Caspian Journal of
Dental Research

Original Article

Total antioxidant property and pH change of dental plaque and saliva in 6-11-year-old children after consumption of flavored milk

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Abstract

Introduction: The antioxidant properties of chocolate and other flavored additives besides the sugar added to milk raises the question about the acidogenecity of flavored milk. This study was conducted to measure the pH changes of dental plaque and saliva after the consumption of flavored milk and evaluate the antioxidant property of them.

Methods: This study was performed on 42 samples of dental plaque and 42 samples of saliva in 6-11 year old school going children. Milk with flavors of strawberry, chocolate, banana, honey and slim milk were evaluated, all from the same manufacturer with a similar production date. At the beginning of the study on the first day, children were given thorough oral propHylaxis and they were instructed to avoid any method of oral hygiene for 48 hours to permit enough plaque deposition. On the third day the children were divided into 7 groups, 6 children in each group. The supra-gingival plaque was collected through the help of an excavator #3 which was pulled twice with the same force on the tooth surface. The saliva was collected using spitting technique. Each child swished 10 cc of milk for 1 minute in his/her mouth. Fresh plaque samples after 5, 10, 20 and 30 minutes and saliva samples immediately, after 5, 10, 20 and 30 minutes were collected. The pH of the samples were recorded by a pH testing apparatus (Basic 20+, Crisom). To evaluate the antioxidant property of studied milk, Frap test was performed. The collected readings were reported as mean±SD and analyzed by ANOVA repeated measures, Post hoc Tukey and Paired T-test. In this study, $p \leq 0.05$ was considered as significant.

Results: After 30 minutes, honey milk caused the least drop 0.74±0.30 and banana milk caused the highest drop 1.38± 0.25 in plaque pH ($p \leq 0.05$). After 30 minutes, the pH of saliva showed no significant difference compared to the initial pH. Chocolate milk contained the highest (1000 micromol/liter) and banana milk the lowest (706.25 micromol/liter) antioxidant concentration.

Conclusions: Because of the highest antioxidant properties and reduction of dental plaque PH to a lesser extent, milk with honey, chocolate and coffee is more recommended for children.

Keywords: Milk, Dental plaque, Saliva, Antioxidant capacity, pH

Received: 25 May 2013 Accepted:16 Jul 2013

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[DOR: 20.1001.1.22519890.2013.2.2.7.2]
[DOI: 10.22088/cjdr.2.2.15]

خاصیت آنتی اکسیدانی تام و تغییرات pH بزاق و پلاک دندانی کودکان ۱۱-۶ سال به دنبال مصرف شیرهای طعم دار

چکیده

مقدمه: خاصیت آنتی اکسیدانی کاکائو و دیگر افزودنی های طعم دار و شکر افزوده به شیر در شیرهای طعم دار اسیدوژنیسیتی این شیرها را تحت سوال می برد. این مطالعه به هدف بررسی تغییرات pH پلاک و بزاق و میزان آنتی اکسیدانی شیرهای طعم دار در کودکان انجام شد.

مواد و روش ها: این مطالعه تجربی روی ۴۲ نمونه پلاک دندانی و ۴۲ نمونه بزاق کودکان ۱۱-۶ سال انجام شد. شیرهایی با طعم های توت فرنگی، کاکائو، عسل، موز و شیر معمولی همه از یک کارخانه و با یک تاریخ تولید بررسی شدند. قبل از مطالعه کودکان تحت پروفیلاکسی قرار گرفتند و سپس جهت تجمع پلاک برای ۴۸ ساعت بهداشت دهانی را رعایت نکردند. در روز سوم کودکان به ۷ گروه ۶ نفره تقسیم شدند. ابتدا پلاک بالای لثه ای با استفاده از اکسکاوتور شماره ۳ که با نیروی یکسان ۲ بار بر سطح دندان های مورد نظر کشیده شد و سپس بزاق آنها به روش Spitting جمع آوری شد. هر کودک ۱۰ سی سی شیر طعم دار را ۱ دقیقه در دهان قرقره کرد. سپس نمونه های تازه پلاک بعد از ۵، ۱۰، ۲۰، ۳۰ دقیقه و نمونه های بزاق فوراً، ۵، ۱۰، ۲۰، ۳۰ دقیقه جمع آوری شدند و pH آن ها با استفاده از pH متر (Basic20+, Crisom) اندازه گیری شد. جهت بررسی خاصیت آنتی اکسیدانی شیرهای مورد مطالعه از روش Ferric Reducing Ability Of Plasma(FRAP) استفاده کردیم. اطلاعات بدست آمده با استفاده از آزمونهای آماری آنالیز واریانس و تست تعقیبی توکی و آزمون تی زوج و Anova Reapeted Measures مورد تجزیه و تحلیل قرار گرفت

