ORIGINAL ARTICLE

Enamel Erosion: A Possible Preventive Approach by Casein Phosphopeptide Amorphous Calcium Phosphate—An *In Vitro* Study

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Abstract

Context: Several efforts were made in order to alter the compositions of acidic food items concerning their properties to be able to reduce their erosivity potential. The addition of combinations of calcium and phosphate salts to these food products has grabbed great interest.

Aim: *In vitro* evaluation of the effect of the addition of 0.2% w/v casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) to four commonly available beverages (of which two were carbonated) on enamel erosion.

Materials and methods: Sound-extracted human third molar teeth were taken, and enamel sections (n = 270) were made and polished. Acid-resistant nail varnish was painted to create an exposed enamel window of 1 mm^2, followed by testing of the four soft drinks and distilled deionized water (DDW). Every drink was evaluated with and without the addition of 0.2% w/v CPP–ACP. The enamel specimens were kept in 50 mL solution at 37°C for 30 minutes, rinsed, and then varnish was removed. All samples were then profiled using white-light profilometer, and erosive depths were recorded.

Statistical analysis: One-way analysis of variance test and *post hoc* Tukey test.

Results: Enamel erosion was created by all the soft drinks tested, but the addition of 0.2% w/v CPP–ACP has remarkably reduced (p value < 0.05) erosive depths in all test solutions compared to solutions without CPP–ACP. The erosive depths for solutions with DDW did not vary much from those with 0.2% CPP–ACP.

Conclusion: Addition of 0.2% w/v CPP–ACP to the soft drinks has remarkably reduced their erosivity potential.

Keywords: Casein phosphopeptide–amorphous calcium phosphate, Enamel erosion, Profilometer, Soft drinks.

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Introduction

In the past few years, there has been substantial fall in caries prevalence worldwide which has been followed by a significant rise in the incidence of noncarious lesions such as dental erosion.[1]

The National Diet and Nutrition Survey undertaken in 1997, reported among young people aged 4–18 years in the UK, reported that 58% of 4–6-year-olds and 42% of 11–14-year-olds have dental erosion affecting the palatal surfaces of their incisors.[2]

Dental erosion is potentially more complex than abrasion, with factors such as titratable acidity(TA), pH, calcium chelating properties, type of acid (pK_a), concentration of inorganic elements (calcium, phosphate and fluoride), chemical and physical properties influencing the adherence to enamel surface and stimulation of salivary flow.[3] Diet in children is principal in this aspect as consumption of natural acidic fruit juices or carbonated drinks as well as liquid pediatric medications correspond with great risk of dental erosion.[4]

An important part in dental erosion process is the ability of a drink to resist pH changes created by salivary buffering action which leads to a prolonged period of oral acidity.[4] Nevertheless, Barbour and Lussi quoted that "a ranking for the *in vivo* erosivity of different acidic drinks is rather complicated if not impossible".[5]

Several efforts were made in order to alter the compositions of acidic dietary products concerning their properties to reduce their erosive potential. The addition of combinations of calcium and phosphate salts to these drinks has grabbed great interest.[3]

In recent times, Tooth Mousse (GC Corporation, Japan) has been recommended in the management of dental erosion. Its principal ingredient is CPP–ACP (casein phosphopeptide–amorphous calcium phosphate nanocomplexes), an anticariogenic remineralizing agent.[6] In metastable solutions, casein phosphopeptides (CPP) has an exceptional ability to stabilize calcium phosphate (ACP).[7]

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This information would help the dental professionals to become more proactive in dental erosion preventive programs to be passed on to patients at an earlier stage before the damage is done.[3]

Hence, the purpose of the present study is to evaluate effect of addition of CPP–ACP 0.2% w/v to acidic beverages in order to reduce the potential enamel erosion.

Materials and Methods

The present study was conducted in the Department of Pedodontics and Preventive Dentistry, Mamata Dental College and Hospital, Khammam. Ethical clearance was obtained. Sound-extracted human third molars with fairly plane buccal and lingual surfaces, without any artefacts or cracks, were collected and stored for 2 weeks in 10% neutral-buffered formalin (Fig. 1). Enamel slabs ($n = 270$) were sawn using a diamond disk under water irrigation, including at least 1 mm of underlying dentin. These slabs were inserted in acrylic resin in a mold of approximately 1.5 mm × 1.5 mm × 0.5 mm dimensions, and approximately 50 µm of enamel surface was grounded using a silicon carbide paper to make a flat enamel surface (Fig. 2). On the polished enamel surface, approximately 1 mm^2 enamel windows were made by painting the surface with acid-resistant nail varnish (Fig. 3).

