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Comparison Between The Effects Of Two Recaldent® Products And Artificial Saliva On The Hardness Of Enamel Exposed And Not Exposed To A Carbonated Beverage – An In Vitro Study

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Abstract

This in vitro experimental study on 48 extracted sound human maxillary central incisors was conducted to evaluate the effects of two Recaldent® products and artificial saliva on the hardness of enamel not exposed to and exposed to a carbonated beverage. Coca-cola was the test beverage. All hardness measurements were taken with Vickers hardness tester.

Three groups of samples (groups B1,B2 and B3) were subjected to the erosion process with the carbonated beverage. Groups A1, A2 and A3 were not subjected to the erosion process. For the remineralisation process, a thin layer of test material I (CPP-ACFP) was applied on the enamel surfaces of the samples in groups A1 and B1 for three minutes and then stored in artificial saliva for 6 hours.

A two stage blinding procedure was performed. Data was entered into MS Excel spreadsheet and processed using SPSS software version 15.0. One way analysis of variance (ANOVA), Student t test, Post Hoc (Tukey) test and Bonferroni test were performed.

Results showed that differences between the sub groups at baseline was statistically not significant. The difference in enamel microhardness between baseline and demineralization in the groups A_1, A_2, A_3, B_1, B_2, B_3 were 1.1, 0.4, 1.1, —44.4, —44.5 and —46.3 respectively. The mean difference in enamel microhardness measured after demineralisation and after remineralisationin the groups A_1, A_2, A_3, B_1, B_2, B_3 were 3.8, 2.9, 0, 14.3, 13.2, 2.7 respectively. Post Hoc (Tukey test) test performed between the groups B_1 and B_2 after remineralisation did not show statistically significant difference between the groups ($p = 0.888$).

Through this in vitro study, it can be concluded that carbonated beverages decrease the microhardness of tooth enamel. CPP-ACP and CPP-ACFP increase the microhardness of enamel. Artificial saliva increases the hardness of enamel, but to a lesser extent than CPP-ACP and CPP-ACFP. There is no difference between the remineralising capacity of CPP-ACP and CPP-ACFP.

INTRODUCTION:

Carbonated beverages are an important factor in tooth erosion, because they contain acids such as citric acid, maleic acid and phosphoric acid, which decrease the pH of the oral environment. They also contain sugars that plaque microorganisms utilize for the fermentation process and

produce acid that leads to dissolution of the tooth enamel. Furthermore, a 2 to 3% increase in carbonated beverage consumption per year depicts an alarming trend[1].

Dietary changes such as the consumption of carbonated beverages have led to erosion becoming more frequent among the younger population. Now, tooth wear is almost a universal condition[2].It primarily affects the upper enamel layers, eventually progressing to involve the dentine and demineralises the inorganic phase thereby reducing the hardness of the tooth substrates[3].

Milk and milk products, such as cheese, have been shown to exhibit remineralising properties in human and animal models[4]. Casein is the predominant phosphoprotein in bovine milk and accounts for almost 80% of its total protein. Casein Phosphopeptide – Amorphous Calcium Phosphate (CPP-ACP) shows an anti caries effect by suppressing demineralization, enhancing remineralisation, or possibly a combination of both[5].

Reynolds and colleagues proposed that under acidic conditions as occurring during a cariogenic challenge, localized calcium and phosphate ions substantially increase the amount of calcium phosphate in plaque. Thus a state of supersaturation is maintained, inhibiting enamel demineralization and enhancing remineralisation[6].

In 2006, Sukasaem.H et al concluded that CPP-ACP can increase hardness of enamel eroded by cola and that the remineralising effect of CPP-ACP is significantly higher than that of artificial saliva in vitro[7]. These studies have primarily focused on the remineralizing capabilities of CCP-ACP on demineralized enamel. In accordance with the principle of minimum intervention dentistry, optimal noninvasive strategies are preferred not only for the management of oral lesions after their occurrence, but also to enhance the existing state of health.

Hence, the present study aims to compare between the effects of two Recaldent® products (Tooth Mousse, Tooth Mousse Plus) and artificial salivaon the hardness of enamel not exposed to and exposed to a carbonated beverage.

MATERIALS AND METHODS:

Study Design:

An in vitro experimental study on extracted sound human maxillary central incisors.

