ORIGINAL RESEARCH

Anticariogenic Effects of the Dairy Products on Human Saliva: An *In Vivo* Study

Yatendra Gautam[1], Manvi Srivastava[2], Priya Verma[3], Sulekha Doley[4]

Abstract

Aim: The aim of this study was to determine whether the intake of cheese, paneer, and yogurt may help in reversing the acidogenic challenge after the consumption of chocolate at different time intervals.

Materials and methods: A total of 120 caries-free children between the ages of 4 and 13 years, with no decayed, missing, and filled teeth (DMFT), were randomly selected from the school. Baseline pH was obtained, and the subjects were asked to eat chocolate. pH was measured again after 10 minutes. Following chocolate exposure, the subjects were then randomly assigned to three groups—cheese, paneer, and yogurt. Salivary pH was measured at intervals of 15, 30, and 60 minutes using the same procedures for all subjects. The data thus obtained were subjected to statistical analysis, which included analysis of variance (ANOVA) test, *post hoc* tests for multiple comparisons using Tukey's test, and paired *t*-test.

Results: The highest pH value after 15 minutes was seen in cheese (6.43), followed by paneer (6.31) and yogurt (6.30). After 30 minutes, the maximum pH value was seen in the cheese group (6.63), followed by yogurt (6.46) and paneer (6.34). The mean pH value 60 minutes after the consumption of dairy products was highest in paneer (6.53), followed by cheese (6.43) and yogurt (6.37).

Conclusion: All the categories were observed in the zone of remineralization. It should be noted that the maximum pH value overall was seen in the cheese group.

Clinical significance: Dairy products help maintain salivary pH, which aids in improving enamel health. Hence, dietary dairy recommendations can be incorporated into patient education and caries prevention plans.

Keywords: Critical pH, Dental Caries, Remineralization.

International Journal of Clinical Pediatric Dentistry (2024): 10.5005/jp-journals-10005-2849

Introduction

Dental caries is a multifactorial disease caused by four main factors—the host (primarily saliva and teeth), the microflora, the substrate (diet), and time. The role of diet deserves special consideration in the modern era, as soft and refined foods tend to adhere tenaciously to the tooth surface and are not removed due to a lack of roughness. In contrast, the diet of primitive man consisted of raw, unrefined foods with a great deal of roughage, which cleansed the teeth during normal chewing movements.[1]

Milk and dairy products have been claimed to be cariostatic in nature. The ability of milk and its products to prevent tooth decay is attributed to the direct chemical effects of casein phosphopeptides, calcium, and phosphate.[2] Based on available evidence, chewing hard cheeses, especially cheddar cheese, may help prevent enamel demineralization by stimulating salivary flow and promoting remineralization.[3] Paneer is a sugar-free and unaged form of cheese, containing higher protein and phosphate content compared to many other types of cheese.[4] Yogurt is a milk product formed by the fermentation of lactic acid in milk through *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria, and it is rich in probiotics.[5]

Therefore, the aim of this study was to investigate whether consuming cheese, paneer, and yogurt could help mitigate the acidogenic effects following the consumption of chocolate at various time intervals.

Materials and Methods

Before starting the study, ethical approval was obtained from the Institutional Review Board, and official permission was obtained from the school principal/headmaster/headmistress of the concerned school in Mathura. The sample size was calculated based on a pilot study conducted on a sample of 15 children who were not included as part of the final sample.

School-age children ranging from 4 to 13 years old who were healthy, willing to participate, and had a decayed, missing, and filled teeth (DMFT) score of 0 were eligible for inclusion in this study. Children with a history of food allergies, particularly to milk products, those undergoing orthodontic treatment or taking any medications, and those exhibiting dental fluorosis were excluded from the study.

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How to cite this article: Gautam Y, Srivastava M, Verma P, *et al.* Anticariogenic Effects of the Dairy Products on Human Saliva: An *In Vivo* Study. Int J Clin Pediatr Dent 2024;17(4):456–460.

Source of support: Nil

Conflict of interest: None

Materials used in this study were (Fig. 1):

- Chocolate (Cadbury Dairy Milk Chocolate 20 gm).
- Paneer (Amul Fresh Paneer 20 gm).
- Cheese (Amul Cheese Slices 20 gm).
- Sugarless yogurt (Amul Yogurt 20 gm).