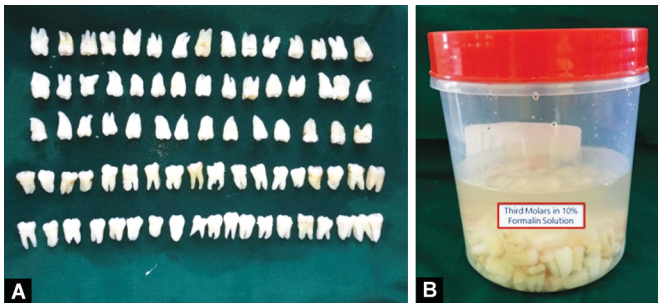


Fig. 1: Sound extracted human third molars stored in 10% neutral buffered formalin

The prepared 270 enamel slabs were divided among the four test groups ($n = 240$), i.e., Group I (Fanta), Group II (Coke), Group III (Minute Maid), and Group IV (Sprite), and distilled deioinized water which served as a negative control ($n = 30$). Each test group was further subdivided randomly into two subgroups depending upon the addition of CPP–ACP, 0.2% w/v.

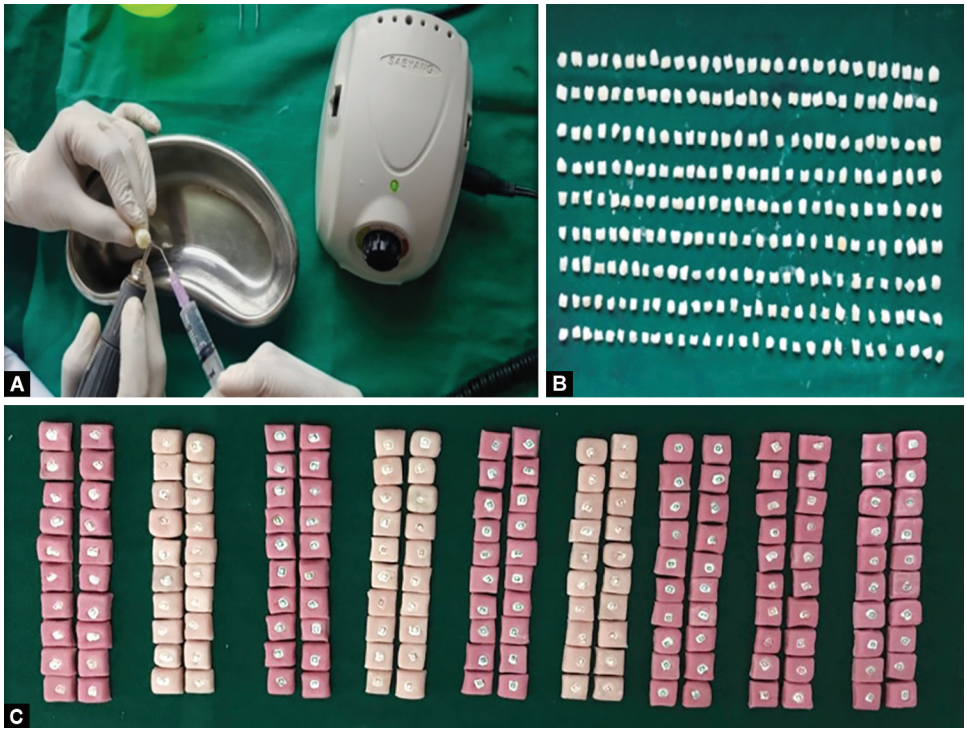
Subgroup A – no addition of CPP–ACP, 0.2% w/v (positive control)

Subgroup B – with addition of CPP–ACP, 0.2% w/v (Fig. 4 and Table 1).