Sample Size Calculation:

Sample size was calculated based on the difference between two group means derived from a similar study conducted by Muratha Panich et al(2009)[3]. The required sample size is **48 teeth**. This should include 8 teeth in each of the six sub groups.

Inclusion Criteria:

Extracted sound human maxillary central incisors.

Exclusion Criteria:

1. Endodontically treated teeth.
2. Teeth affected by Fluorosis,wasting diseases,Fractured teeth

Materials used :

Material	Composition	pH
Coca-cola	Carbonated water, 10% sugar, flavours	3.3
Tooth Mousse	Pure water, glycerol, Caseinphosphopeptide-Amorphous calcium phosphate, d-sorbitol, silicon dioxide, sodium carboxymethyl cellulose, propylene glycol, titanium dioxide, xylitol, phosphoric acid, guar gum, zinc oxide, sodium saccharin, ethyl p-hydroxybenzoate, butyl p-hydroxybenzoate, propyl p-hydroxybenzoate, magnesium oxide.	6.6
Tooth Mousse Plus	Pure water, glycerol, Caseinphosphopeptide-Amorphous calcium fluorophosphate, d-sorbitol,	6.6

	silicon dioxide, sodium carboxymethyl cellulose, propylene glycol, titanium dioxide, xylitol, phosphoric acid, guar gum, zinc oxide, sodium saccharin, ethyl p-hydroxybenzoate, butyl p-hydroxybenzoate, propyl p-hydroxybenzoate, magnesium oxide.	
Artificial Saliva	Potassium chloride, magnesium chloride, calcium chloride, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, sodium benzoate, sodium carboxymethyl cellulose, deionized water.	6.8

Study methodology:
Group allocation:

Stage of treatment	A group (24 teeth)			B group (24 teeth)		
	A_1	A_2	A_3	B_1	B_2	B_3
Baseline	No treatment	No treatment	No treatment	No treatment	No treatment	No treatment
Demineralisation	Artificial saliva	Artificial saliva	Artificial saliva	Coca - cola	Coca - cola	Coca - cola
Remineralisation	Test material I and Artificial saliva	Test material II and Artificial saliva	Artificial saliva (inbuilt control)	Test material I and Artificial saliva	Test material II and Artificial saliva	Artificial saliva (inbuilt control)

Baseline Measurements:
Baseline micro hardness measurements were taken on the labial surface by means of a Vickers indenter with 100 grams of force for 15 seconds. Five indentations were made on the left hand side of the middle one third of the labial surface. The indentations were 120 micro meters apart from each other. The hardness value of each sample at baseline was calculated by averaging the value of all five indentations.

Erosion Process :
The pH of the carbonated drink (Coca-Cola, USA) and artificial saliva were measured with a portable pH meter (Hannah instruments, Germany). Three groups of samples (groups B1,B2 and B3) were subjected to the erosion process. Each sample was immersed in adequate milliliters of the carbonated beverage to completely cover the samples, and left to remain for five seconds. Then they were placed in a comparable amount of artificial saliva for another five seconds. Ten cycles of the immersion process were conducted at room temperature. This process was repeated two more times at six hour intervals. During each six hour interval, the samples were stored in artificial saliva at room temperature.
After the erosion process was completed, the samples were washed in distilled water and blotted dry. The hardness of the enamel surfaces was measured using Vickers indenter with 100grams of

force for 15 seconds. Five indentations were made, 120 micrometer apart from each other in the center of the middle one third of the labial surface. The hardness value of each sample, after the erosion process was obtained by averaging the values of all five indentations.
Groups A1, A2 and A3 were not subjected to the erosion process but remained immersed in artificial saliva for a comparable period.

Remineralisation Process :
A thin layer of test material I (CPP-ACFP) was applied on the enamel surfaces of the samples in groups A1 and B1 for four minutes, as recommended by the manufacturer and then stored in artificial saliva for 6 hours. In groups A2 and B2, a thin layer of test material II (CPP-ACP) was applied on the enamel surface for four minutes and stored in artificial saliva for 6 hours. Groups A3 and B3 were immersed in artificial saliva for 6 hours and served as controls.
After the remineralisation process was completed, the samples were washed in distilled water and blotted dry. The hardness of the enamel surfaces were measured using Vickers indenter with 100grams of force for 15 seconds. Five indentations were made, 120 micrometer apart from each other in the right hand side of the middle one third of the labial surface. The hardness value of each sample, after the remineralisation process was obtained by averaging the values of all five indentations.

