutans and Streptococcus Sanguinis in a media containing different types of fermentable sugars demonstrated that addition of KCl to their culture medium resulted in greater pH drop and concluded that potassium ions reinforce the acidogenicity of Streptococcus mutans and Streptococcus Sanguinis. This is attributed to the role of potassium in preserving the membrane energy [22]. Iwami et al, in their in-vitro study mentioned that in low pH, potassium ions increase the amount of titratable acid produced by the understudy bacteria [23]. Wang and Germaine in their study reported that potassium ions neutralize the inhibitory effect of lysozyme enzyme present in dental plaque and saliva on glucose fermentation by Streptococcus mutans [24]. However, this finding needs further laboratory and clinical investigations.

It should be noted that explaining the present study findings is rather complex because industrial fruit juices are not simple chemical solutions. They have numerous ingredients that can affect their properties from different aspects. For instance, calcium and phosphorous content, or level of protein can explain the differences in pH curves due to their buffering capacity or tendency to gradual release of destructive acids through the fermentation of carbohydrates [6]. Presence of preservatives and additives in fruit juices can also affect changes in plaque pH [8].

In general, our study showed that industrial fruit juices could quickly drop the plaque pH and maintain it for a long time below the critical pH level. They can initiate demineralization in tooth enamel as well.

The present study had some advantages: all the participants used one type of toothpaste throughout the study, did not use any fluoride-containing products or mouth rinses and subjects were matched in terms of buffering capacity of the saliva, lack of carious lesions, number of cariogenic bacteria in the saliva and etc. Thus, the baseline plaque pH was not significantly different between groups. Koparal and Akay believe that this finding is indicative of the reliability of study design [12, 25].

Also, standardizing and matching the fluoride content of the saliva and dental plaque were carried out in our study according to a study by Heijnsbroc [26]. None of the similar studies considered this issue. Furthermore, it should be noted that the Ω Metrohm microelectrode used in our study is much more accurate and sensitive than those requiring a separate reference electrode and salt bridge [5, 11, 14]. Additionally, all phases of plaque pH measurement and calibration of electrodes were carried out under direct supervision of the Chemistry Department at Shahid Beheshti University.

Conclusion

1.Drop in plaque pH after the consumption of understudy beverages was greater compared to 10% sucrose solution.

2.The mean plaque pH following the consumption of orange nectar with pulp at all time points was lower in comparison to other fruit juices.

3.The understudy fruit juices are all considered harmful to tooth structure due to droppingthe plaque pH and long duration of time spent below the critical pH level.

References

1- Sudhanshu S, Oberoi SS, Tiwari S. Effect of fresh fruite juices on pH of dental plaque. Annals and Essence of Dent, 2010; 2(4):36-40.

2- Banan LK, Hegde AM. Plaque and salivary PH changes after consumption of fresh fruit juices. J Clin Pediatric Dent. 2005 Fall;30(1):9-13.

3- Huang GF, Chang H, Wang Y, Guo MK. Effect of oral rinse with soft dirnks on human plaque pH. Chin Dent J. 2001 June;20(2):83-92.

4- Axelsson Per. Diagnosis and risk prediction of dental caries. Illinoise Quintessence: Pub; 2000, 55-76.

5- Lingstrom P, Imfeld T, Birkhed D. Comparison of three different methods for measurement of plaque-PH in humans after consumption of soft bread and potato chips. J Dent Res. 1993May; 72 (5):865-870.

6- Grenby TH, Phillips A, Deasi T, Mistry M. Laboratory studies of the dental properties of soft drinks. Br J Nutr. 1989 Sep; 62(2):451-64.

7- Saeed S, Al-Tinawi M. Evaluation of acidity and total sugar content of children's popular beverages and their effect on plaque pH. J Indian Soc Pedodo & preven Dent. 2010 Jul-Sep; 28(3):189-192.