یافته ها: پس از ۳۰ دقیقه شیر عسل به کمترین مقدار (۰/۳۰±۰/۷۴) و شیر موز به بیشترین میزان (۰/۲۵±۱/۳۸) سبب کاهش pH پلاک شدند ($p\leq 0.05$). در پایان ۳۰ دقیقه تغییرات pH بزاق نسبت به pH اولیه اختلاف معنی داری نداشت. شیر کاکائو دارای بیشترین (۱۰۰۰ میکرو مول/لیتر) و شیر موز دارای کمترین (میکرو مول/لیتر۷۰۶/۲۵) غلظت آنتی اکسیدانی هستند.

نتیجه گیری: مصرف شیر عسل, شیر کاکائو و شیر قهوه به دلیل خاصیت آنتی اکسیدانی بالاتر و کاهش کمتر pH پلاک برای کودکان توصیه می شود.

واژگان کلیدی: شیر، پلاک دندانی، بزاق، کودکان، ظرفیت آنتی اکسیدانی،pH

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Introduction

Dental caries is among the most common childhood disease (1-3). This disease causes demineralization of mineral structures of teeth. It is an infective, multifactorial and transmittable disease (1). The factors including dietary habits, quality and quantity of dental plaque, quantity and quality of saliva, age and immunity of body, oral hygiene habits, oral microflora, condition of the teeth and genetic factors are among the effective invoices on dental caries (2-5).

R.S.Levine in his study revealed that milk is one of the most important sources of calcium and phosphate. It causes a considerable reduction in plaque pH. The plain milk is non-cariogenic. The anticariogenicity of milk lies within its high buffering capacity and its components i.e, calcium, phosphate, proteins and phosphoproteins (6). Masih et al. and Danchaivijitr et al. showed that milk can be cariostatic and its consumption with cariogenic food dominantes this property (7,8).

Flavored milks contain 5% or more added sugar. Increasing the consumption of sweetened milk in the last two decades raises this question about the carcinogenicity of these kinds of milk (6). The Study performed by Nassar et al., revealed the antibacterial property of honey and its prominent role in the prevention of s.mutans growth and biofilm formation (9). Antonio et al. discussed that components containing soluble coffee have antioxidant property. It is related to the concentration of phenolic components, caffeine and melanoidin present in these foods (10).

Frazzano et al. performed a study on plant polyphenols. They showed that the ability of s. mutans to attach to tooth surface and biofilm formation, and also the ability to metabolize carbohydrates are among the important factors in the induction of decay. Plant polyphenols have an important effect on S. mutans (11).

Srikanth et al. revealed that the cocoa husk bean extract has an antibacterial and antiglucosyl transferase properties and it plays an important role in the attachment of bacteria to acquired pellicle. Therefore, consumption of beverages containing cocoa causes a considerable reduction in S. mutans and deposition of dental plaque (12). This study aimed to investigate the changes in saliva and plaque pH after consumption of flavored milk and the antioxidant property of these kinds of milk in 6-11- year -old children.

Methods

This study was conducted in Babol on 42 samples of dental plaque and 42 samples of saliva of school children in the age group (6-11), who were randomly selected. The following different kinds of flavored milk was investigated i.e. chocolate milk, strawberry milk, honey milk, coffee milk and banana milk along with the plain milk, all were produced by the same company on the same date.

A letter including all the information about the study was given to the guardians and the study was carried out only after permission was granted. Inclusion criteria: Healthy, co-operative children who had teeth with labial, buccal and lingual surfaces free from any restoration and dft/DFT of 3 or less than that. Exclusion criteria: children having xerostomia, lactose intolerance, allergy to milk and children on antibiotic therapy in the past one month.

While the intra-oral examination of the children, those with clinical features of xerostomia i.e. dryness and burning sensation in oral cavity, pale or cracked oral mucous membrane, fissured oral mucosa, atrophic tongue were excluded from this study. Selected tooth surfaces including: a) Palatal surface #16 (maxillary first permanent right molar) b) lingual surface #46,44/84 (mandibular first permanent right molar, mandibular first permanent right premolar/mandibular first deciduous right molar) c) lingual surface #34/74, 36 (mandibular first permanent left premolar/mandibular first deciduous left molar, mandibular first permanent left molar).