At 4°C, the titratable acidity and pH were measured with a pH electrode. Solutions were warmed to 37°C in an incubator, and pH and titratable acidity were measured in the same way as done at 4°C (Figs 5 and 6). Using a scalpel, the nail varnish was gently removed by "peeling off" while avoiding test area. All the specimens were immersed for 30 minutes in 50 mL of each solution and then removed and thoroughly washed using distilled deionized water (Fig. 7). A non-contact white light profilometer was used for profiling the enamel window and adjacent enamel (Fig. 8). The associated software has generated the erosion cavities depths of each enamel specimen that was profiled.

Results

Statistical analysis of the measurements was done using one-way analysis of variance test and *post hoc* Tukey test with SPSS version 24.0 software. The pH of the beverages at 4°C was in the range of 2.65–2.75. Group IIA (Coke without CPP–ACP) shows the least pH at 4°C, i.e., 2.65, followed by Group IIIA and VIA, respectively. The pH of all the test beverages dropped with rise of the temperature to 37°C; the pH at 37°C ranged from 2.35 to 2.45. Adding CPP–ACP



Figs 2A to C: Enamel slabs including at least 1 mm of underlying dentin were sawn from the teeth using a diamond disk under water irrigation. The slabs were embedded in acrylic resin

(0.2% w/v) to the soft drinks resulted in a remarkable rise in pH at both the temperatures (Table 2 and Fig. 9).

The TA of the beverages at 4°C was in the range of 3.7 mL to 5.5 mL. Group IIA had the highest titratable acidity of 4.26 mL, followed by Group IIIA, IVA, and IVA with TA, respectively. The titratable acidity of all the test beverages increased significantly by almost 1 unit at 37°C. Adding CPP–ACP (0.2% w/v) to the beverages resulted in decrease in titratable acidity of all the test beverages at both the temperatures almost by 1 unit (Table 2 and Fig. 10).

The erosive depths of enamel specimens were analyzed using a non-contact white light profilometer. Mean surface depths of the specimens were in the range of 8.13 to 12.3 µm. Group IIA shows the highest mean erosive depth i.e., 12.38 µm followed by Group IIIA, IVA, and IA with mean erosive depths of 10.35 µm, 9.38 µm, and 8.13 µm, respectively. By adding CPP–ACP (0.2% w/v) to the beverages, the erosivity potential of the beverages reduced significantly which was seen with the decrease in the surface roughness of the enamel specimens that was in the range of 0.6 µm to 1.12 µm (Table 2 and Fig. 11).