Data Analysis:
Data was entered into MS Excel spreadsheet and processed using SPSS software version 15.0
Statistical analysis:

- One way analysis of variance (ANOVA) ,Student t test ,Post Hoc (Tukey) test,Bonferroni test.

RESULTS

Figure 1: Enamel microhardness (Vicker's Hardness Measurement) at baseline, after demineralisation and after remineralisation for artificial saliva group (Group A)

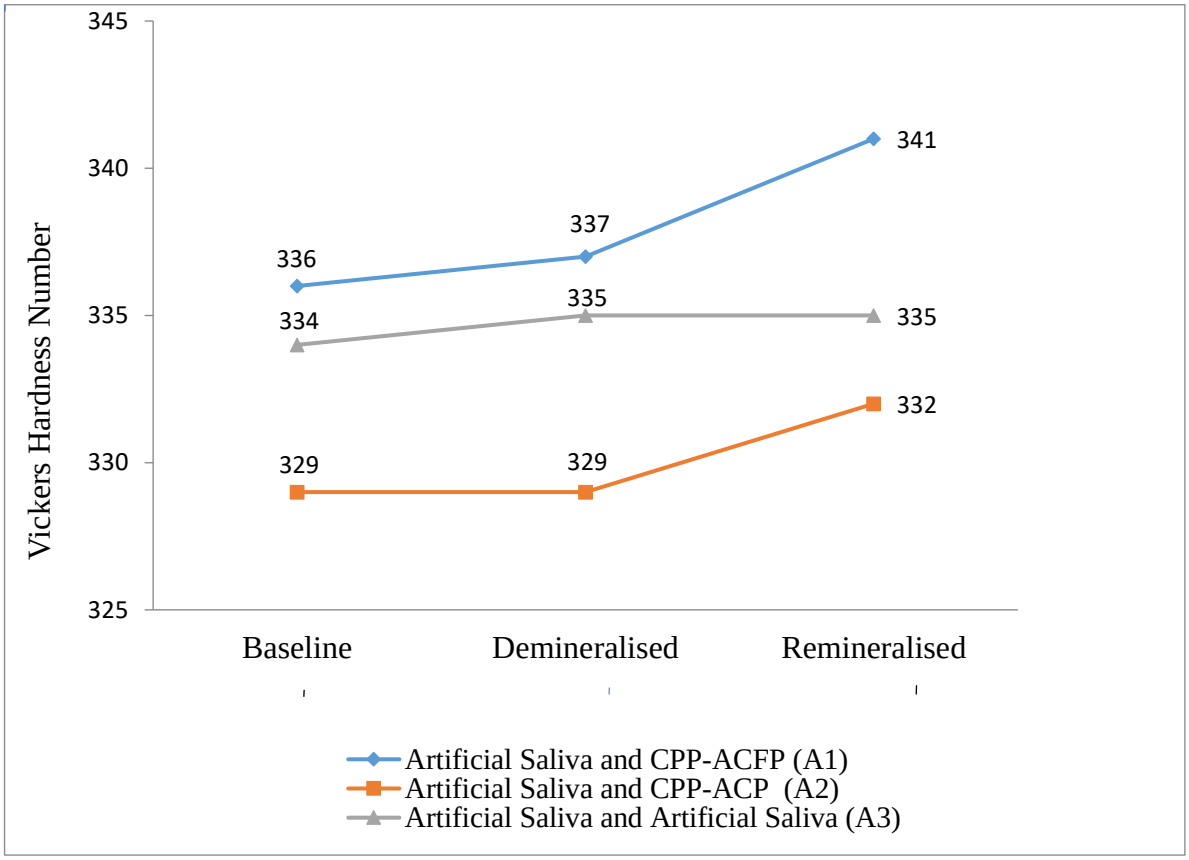


Figure 2: Enamel microhardness (Vicker's Hardness Measurement) at baseline, after demineralisation and after remineralisation for beverage group (Group B)