The study was performed under the following steps: step 1- At the beginning of study on the first day, the children underwent oral prophylaxis to obtain a uniform baseline of zero for plaque score and to determine that a disclosing solution was used. The children were asked to avoid any method of oral hygiene for 48 hours.

It provides enough time for the adequate deposition of dental plaque. They were asked to attend school without eating any form of breakfast on the third day. They were only allowed to drink one or more glasses of water. At the end of the study, scaling and fluoride therapy were performed for these children. Step 2: 48 hours after oral prophylaxis between 8-11 am, supragingival plaque samples were collected. The readings obtained from pH meter were served as baseline or resting plaque pH.

After collecting the resting plaque, the children were divided into 7 groups, 6 persons in each group. Each child was given 10 cc of flavored or plain milk and they were asked to swish it around the mouth for 1 minute and depend on their preference either swallow or spit it out. Fresh plaque samples were collected by plaque sampling technique to measure their pH by pH meter. The samples were placed in the test tube and changes in plaque pH were recorded according to the following: a) After 5 minutes from #46 b) After 10 minutes from #44/84 (based on the presence of any of these teeth in the mouth) c) After 20 minutes from #36 d) After 30 minutes from #74/34. Unstimulated salivary samples were collected before and immediately, 5, 10, 20 and 30 minutes after drinking the test drinks.

They were placed in test tubes and during sample collection the samples were coded and the time of collection of each sample was inserted on the test tubes. After collection, the samples were immediately placed in the refrigerator at 0-4 C and at the end of collection they were carried in a container filled with samples and dry ice to the biochemistry laboratory of Babol University of Medical Sciences.

Step 3: The pH of salivary and plaque samples were measured by a calibrated pH meter with H+ ion sensitive electrode. (pH meter Basic 20+, Crisom). Equal weight of plaque samples were dissolved in 2 ml of distilled water. 60 seconds after placing the plaque suspension in electrode system, the pH was read. The collected readings were reported as mean±SD and analyzed by ANOVA repeated measures, Post hoc Tukey and Paired T-test. In this study, $p<0.05$ was considered as significant.

FRAP test (Ferric Reducing Antioxidant Power) was used to calculate the total antioxidant activity of plain and flavored milk. The samples were transported out of -20°C freezer to reach to the laboratory temperature and they underwent centrifugation at 4000 rpm for 10 minutes. After deposition of impurities, the supernatant was used to measure total protein and total antioxidant capacity (TAC). FRAP reagent contains (TPTZ 2, 4, 6, Tripyridyl-s-triazine) 10 mmol/L in 40 mmol/L HCl with FeCl3 20 mmol/L and buffer acetate 0.3 mol/L. The principal of FRAP test is based on the reduction of Fe3+ to Fe2+ in the presence of antioxidants. The prepared FRAP reagent was heated at 37°C for 5 minutes. Then 1.5 ml of working FRAP reagent was transferred to test tubes, and 50 microliter of milk was added and mixed. After incubation at 37°C for 10 minutes the absorption was read at 593 nm and compared with the standards. The standard solution for FRAP test is FeSO4 (125, 250, 500 and 1000 micromol/liter). The standard curve was plotted based on that. The collected readings were reported as mean±SD and analyzed by ANOVA repeated measures, Post hoc Tukey and Paired T-test. In this study, $p\leq0.05$ was considered as significant.

Results

This study was performed on 42 plaque samples and 42 salivary samples. The samples were divided into 7 groups, 6 persons in each. After collection of samples, their pH were measured, and the course of changes of pH was compared among the different groups. The course of changes in dental plaque pH (obtained by ANOVA test) reported as mean±SD is shown in each group at 5 minutes before, 5, 10, 20 and 30 minutes after consumption of flavored milk ($p<0.05$). A gradual decrease in pH of dental plaque was observed over 30 minutes. Banana milk caused the highest (1.38±0.25) and honey milk caused the lowest (0.74±0.30) drop in pH of dental plaque (table and figure 1). The course of changes in salivary pH (obtained by ANOVA repeated measures) reported as mean±SD is shown in each group at 5 minutes before, immediately and 5, 10, 20 and 30 minutes after consumption of flavored milk ($p\leq0.05$). After the consumption of milk, a sudden decrease and then a gradual increase in pH was observed. Honey milk caused the highest (0.27±0.37) increase in salivary pH ($p>0.05$). All the other milk except chocolate milk reduced the pH (0.11±0.27) ($p>0.05$). (table and figure 2).