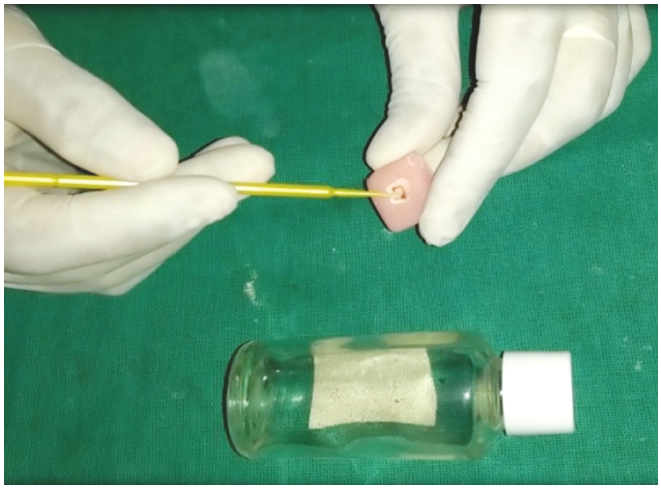


Fig. 3: Painting the enamel window with acid-resistant nail varnish

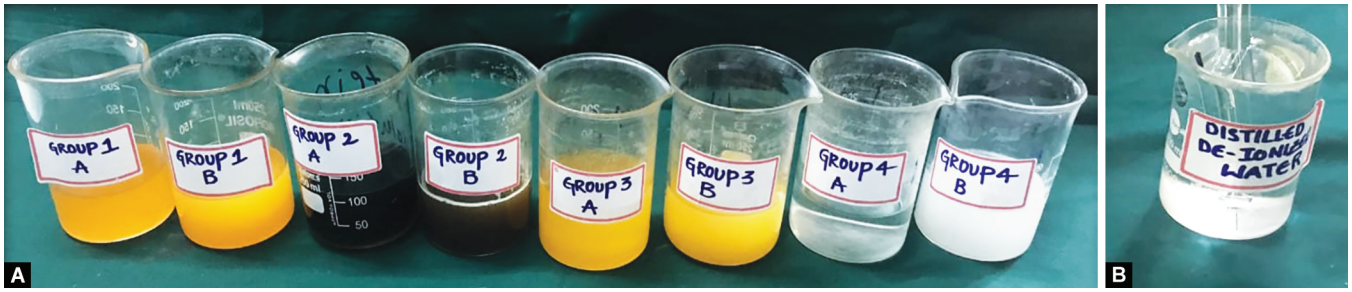
Discussion

Noncarious lesions are becoming a progressively significant problem in oral health.[8] Generally, unless the final intra-oral maturation of enamel surface takes place, the young and immature

Table 1: Distribution of test solutions

Groups	*N = 270*	*Test solutions*
Group IA	*n* = 30	Fanta without CPP–ACP
Group IB	*n* = 30	Fanta with CPP–ACP
Group IIA	*n* = 30	Coke without CPP–ACP
Group IIB	*n* = 30	Coke with CPP–ACP
Group IIIA	*n* = 30	Minute maid without CPP–ACP
Group IIIB	*n* = 30	Minute maid with CPP–ACP
Group IVA	*n* = 30	Sprite without CPP–ACP
Group IVB	*n* = 30	Sprite with CPP–ACP
DDW	*n* = 30	Distilled deioinized water

N, no. of samples; CPP–ACP, casein phosphopeptide–amorphous calcium phosphate



Figs 4A and B: Four different commercially available test beverages and deionized distilled water served as a negative control

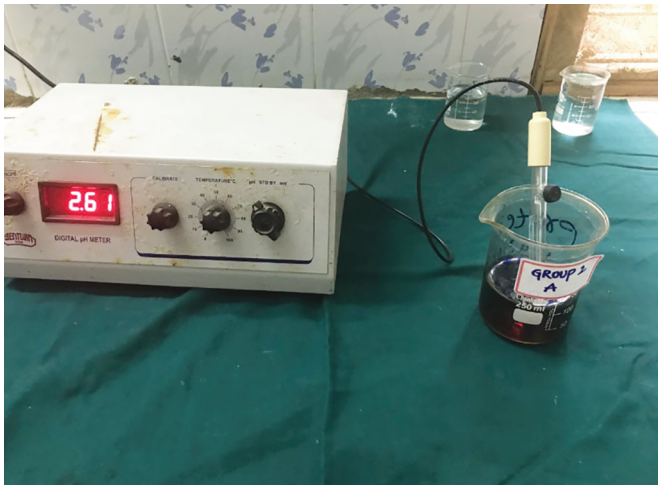


Fig. 5: Measuring pH using a pH electrode

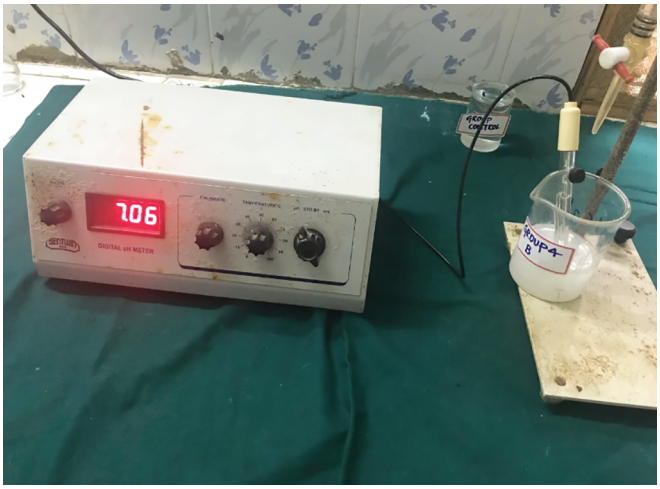


Fig. 6: Measuring titratable acidity

enamel which is porous gets easily dissolved by acids. It becomes progressively harder and gets fairly resistant to acid attack and less penetrable as the immature enamel is bathed by salivary ions.[9]