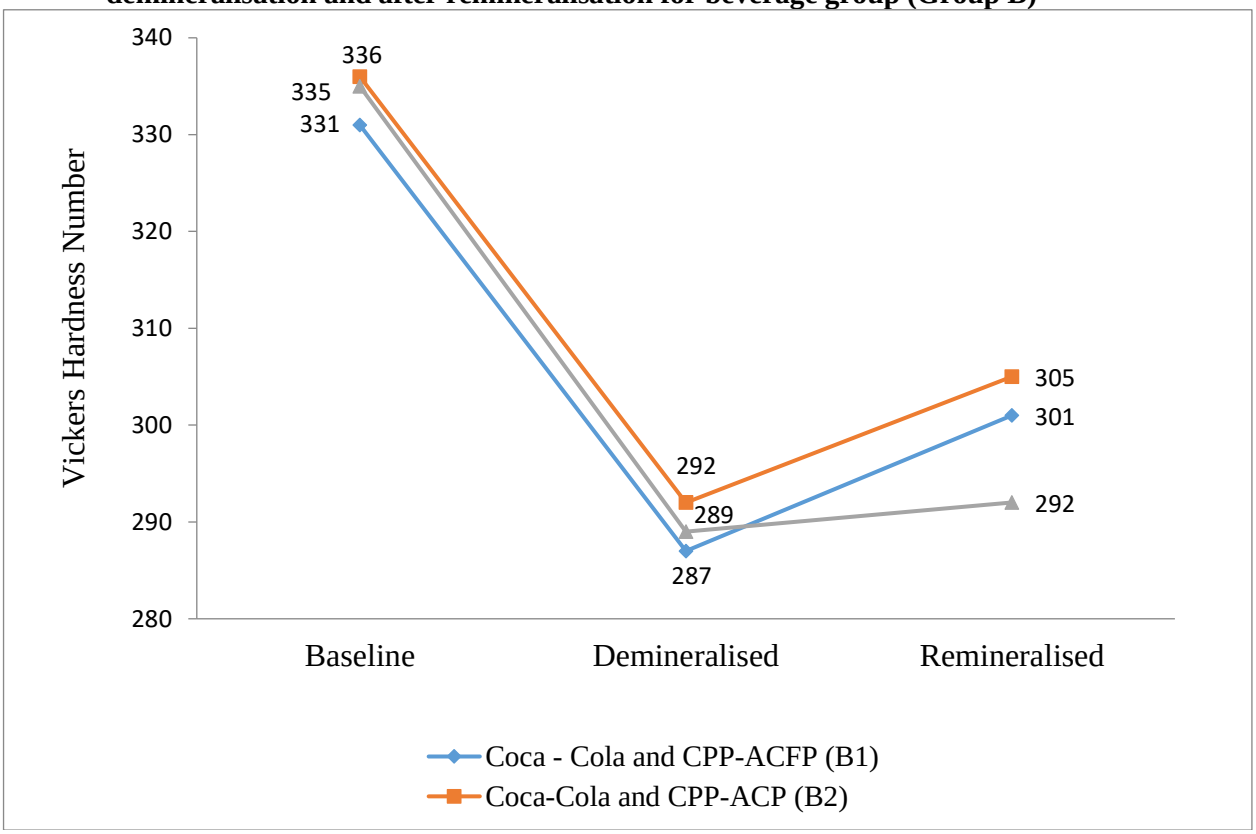


Figure 3: Enamel microhardness (Vicker's Hardness Measurement) at baseline, after demineralisation and after remineralisation for Artificial Saliva and CPP-ACFP group (Group A_1)