A total of 120 healthy subjects were randomly allocated to the study groups—cheese (40), paneer (40), and sugarless yogurt (40). The subjects were randomized using a simple random sampling method through a lottery system. All 120 children were instructed to abstain from eating or drinking for at least 2 hours before sample collection. Before saliva collection, they rinsed their mouths with distilled water. The subjects were then instructed to spit saliva into a fresh container every minute for up to 10 minutes. Saliva was collected by tilting the head forward and allowing saliva to flow naturally to the front of the mouth. Around 5 mL of saliva was collected in sterile 100 mL beakers, which were later thoroughly mixed with 30 mL of distilled water to obtain a salivary baseline pH. pH was measured using a glass electrode connected to a display for at least 30 seconds.[4]

Fig. 1: Dairy products used in the study

The readings were recorded, and thereafter, the electrode was rinsed with distilled water and dried after every use. The electrode was calibrated (Fig. 2) before starting the test and between measurements using two buffering solutions of pH 4.0 and 7.0 and stored in a reference buffer (pH 7).[2]

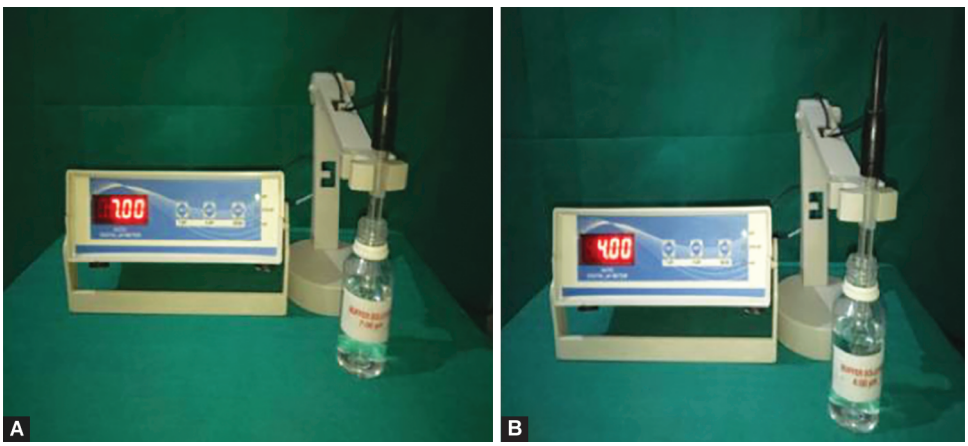
After obtaining the baseline pH, the children were asked to consume chocolate, and the pH was measured after 10 minutes. Subsequently, the subjects were instructed to consume their respective dairy products containing 20 gm of cheese, paneer, and yogurt for 3 minutes each. Salivary pH was measured again at 15, 30, and 60 minutes using the same procedures for all subjects. pH readings were taken after inserting the pH meter electrode into the container containing saliva for 30 seconds. pH is the logarithm of the reciprocal of hydrogen ion concentration in gram atoms per liter, providing a measure on a scale from 0 to 14 of the acidity or alkalinity of a solution (where 7 is neutral, >7 is more basic, and <7 is more acidic).

- Acidity—pH value below <7.
- Alkalinity—pH value above >7.
- Neutrality—pH value of 7.

The data obtained were subjected to statistical analysis using analysis of variance (ANOVA), followed by *post hoc* tests for multiple comparisons using Tukey's test and paired *t*-tests. The significance level for the entire statistical analysis was predetermined at $p < 0.05$.

Results

Table 1 shows the mean pH values of the participants at baseline, 10 minutes after chocolate consumption, and after consuming



Figs 2A and B: Calibration of the electrode using a buffering solution of (A) pH 7.0; (B) pH 4.0

Table 1: The mean pH values of chocolate, cheese, paneer, and yogurt with the objective to determine the most anticariogenic dairy products after the consumption of chocolate at different time intervals

	At baseline			*After chocolate consumption*			*15 minutes after dairy products*			*30 minutes after dairy products*			*60 minutes after dairy products*		
Groups	*I*	*II*	*III*	*I*	*II*	*III*	*I*	*II*	*III*	*I*	*II*	*III*	*I*	*II*	*III*
Mean ± standard deviation (SD)	6.30 ± 0.77	6.60 ± 0.60	6.61 ± 0.53	6.20 ± 0.55	5.90 ± 0.45	6.00 ± 0.40	6.43 ± 0.57	6.31 ± 0.65	6.30 ± 0.70	6.63 ± 0.43	6.34 ± 0.62	6.46 ± 0.74	6.43 ± 0.58	6.53 ± 0.60	6.37 ± 0.47
Minimum	5.13	5.50	5.71	5.22	5.15	5.25	5.52	5.33	5.13	5.74	5.44	5.31	5.35	5.47	5.58
Maximum	7.48	7.41	7.49	7.04	6.60	6.72	7.26	7.38	7.50	7.24	7.50	7.74	7.48	7.39	7.18

cheese, paneer, and yogurt, with the objective of determining the most anticariogenic dairy products at different time intervals following chocolate consumption. The mean pH range for all groups at baseline was between 6.30 and 6.61, indicating that all three categories were within the zone of remineralization, with pH values consistently >6. After chocolate exposure, pH readings were recorded 10 minutes before the subsequent consumption of dairy products (cheese, paneer, and yogurt). Similar dairy product exposures were repeated at 15, 30, and 60 minutes.