Therefore, children are the main focus to understand the relationship between soft drinks and teeth. Periods pertaining to the ages are very young or less than 3 years old and school age until the completion of full complement of permanent teeth. The time after the eruption of the primary and permanent teeth is crucial to know if a tooth is damaged by a cariogenic or acidogenic challenge.[9]

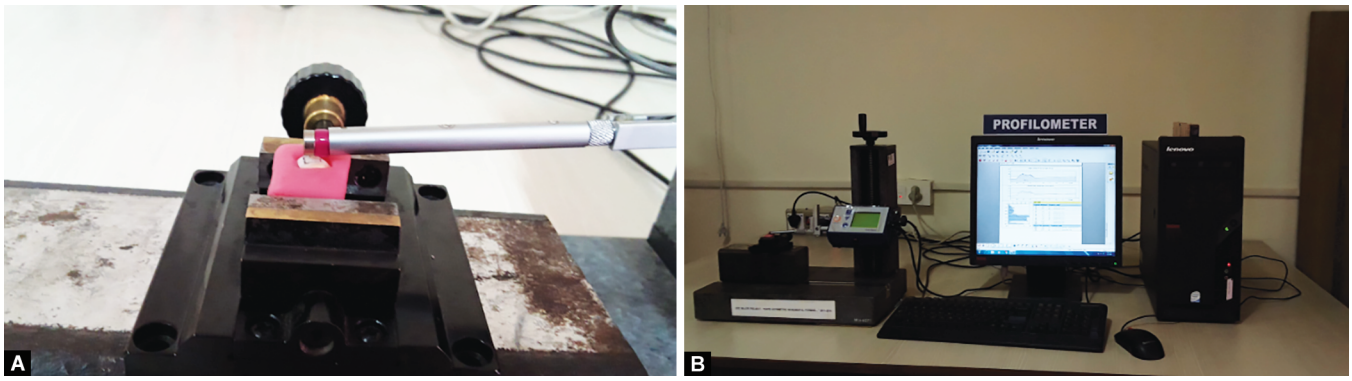
Fig. 7: Enamel samples immersed in test solutions and negative control group

The potential of enhanced demineralization and limited remineralization is because the citrate anions can chelate calcium ions, thereby limiting amount of free ionic calcium available in both enamel surface and saliva.[10]

Nevertheless, many previous studies consider that to understand the erosivity potential of acidic beverages, TA and pH are most appropriate parameters. These are regarded as better for characterizing the erosivity potential with longer exposure times because titratable acidity can measure the total acid content of a beverage, and pH identifies the erosivity potential within few minutes of an erosion test.[11] As per Edwards M, in establishing erosivity potential of beverages titratable, acidity is a more valuable indicator compared to the pH value as it gives the measurement of total acid content in beverage.[12]

There is a rising concern among the dental professionals, owing to wide usage of cold drinks, especially in children, concerning the risk of erosiveness and demineralization that eventually leads to caries. Many ways have been explored to find the best possible way to reduce dental erosion. Modification in the product composition is one of the many ideas explored like adding of compounds or combinations supplying calcium and phosphate.[9]

Therefore, the purpose of the current study is to evaluate the effect of adding CPP–ACP (0.2% w/v) to acidic beverages on dental erosion.



Figs 8A and B: Profilometric analysis of the enamel samples

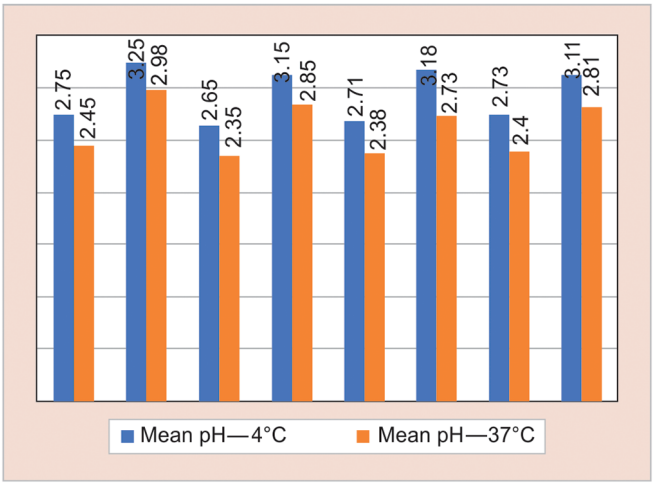


Fig. 9: Mean pH. *CPP–ACP = casein phosphopeptide–amorphous calcium phosphate, pH = potential of hydrogen