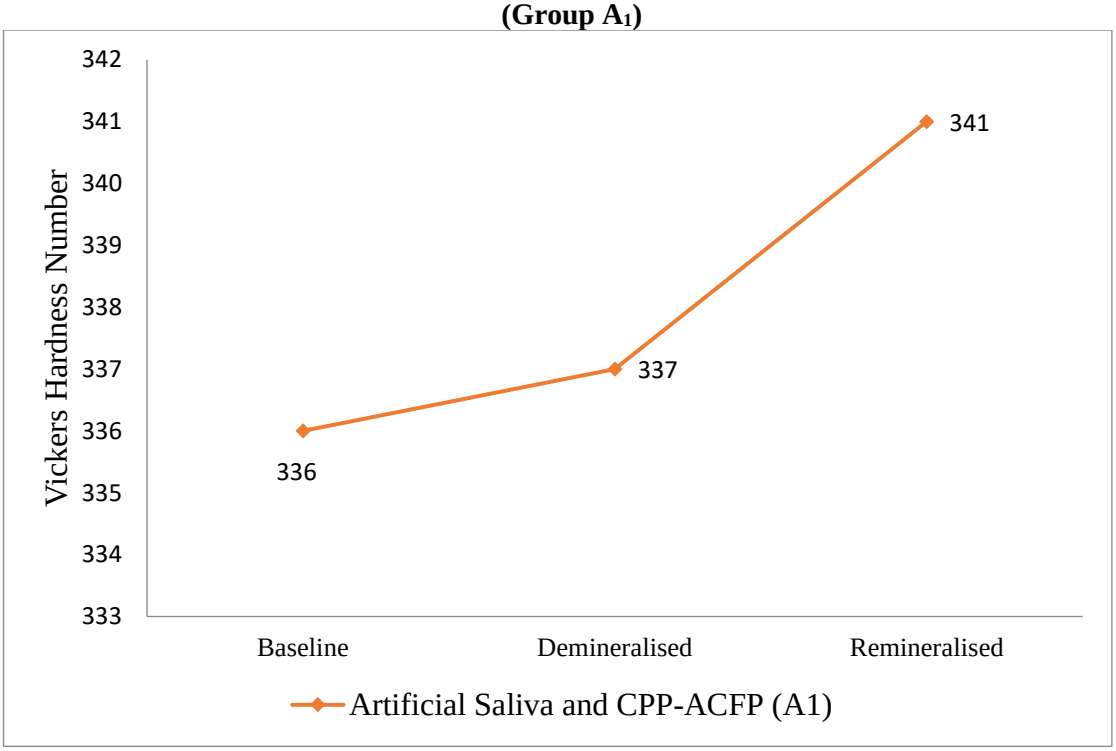


Figure 4: Enamel microhardness (Vicker's Hardness Measurement) at baseline, after demineralisation and after remineralisation for Artificial Saliva and CPP-ACP group (Group A_2)

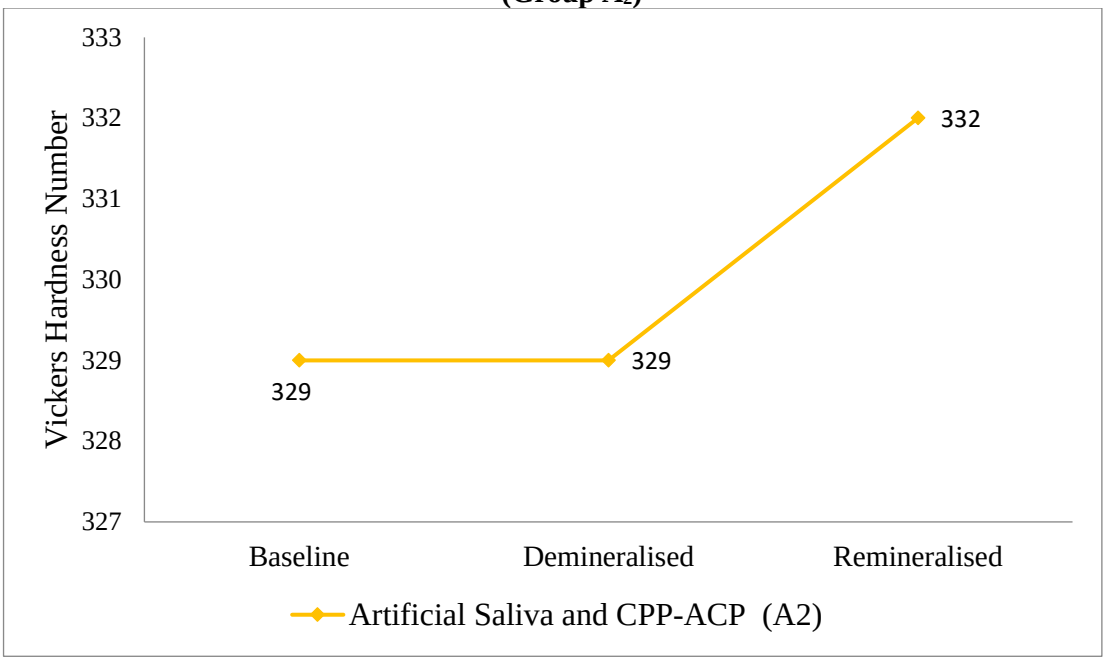


Figure 5: Enamel microhardness (Vicker's Hardness Measurement) at baseline, after demineralisation and after remineralisation for Artificial Saliva and Artificial Saliva group (Group A_3)