The differences in pH of dental plaque and saliva between the time before and 30 minutes after consumption are reported as mean±SD. (Obtained by ANOVA repeated measures and post hoc Tukey) (table 3). FRAP test is used to determine the antioxidant activity of plain and flavored milk (Error Bars: ±2SD) ($p=0.05$). Chocolate milk has the highest (1000 micromol/liter) and banana milk contains the lowest (706025 micromol/liter) antioxidant concentration (figure 3).

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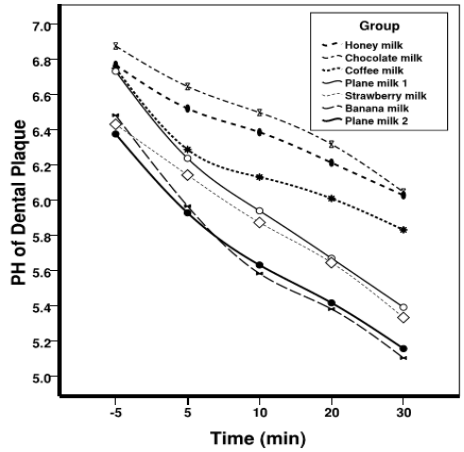


Figure 1. Course of changes in dental plaque pH at different time intervals

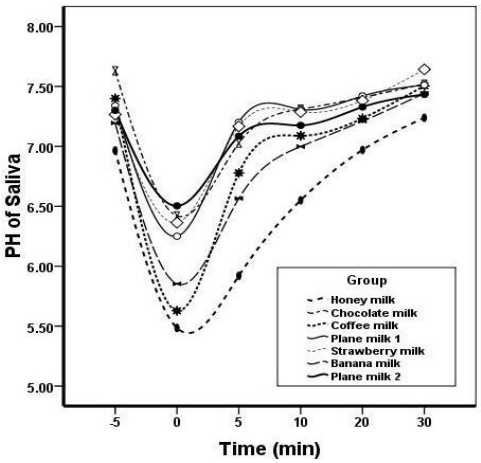


Figure 2. Course of changes in salivary pH at different time intervals

Table 1. Course of changes in dental plaque pH at 5 minutes before and 5-30 minutes after consumption of flavored and plain milk

Time (min)	Honey milk Mean±SD	Chocolate milk Mean±SD	Coffee milk Mean±SD	Plain milk1 Mean±SD	Strawberry milk Mean±SD	Banana milk Mean±SD	Plain milk2 Mean±SD	P-Value
-5	6.77±0.22	6.78±0.14	6.75±0.15	6.73±0.05	6.43±0.23	6.48±0.13	6.37±0.11	<0.001
5	6.52±0.23	6.64±0.16	6.29±0.5	6.24±0.15	6.14±0.23	5.96±0.04	5.93±0.12	<0.001
10	6.38±0.2	6.5±0.16	6.13±0.45	5.94±0.15	5.87±0.32	5.58±0.17	5.63±0.11	<0.001
20	6.21±0.31	6.32±0.17	6.01±0.43	5.67±0.18	5.47±0.31	5.38±0.16	5.41±0.15	<0.001
30	6.03±0.43	6.04±0.24	5.83±0.45	5.39±0.23	5.33±0.32	5.10±0.17	5.15±0.14	<0.001

Table 2. Course of changes in salivary pH at 5 minutes before and 5-30 minutes after consumption of flavored and plain milks

Time (min)	Honey milk Mean±SD	Chocolate milk Mean±SD	Coffee milk Mean±SD	Plain milk1 Mean±SD	Strawberry milk Mean±SD	Banana milk Mean±SD	Plain milk2 Mean±SD	P-Value
-5	6.69±0.38	7.63±0.27	7.4±0.33	7.34±0.25	7.26±0.21	7.19±0.37	7.30±0.30	0.30
0	5.48±0.79	6.42±0.75	5.62±0.59	6.25±0.58	6.36±0.79	5.85±0.49	6.50±0.42	0.48
5	5.9±0.86	7.02±0.33	6.77±0.58	7.20±0.42	7.16±0.25	6.56±0.47	7.08±0.43	0.01
10	6.5±0.74	7.31±0.18	7.08±0.39	7.30±0.15	7.28±0.12	6.99±0.20	7.17±0.14	0.006
20	6.97±0.39	7.40±0.20	7.23±0.35	7.42±0.30	7.38±0.10	7.21±0.17	7.32±0.21	0.078
30	7.23±0.37	7.52±0.20	7.50±0.31	7.51±0.29	7.64±0.22	7.44±0.35	7.43±0.19	0.392