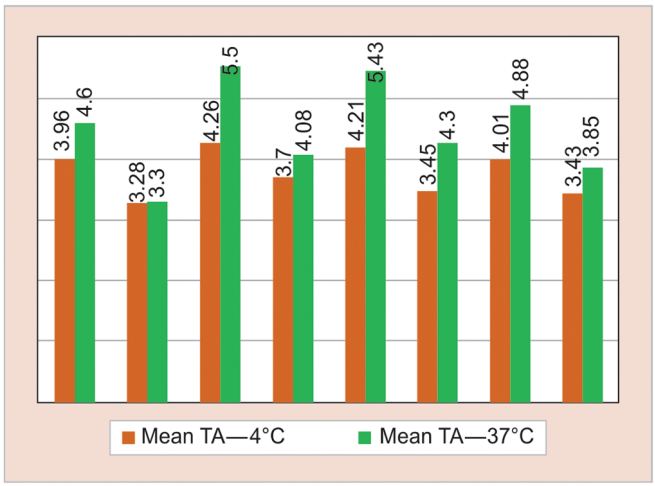


Fig. 10: Mean TA. *CPP–ACP = casein phosphopeptide–amorphous calcium phosphate, TA = titratable acidity

Table 2: Mean pH, mean TA, mean erosive depths

Groups	*Mean pH—4°C*	*SD pH—4°C*	*Mean pH—37°C*	*SD pH—37°C*	*Mean TA—4°C*	*SD TA—4°C*	*Mean TA—37°C*	*SD TA—37°C*	*µm mean*	*µm SD*
Group IA	2.75	0.03	2.45	0.06	3.96	0.48	4.6	0.83	8.13	1.4
Group IB	3.25	0.01	2.98	0.04	3.28	0.76	3.3	0.33	0.72	0.28
Group IIA	2.65	0.02	2.35	0.07	4.26	0.85	5.5	0.95	10.35	1.32
Group IIB	3.15	0.03	2.85	0.08	3.7	0.71	4.08	0.25	1.12	0.15
Group IIIA	2.71	0.05	2.38	0.03	4.21	0.83	5.43	0.89	12.38	2.02
Group IIIB	3.18	0.01	2.73	0.05	3.45	0.55	4.3	0.31	0.62	0.41
Group IVA	2.73	0.02	2.4	0.04	4.01	0.51	4.88	0.78	9.38	3.6
Group IVB	3.11	0.04	2.81	0.08	3.43	0.43	3.85	0.28	0.96	0.33
DDW									0.59	0.07

pH, potential of hydrogen; TA, titratable acidity; SD, standard deviation; CPP–ACP, casein phosphopeptide–amorphous calcium phosphate; DDW, distilled deionized water

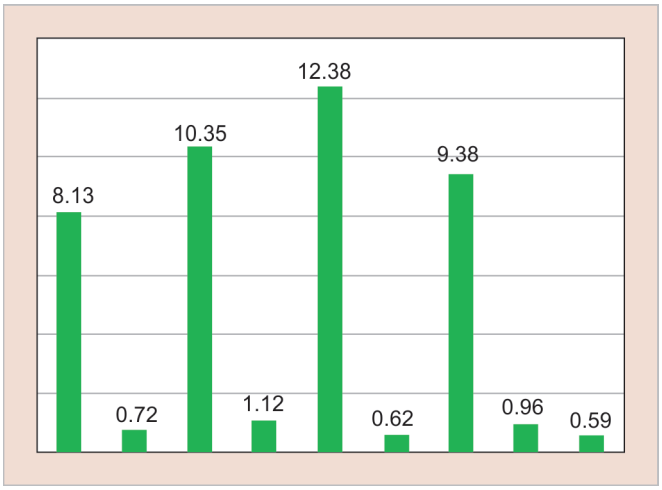


Fig. 11: Mean erosive depths. *CPP–ACP = casein phosphopeptide–amorphous calcium phosphate, DDW = distilled deionized water