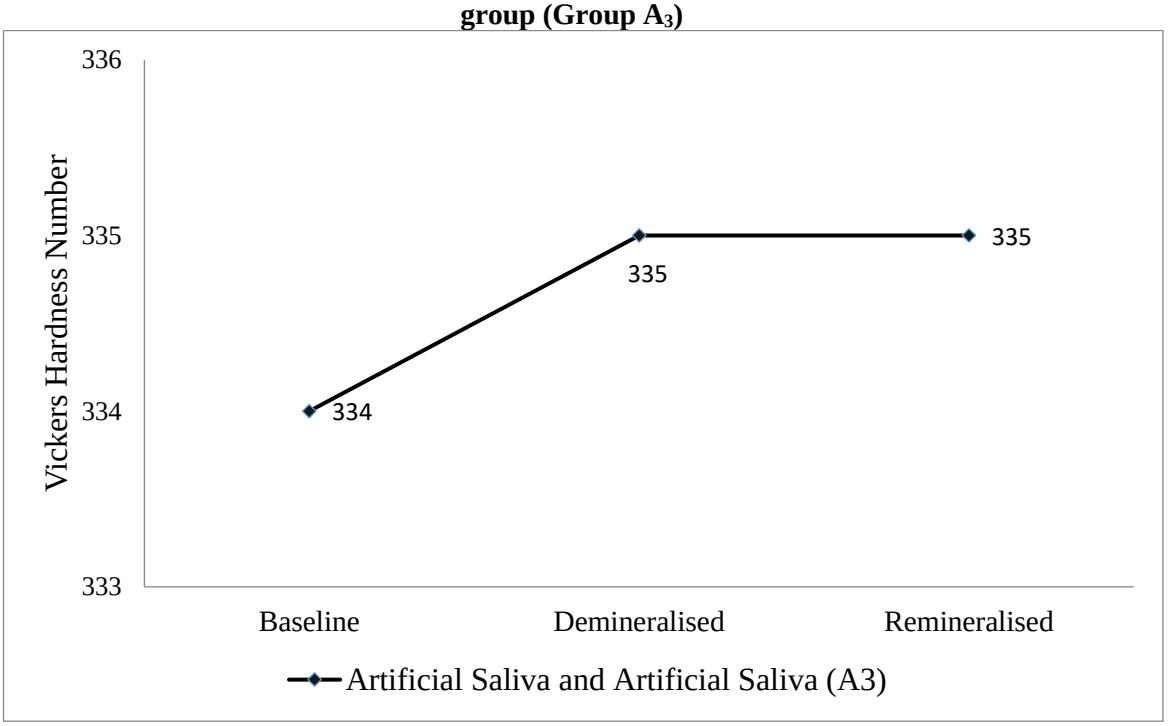


Figure 6: Enamel microhardness (Vicker's Hardness Measurement) at baseline, after demineralisation and after remineralisation for Coca-cola and CPP-ACFP group (Group B_1)