It was observed that after chocolate exposure, there was a dip in pH to 5.90 from the highest baseline value of 6.6. The pH levels 15 minutes after consumption of dairy products by children showed that among all three evaluated categories, cheese had the highest pH (6.43), followed by paneer (6.31) and yogurt (6.30). After 30 minutes, the pH levels showed that cheese had the highest pH (6.63), followed by yogurt (6.46) and paneer (6.34). All three categories remained within the zone of remineralization. Saliva from subjects who consumed cheese had the highest pH observed. After 60 minutes, the pH levels showed that paneer had the highest pH (6.53), followed by cheese (6.43) and yogurt (6.37). Again, all three categories were within the zone of remineralization. Subjects who consumed paneer had the highest observed pH.

Figure 3 shows a line diagram demonstrating the mean pH values of cheese, paneer, and yogurt with the objective of determining the most anticariogenic dairy products after the consumption of chocolate at different time intervals. The highest pH value after 15 minutes was observed in the cheese group (6.43), followed by paneer (6.31) and yogurt (6.30). After 30 minutes, the maximum pH value was observed in the cheese group (6.63), followed by yogurt (6.37) and paneer (6.34). The mean pH value 60 minutes after the consumption of dairy products was highest in paneer (6.53), followed by cheese (6.42) and yogurt (6.37). All categories were within the zone of remineralization, and it should be noted that the overall maximum pH value was observed in the cheese group.

Table 2 shows the comparison of salivary pH levels in the cheese group at different time intervals. The mean pH value at baseline was 6.30, which reduced to 6.20 at 10 minutes after exposure to chocolate. Although there was a dip in pH, it was not significant enough to push it into the zone of demineralization. After the consumption of cheese, there was a significant increase in salivary pH values observed at 15 minutes (6.43) and 30 minutes (6.63). By 60 minutes, the salivary pH levels started to decline gradually. The highest mean pH value was observed at 30 minutes.

Table 3 shows the comparison of salivary pH levels in the paneer group at different time intervals. The mean pH value at baseline was 6.00, which was reduced to 5.9 at 10 minutes after exposure to chocolate; these values were found to be significant. After the consumption of paneer, there was a significant increase in salivary pH values observed at 15 (6.31), 30 (6.34), and 60 minutes (6.50). The highest mean pH value was observed at 60 minutes.

Table 2: Comparison of pH levels in group I (cheese) at different time intervals

Comparisons	*Mean difference*	*SD*	*t-value*	*p-value*
Baseline to 10 minutes	0.12	0.78	0.779	0.441 NS
10–15 minutes	−0.28	−2.18	−2.180	0.035 S
10–30 minutes	−0.48	−4.56	−4.564	0.000 S
10–60 minutes	−0.28	−2.13	−2.127	0.040 S
15–30 minutes	−0.20	−1.79	−1.792	0.081 NS
15–60 minutes	0.00	0.01	0.011	0.991 NS
30–60 minutes	0.20	1.63	1.633	0.111 NS

Statistical analysis, paired *t*-test; statistically significant if $p < 0.05$

Table 3: Comparison of pH levels in group II (paneer) at different time intervals

Comparisons	*Mean difference*	*SD*	*t-value*	*p-value*
Baseline to 10 minutes	0.64	0.76	5.364	0.000 S
10–15 minutes	−0.4	0.77	−3.306	0.002 S
10–30 minutes	−0.44	0.72	−3.861	0.000 S
10–60 minutes	−0.63	0.82	−4.816	0.000 S
15–30 minutes	−0.03	0.9	−0.238	0.813 NS
15–60 minutes	−0.22	1.01	−1.389	0.173 NS
30–60 minutes	−0.19	0.9	−1.318	0.195 NS