In the present study, four commercially available test beverages, namely Fanta, Coke, Sprite, and Minute Maid, were chosen. Coca cola was considered as it has a lowest pH and is most popular and highly consumed drink in the market. Through the years with changing lifestyles and habits, many people are looking forward to a healthier diet that has increased the demand for of natural juices and fruits. Particularly, orange juices are more frequently drank by children or teenagers. Hence, Minute Maid-pulpy orange that simulates fresh orange juice is chosen, and moreover it has no preservative content in it. In this study, Coke was observed to be highly acidic with pH of 2.65 and 2.35 at 4°C and 37°C, respectively; highest pH was shown by Fanta, i.e., 2.75 and 2.45 at 4°C and 37°C, respectively. It was seen that by adding 0.2% w/v CPP–ACP to acidic beverages, there was remarkable rise in pH.

Basically, CPP–ACP is a bank for calcium and phosphate ions and can maintain these ions in a supersaturated state with respect to enamel. Thus, in the present study, it was assumed that the erosion was limited although the pH values seen were all less than the critical limit needed for dissolution of hydroxyapatite and fluorapatite is because of presence high concentrations of calcium and phosphate ions.[13]

The results of the current study were similar to the previous studies done by Bamise et al. and Naziya et al., where the pH values of all the test beverages were below 2.8 which is far below the critical pH. The mean pH of fruit juices was greater than that of carbonated juices, and beverages with low pH were regarded as more destructive for enamel and restorations.[1,14]

In this study, titratable acidity measured was in the range of 3.96 mL to 4.26 mL at 4°C, which represents the total KOH required to increase the pH of drinks to pH 7. Maximum amount of KOH required for Coke was 4.26 mL and Fanta was 3.96 mL to reach pH 7. Thus, it can be said that Coke required more base for neutralizing its acidity.[15] The titratable acidity decreased with increase in temperature to 37°C by two units in Fanta and Coke whereas increased by one unit in case of Sprite and Minute maid. With the addition of CPP–ACP, it was seen that titratable acidity significantly decreased in all the test beverages at 4°C and 37°C, respectively.

These results were in accordance with Singh et al. and Tenuta et al., showing that after drinking acidic beverages, the titratable acidity of the drink influences salivary pH values more than its pH. Thus, pH influences the erosivity potential of the drink during

drinking and after ingesting, the titratable acidity is accountable for maintaining low salivary pH level in mouth.[15–17]

The results of the current study are not in agreement with the results of Awasthi et al. and Bamaise CT et al., where the titratable acidity of the soft drinks was found to be in the order, fruit juices > fruit based carbonated drinks > non fruit-based carbonated drinks, where fruit juices had higher erosivity potential than the cola and the non-cola beverages as it required more base for neutralization.[12,18]

The present study was conducted at 37°C to simulate the temperatures in the oral cavity as it could enhance the erosivity potential of the beverages in comparison to their usual drinking temperature from a refrigerator of 4°C to 6°C. When the temperature was dropped from 37°C to 4°C, the pH of all beverages raised markedly, matching the observations of Barbour et al. There was also a significant drop in pH and rise in the titratable acidity in the beverages with increase in temperatures, the erosivity potential of the beverages increased with the rise in temperature. This suggests a strong correlation between temperature of beverages and erosion.[19]

In the present study, 30 minutes of exposure time is used to create enough enamel loss that can be measured with profilometer. There is evidence for acceptance of this immersion time in previous literatures although it is longer than the time taken normally in mouth.[11] Jensdottir et al. stated that within few minutes of exposure, the pH of beverage can determine its erosivity potential.[20]

In this study, enamel sections were made systemically by taking buccolingual sections that are cut mid-coronally. Generally, enamel surfaces show many variations in susceptibility to erosion; for instance, prismatic enamel erodes more unevenly compared to aprismatic enamel and thus more prone for erosion. As the erosivity increases *in vitro*, gradual dissolution of enamel prisms takes place appearing first in prism sheaths, followed by prism cores, and lastly in the interprismatic substance. After the dissolution of the sheaths, next the prism's heads and then the tails dissolve and eventually the whole of prismatic structure vanish.[13]