8- Toumba KJ, Duggal MS. Effect on plaque PH of fruit drinks with reduced carbohydrate content. Br Dent J. 1999 Jun 26;186(12):626-29.

9- Journal of sarmayeh. Consumption of fruit juices in Iran is 32 liters less than Europe annually. magiran.co, [No 178-P15]. Available at: www. Magiran/ npview. asp, 2010 (Persian).

10-Harper Ds, Abelson DC, Jensen ME. Human plaque acidity models. J Dent Res. 1986; 65:1503-10.

11-Mortazavi S, Noin S.Plaque pH changes following consumption of two types of plain and bulky bread. Dent Res J. 2010 Spring; 8(2):80-4.

12-Koparal E, Eronat C, Eronat N. Invivo assessment of dental plaque pH changes in children after ingestion of snack foods. J Dent Child. 1998, 4(Nov–Dec); 65(6): 478-483.

13-Harris NO, Garcia-Goday F, Nielsen NC. Primary preventive dentistiry.7th ed. Newjersy: Pearson; 2009,40-1.

14-Johansson A, Lingstrom P, Birkhed D. Effect of soft drinks on proximal plaque PH at normal and low salivary secretion rates. Acta Odontol Scand. 2007 Nov; 65(6):352-6.

15-Jensen ME, Schacthele CF. The acidogenic potential of refrence foods and snacks at interproximal sites in human dentition. J Dent Res. 1983 Aug;62(8):889-892.

16- Beighton D, Brailsford SR, Gilbert SC. Intra–oral acid production associated with eating whole or pulped raw fruits. Caries Res. 2004 Jul-Aug; 38(4):341-49.

17-Odebunmi EO, Dosumu OO. Fermentation studies and nutritional analysis of drinks made from water extract Hiscus sabdariffa Galyx (SOBO), juices of citrus science (Orange), Ananas comosus (pineapple). J Food Technol. 2007;5(3):198-204.

18-Sairi MS, Yih LJ, Sarmidi MR. Chemical composition and sensory analysis of fresh pineapple and decidified pineapple juice using electrodialysis. Regional symposium on Membrane science and technology. 21-25 April, Johor/Malaysia. 2004.

19-Camara MM, Diez C, Torija ME, Cano MP. HPLC determination of organic acids in pine apple juices and nectars. Europ Food Res & Technol. 1994;198(1):52-56.

20-Murray jj, Nunn JH, G. Steel J. Prevetion of oral disease. 4th ed. New York: Oxford university Press; 2003, 17-25.

21-Suhg HK, Kim Yk, Chung HJ, Nam MO, Jun KM, Kim HG. Relationship between nutritional intake and dental caries experience of junior high students. Yonsei Med J. 1997Apr; 38(2):101-110.

22-Marsh PD, Willamson MI, Keevil CW. Influence of sodium and potassium Ions on acid production by

washed cells of streptococcuss mutans ingbritt and streptococcus sanguis NCTC 7865 grown in a chemostat. J Infection Immunity. 1982 May;476-483.
23-Iwami Y, Guha-Chowdhury N, Yamada T. Effect of sodium and potassium ions on intracellular pH and proton excretion in glycolysing cells of streptococcus mutans NCTC 10449 under strictly anaerobic conditions. Oral Microbiol Immunol. 1997 Apr;12(2):77-81.
24- Wang YB, Germaine GR. Effects of pH, Potassium, Magnesium and Bacterial growth phase on lysozyme inhibition of glucose fermentation by streptococcus mutans 10449. J Dent Res. 1993 May;75(5):907-911.
25- Atar Akay G, Altay N. Effect of chewing gum containing calcium fluoride on salivary acidogenecity. Caries Res. 2007; 41:208-211.
26-Heijnsbroc M. Geroduva, Bui Jsmj, Van loveren C, Tencate JM. Timmerman MF, etal. Increased salivary fluoride concentrations after post-brush fluoride rising not reflected in dental plaque. Caries Res. 2006; 40 (5):444-8.