Table 3. Dental plaque pH differences and Salivary pH differences between the time before and 30 minutes after consumption of milk

Salivary pH differences between before and 30 minutes after consumption Mean±SD	Plaque pH differences between before and 30 minute after consumption Mean±SD	
0.27±0.37	0.74±0.30	Honey milk
0.11±0.27	0.83±0.29	Chocolate milk
0.10±0.23	0.91±0.33	Coffee milk
0.17±0.30	1.34±0.23	Plain milk 1
0.37±0.30	1.09±0.32	Strawberry milk
0.24±0.24	1.38±0.25	Banana milk
0.13±0.21	1.22±0.13	Plain milk 2
0.139	0.001	P-Value

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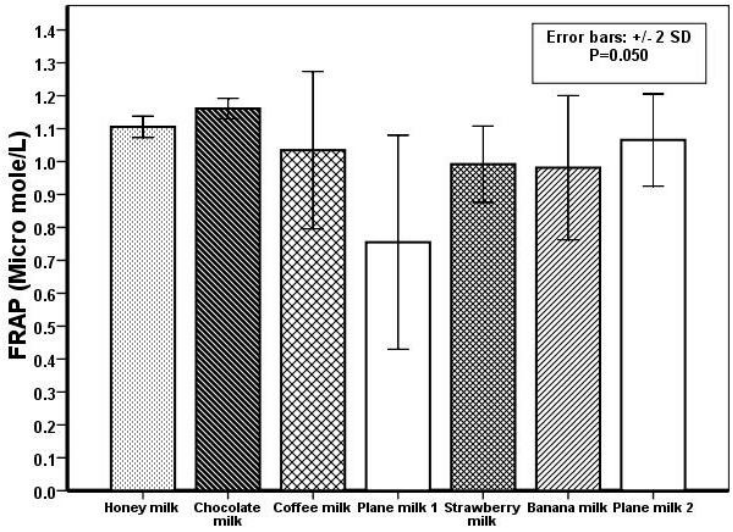


Figure 3. Antioxidant activity of plain and flavored milk (Error Bars:±2SD) (p=0.05)

Discussion

Consumption of plain and flavored milk reduces plaque pH. Except chocolate milk, the rest of the tested milk increased salivary pH. Banana milk and strawberry milk had lower, and chocolate milk, honey milk and coffee milk had higher antioxidant concentration compared with plain milk.

No similar studies have been done on pH changes of saliva and plaque following the intake of flavored milk, and on the evaluation of antioxidant property of these kinds of milk. Masih et al. performed a study on plain milk, sweetened milk, lactodex 2 milk and lactogen 2 milk. They revealed that after the consumption of sweetened milk, a considerable reduction in plaque pH occurred, and it reached to a pH lower than critical 5.5.

The minimum pH for all drinks obtained 10 minutes after intake of test foods. The mean minimum pH was (6.77±0.15) for plain milk and (5.48±0.05) for sweetened milk. The maximum reduction in pH occurred by sweetened milk (7). In our study, consumption of plain and flavored milk caused reduction in plaque pH. The mean pH for plain milk 1 before consumption was 6.73±0.05 and 10 minutes after intake it reached to 5.49±0.15 that was higher than critical pH.

This is in accordance with the mentioned study. The mean pH for plain milk 2 before and 10 minutes after consumption was 6.73±0.11 and 5.63±0.11, respectively. Banana milk and strawberry milk reduced plaque pH to less than critical pH of 5.5. Banana milk caused a higher reduction in pH. Danchaivijitr et al. performed a study and showed that milk formulae containing sugar except lactose tend to reduce plaque pH. Milk formulae containing 10% sucrose reduce plaque pH to critical level (8).

Our study showed that the intake of plain and flavored milk reduces plaque pH. The reduction of plaque pH by honey milk, chocolate milk and coffee milk was less than that obtained by plain milk. Probably it is related to the effect of other additives to these milk formulae such as chocolate, coffee and honey.