Statistical analysis, paired *t*-test; statistically significant if $p < 0.05$

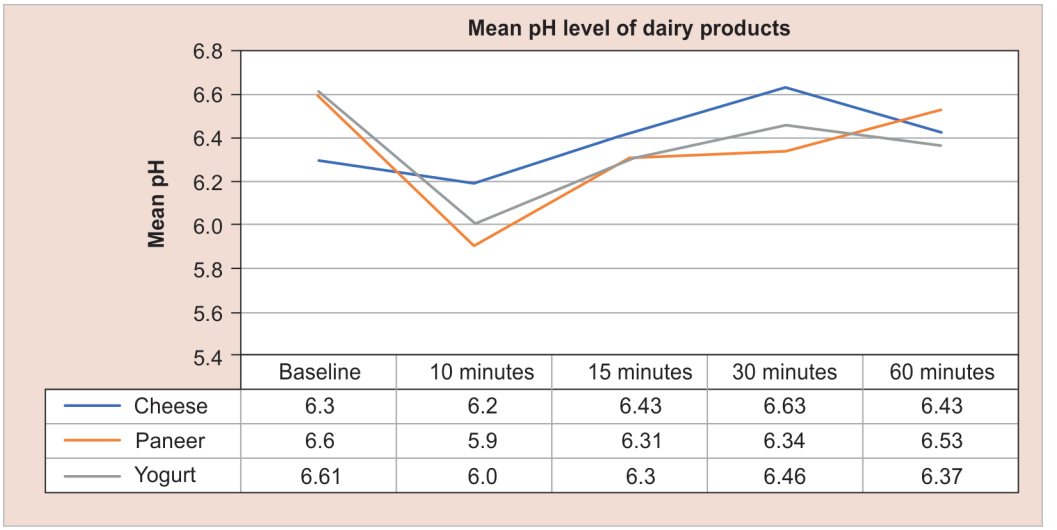


Fig. 3: The mean pH values of cheese, paneer, and yogurt after the consumption of chocolate at different time intervals

Table 4: Comparison of pH levels in group III (sugarless yogurt) at different time intervals

Comparisons	*Mean difference*	*SD*	*t-value*	*p-value*
Baseline to 10 minutes	0.61	0.59	6.543	0.000 S
10–15 minutes	−0.30	0.85	−2.205	0.033 S
10–30 minutes	−0.45	0.76	−3.786	0.001 S
10–60 minutes	−0.36	0.53	−4.298	0.000 S
15–30 minutes	−0.16	0.97	−1.027	0.311 NS
15–60 minutes	−0.06	0.97	−0.419	0.677 NS
30–60 minutes	0.09	0.89	0.665	0.510 NS

Statistical analysis, paired *t*-test; statistically significant if $p < 0.05$

Table 4 depicts the comparison of salivary pH levels in the yogurt group at different time intervals. The mean pH value at baseline was 6.61, which was reduced to 6.00 at 10 minutes after exposure to chocolate; these values were found to be significant. After the consumption of yogurt, there was a significant rise in salivary pH values observed at 15 minutes (6.30) and 30 minutes (6.46). By 60 minutes, the salivary pH levels started to decline gradually. The highest mean pH value was observed at 30 minutes.

Discussion

Dental caries occurs due to repeated acid attacks resulting from the fermentation of dietary substrates by oral microflora over time. This process disrupts the balance between demineralization and remineralization cycles in the oral cavity, leading to decay. This balance is influenced by various factors that can either promote or inhibit dental caries. The critical pH of enamel is 5.5, below which saliva becomes undersaturated with calcium and phosphate, leading to the dissolution of hydroxyapatite in dental enamel.[6]

To estimate the acidogenic potential of food, it is necessary to measure the pH of the oral cavity following food intake. Several methods can be used to measure oral cavity pH, including plaque sampling, touch electrodes, and built-in electrodes.[2] Therefore, in the present study, we selected a glass combination electrode as a sensitive and accurate method for assessing salivary pH.

Dental caries is initiated by the consumption of fermentable carbohydrates, which are metabolized into organic acids by bacteria present in dental plaque. After obtaining baseline pH, the subjects were asked to eat chocolate, resulting in a drop in oral pH measured after 10 minutes. The duration of time that pH remained depressed was significant, as it could indicate food retentiveness and its potential cariogenicity. Results of the present study showed that all study groups exhibited a decrease in salivary pH after a 10-minute chocolate challenge. These findings are consistent with those of previous studies by Bibby et al., Reynolds and Black, and Grenby and Mistry, who also observed a similar pH drop after carbohydrate exposure.[7–9] Certain food items such as dairy products (milk, cheese, paneer, yogurt, etc.) have shown a low cariogenic potential.[10] The anticariogenic activity of dairy and its products is attributed to the direct chemical effects of casein phosphopeptides, calcium, and phosphate.[11,12]

Cheese is a rich source of essential nutrients including proteins, bioactive peptides, amino acids, fats, fatty acids, vitamins, and minerals. Additionally, conjugated linoleic acid and sphingolipids found in cheese may possess anticarcinogenic properties. Drummond et al. found that cheddar cheese can help prevent enamel demineralization by increasing calcium and phosphorus concentrations in dental plaque. Additionally, it stimulates salivary flow, which buffers dental plaque pH and promotes remineralization.[13] The results of the present study showed that consumption of cheese led to an increase in pH after 15 minutes, followed by a consistent rise over time. By 60 minutes, the mean pH value was higher than the baseline value. For all evaluation periods, the difference was statistically significant ($p < 0.001$).