In the present study, profilometer which is the most popular and standard method for measuring erosion was used. The profilometric profiles have shown variations for reference area and eroded area on the enamel surface which was measured as enamel loss and it also gives a difference of surface roughness depths between them. Since Coca–Cola had shown low pH, it created greatest enamel loss or erosion.[11] There were increased mean erosive depths of the enamel samples when treated in solution without CPP–ACP and test solutions with CPP–ACP. The erosive depths of the enamel specimens were in the range from 9.38 to 12.38 µm. Statistically significant decrease in erosive depths of enamel was seen after adding CPP–ACP (0.2%w/v) to the beverages. The present study results are similar to Jager et al. and Larsen et al., where Sprite showed the highest value of enamel loss of 3.74 µm, Coca cola 0.34 µm, and apple juice 1.06 µm. They concluded that the enamel dissolution rises statistically inversely with rise of pH of drink.[5,21]

In the present study, flat and polished sections were used in order to systematize the specimens and eliminate any disparity between teeth and also between various sites and types tooth; else it can lead to differences in responses to acid dissolution. Nonetheless, the polished surfaces tend to erode faster than natural surfaces of tooth.[7]

According to Reynold et al., CPP–ACP deposits a high concentration of ACP near the tooth surface, as it attaches promptly to tooth surface and to plaque bacteria. When there is acidic environment, this concentrated CPP–ACP releases the free calcium and phosphate ions resulting in a significant raise in calcium phosphate levels in the plaque to maintain a supersaturated state, thereby inhibiting enamel demineralization and enhancing remineralization.[22] The present study results show that by adding CPP–ACP (0.2% w/v) to acidic beverages, there is a significant decrease in titratable acidity and rise in the pH at 4°C and 37°C. Adding CPP–ACP (0.2% w/v) also resulted in reduced loss of enamel as observed in the profilometric analysis, where the surface depths of enamel specimens significantly reduced.

In the present study, Tooth mousse (Recaldent) was used as it contains CPP and stabilized nano-clusters of ACP in a metastable solution. Following usage of the paste, it produces high calcium and phosphate concentration in saliva approximately 6.5 times and 7.9 times, respectively. Compared to CPP–ACP complexes, Recaldent is considered to be an effective anticariogenic agent, as it has more calcium, phosphate, and hydroxide ions.[23]

Results of the current study are similar to Vongsawan and Tantbirrojn et al., where teeth treated by cola beverage with CPP–ACP showed hard enamel surface as against untreated enamel; the cavity depths were decreased from 50 µm to 20 µm manifesting the protective effect of CPP–ACP. They concluded that use of Tooth Mousse produced decrease in both enamel and dentin erosion depths.[7,24]

The results of present study are not in accordance with studies of Wang et al. and Wegehaupt et al., where the surface hardness of teeth could not be restored by CPP–ACP, and it did not exhibit any protective effect of on erosion. This could be because CPP–ACP was only used one time in a day contrary to daily multiple, continuous, or intermittent usage of Tooth mousse in other studies (Ranjitkar et al.).[6] The difference in results can also be attributed to reduced affinity of CPP–ACP for the enamel orange juice had less pH of 3.6 and CPP net charge being positive. (Wang et al.). Variations in the rinsing protocol used after applying CPP–ACP paste may also create major difference between studies because the active ingredient would act for more time if not completely rinsed off. That is why it is advised not to aggressively rinse away after the using of fluoride toothpastes while brushing. Therefore, in the present study, deionized distilled water was used to gently clean the samples after the treatment.[25]

When CPP–ACP is added to foods with erosivity potential such as sugary confectionaries or low pH cold drinks, a protective mechanism has always been observed. Thus, they are favourably added into a variety of products starting from oral hygiene products like toothpastes and mouth rinses, to chewing gums, lozenges and even certain food items. This presents a major advantage over other bioactive peptides that are added as dental health enhancement agents. This is because mouth is their site of action where they act immediately on entering mouth and also they have fewer chances to be degraded.[4]

Nonetheless, tooth erosion is a complex condition with multiple factors affecting it, such as intrinsic acids or abrasive and attritional influences that might interfere or be associated with it. Different studies have studied it with different factors and of course not in the same manner, for example, there could be variations in frequencies or quantities of consumption, and thus residing confounding could persist.[4] The current study used an *in vitro* model, and thus the findings cannot be generalized even to an *in vivo* environment, as many crucial intraoral factors such as saliva, pellicle and plaque were not included.

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