The amount of plaque pH reduction produced by banana milk and strawberry milk was similar to that obtained by plain milk. These 2 kinds milk reduced the pH to less than critical pH. Selwitz et al. performed a study on the differences in the reduction of salivary pH after consumption of various drinks. The mean base salivary pH was 7.09±0.07. Consumption of mineral water caused an increase in salivary pH.

In the first 10 minutes, orange juice caused reduction of salivary pH. Immediately after consumption of milk and instant fennel tea, no changes in pH were observed. 5-10 minutes after consumption, all test drinks except mineral water reduced the salivary pH (3). In our study, after the consumption of plain and flavored milk immediately pH was reduced. After that, it started increasing slowly for 10 minutes, between 5 to 10 minutes, a significant increase was

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observed in salivary pH but only a negligible difference occurred between 10-30 minutes.

The differences between these two studies could be due to the differences in milk components such as its percentage of fat. Finally, after the consumption of all the tested drinks no significant difference occurred in pH of saliva over 30 minutes.

According to the study that was performed by Frazzano et al. on plant polyphenols antioxidant activity, they revealed that polyphenols have a high anticariogenic and antibacterial potential (11). In the present study, banana milk and strawberry milk had a lower antioxidant property compare to plain milk. Therefore, the consumption of plain milk is more recommended than banana milk and strawberry milk.

Antonio et al. revealed in a study that coffee reduces bacterial growth and their attachment to tooth surface (10). Our study showed that comparing to plain milk, coffee milk has a higher antioxidant concentration and reduces the plaque pH to a lesser extent, but no significant difference was observed in pH changes of saliva by plain and flavored milk. It is due to the fact that coffee contains a high polyphenol level which prevents the growth of cariogenic bacteria and biofilm formation. Besides that, caffeine and the products of Millard reaction intensify the antibacterial property of coffee. Antibacterial activity prevents the reduction of plaque pH (5).

Srikanth et al. (studied on chocolate drinks revealed that cocoa husk extract has antibacterial property (12). Our study showed that, comparing to plain milk, chocolate milk has the highest antioxidant concentration and it reduces the plaque pH to a lesser extent. Cocoa husk extract has anti-glucosyl transferase (GTF) and antibacterial property.

GTF performs an important role in attachment of bacteria to acquired pellicle. Therefore, antibacterial property of chocolate milk prevents the bacterial activity over the tooth surface, their acidogenicity and reduction in plaque pH . Nassar et al. performed a study about the effect of honey on S. mutans and biofilm formation.

They used different concentrations of natural and artificial honey to evaluate their ability in the prevention of bacterial growth and biofilm formation. Natural honey more than artificial honey is capable of preventing bacterial growth and biofilm formation (9). The present study revealed that after chocolate milk and coffee milk, honey milk has the highest antioxidant concentration. The antibacterial activity of honey caused a lesser amount of plaque pH reduction compared to the other flavored and plain milk. There were no significant differences between the pH changes obtained by plain and flavored milk.

R.S Levine et al. performed a study on plain milk, flavored milk and dental caries. Their study revealed that plain and flavored milk have a low cariogenic potential and they are better replacement compared to sweetened drinks (6).

The present study showed that no considerable changes occur in salivary pH after consumption of these kinds of milk. The course of changes in plaque pH caused by strawberry and banana milk was similar to that of plain milk, but it was different from the course of pH changes obtained by honey milk, coffee milk and chocolate milk. Honey milk, coffee milk and chocolate milk reduced the plaque pH less than plain milk.

Conclusion

After 30 minutes, no significant changes occurred in pH of saliva among the various groups. Immediately after the intake of milk, salivary pH was reduced, but after that, it started to increase and this increase continued until 30 minutes. In the first group, the least pH reduction of dental plaque obtained respectively by honey milk, chocolate milk, coffee milk and plain milk 1. Accordingly, in group 2, strawberry milk, plain milk 2 and banana milk in order, showed the least reduction in dental plaque pH. Chocolate milk contained the highest and banana milk the lowest antioxidant concentration. Therefore, based on the antioxidant property and lesser reduction in plaque pH, consumption of honey milk, chocolate milk and coffee milk is recommended for children

Acknowledgments

Special thanks to the staff members of Department of Pedodontics and laboratory of Biochemistry, Babol University of Medical Sciences.

Funding: This study was a part of thesis and research project (Grant No: 9133417) which was supported and funded by Babol University of Medical Sciences.
Conflict of interest: There was no conflict of interest.

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[DOR: 20.1001.1.22519890.2013.2.2.7.2]
[DOI: 10.22088/cjdr.2.2.15]