Paneer is a soft, nonfermented cheese with higher protein, phosphate, calcium, fats, and vitamins. Chewing paneer after a chocolate challenge resulted in an increase in salivary pH among the subjects.[4] Sampat found that chewing a cube of cheese and paneer increased salivary pH by elevating the salivary concentration of calcium and phosphorus while also reducing the *S. mutans* count. This contributed to the remineralization process and helped control caries.[14] Similar results were observed in our study, where pH levels increased over time after intake of paneer (group II), and at 60 minutes, the change from baseline pH was statistically significant.

In recent years, numerous studies have explored the health effects of yogurt and the bacterial cultures used in its production. Research has focused on the benefits of yogurt and lactic acid-producing bacteria (LAB) on gastrointestinal health, with investigations conducted in animal models and occasionally in human subjects.[15,16] The findings of the present study indicated that after consuming yogurt, the salivary pH increased from baseline values. These results contrast with the findings of Sudhir et al., where the drop in salivary pH observed after consuming both probiotic-containing and normal curd was mainly attributed to the acidic nature of the curds. However, it's noteworthy that the pH levels remained above the critical range (5.2–5.5), which typically indicates a low risk for causing or worsening dental caries. This suggests that curd, despite its acidity, does not pose a threat to children prone to caries. On the contrary, it may offer benefits due to its calcium, phosphorus, protein, and vitamin content.[17]

In an overall comparison of salivary pH levels after 15 minutes of consumption of dairy products in different study groups, group I (cheese) had the highest mean salivary pH, followed by group II (paneer) and group III (yogurt) in descending order. Salivary pH levels after 30 minutes of consumption of dairy products in different study groups showed that group I (cheese) had the highest pH, followed by group III (yogurt) and group II (paneer) in descending order. Johansson concluded that the rise in pH observed after consumption of cheese can be attributed to the peptides and amino acids produced by the hydrolysis of casein. These compounds undergo further catabolism, which raises plaque pH and helps prevent demineralization. This process of alkaline production may counteract the acidity produced from lactose fermentation by neutralizing the acidity.[18]

Although both cheese and paneer led to an alkaline pH, group I (cheese) was more effective in quickly neutralizing acidic saliva, with this beneficial effect lasting longer. Sugarless yogurt (group III) also increased mean salivary pH values, but this increase was slightly less than that seen with cheese. The results of our study are similar to those of Sudhir et al.[17] and Ferrazzano et al.[19]

On comparison of salivary pH levels after 60 minutes of consumption of dairy products in different study groups, the difference in pH levels among the groups was observed to be statistically significant ($p < 0.001$). Group II had the highest pH (PANEER), followed by group I (cheese) and group III (yogurt) in descending order. Results of the present study showed that paneer (group II) exhibited the maximum rise in salivary pH after 60 minutes of chocolate challenge, consistent with the study conducted

by Tayab et al., which suggested that the increase in pH may be attributed to the buffering capacity of saliva, as well as the higher protein, calcium, and phosphorus content in paneer compared to processed cheese.[4]

These findings confirm that cheese, sugarless yogurt, and paneer are noncariogenic and somewhat cariostatic. Regular consumption of these dairy products offers health benefits, including enhanced oral health. Dairy foods supply essential nutrients such as calcium, potassium, vitamin D, and protein, which are crucial for overall health and body maintenance. Furthermore, they provide caries protection for individuals who are highly susceptible to dental cavities. However, further studies with a longer time interval, larger sample size, and a different control group, such as sugar candies, are suggested, as the dissolution rate of milk chocolate is very high.

Conclusion

Based on the results obtained from the present study, the following conclusions can be drawn after the consumption of chocolate, the baseline salivary pH levels decreased in all three groups. When comparing the anticariogenic properties of various dairy products, cheese showed the highest anticariogenic properties, followed in descending order by sugarless yogurt and paneer.

Clinical Significance

This study's results showed that dairy products help maintain salivary pH, which aids in improving enamel health. Hence, dietary dairy recommendations can be incorporated into patient education and caries prevention plans.

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