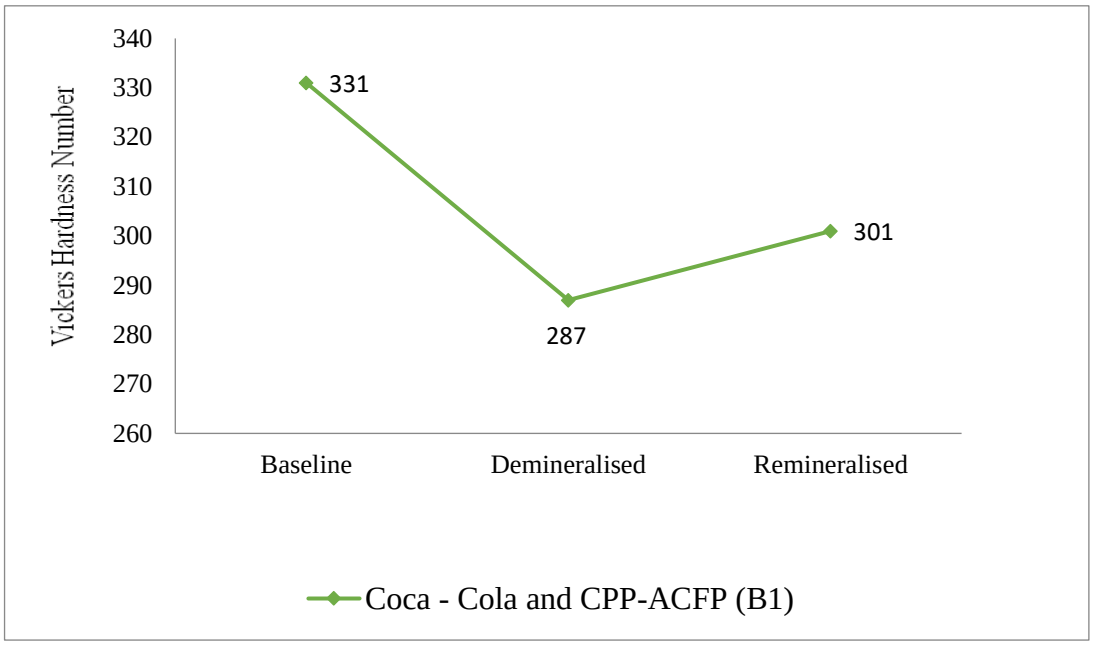


Figure 7: Enamel microhardness (Vicker's Hardness Measurement) at baseline, after demineralisation and after remineralisation for Coca-cola and CPP-ACP group (Group B_2)

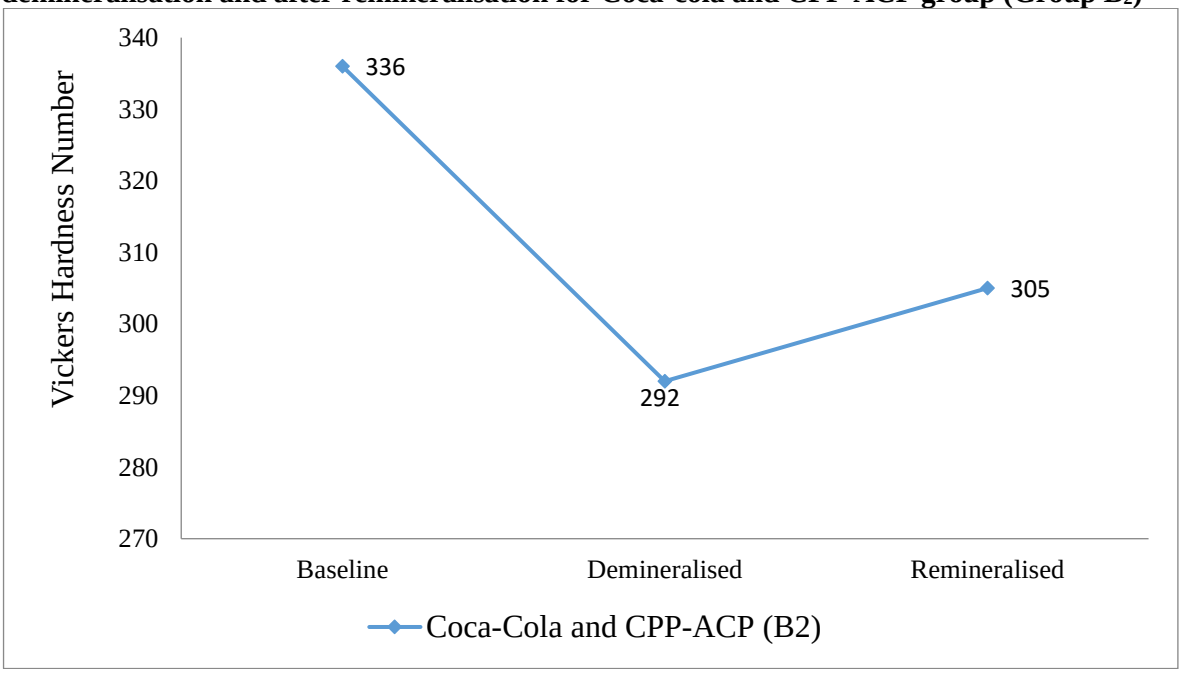
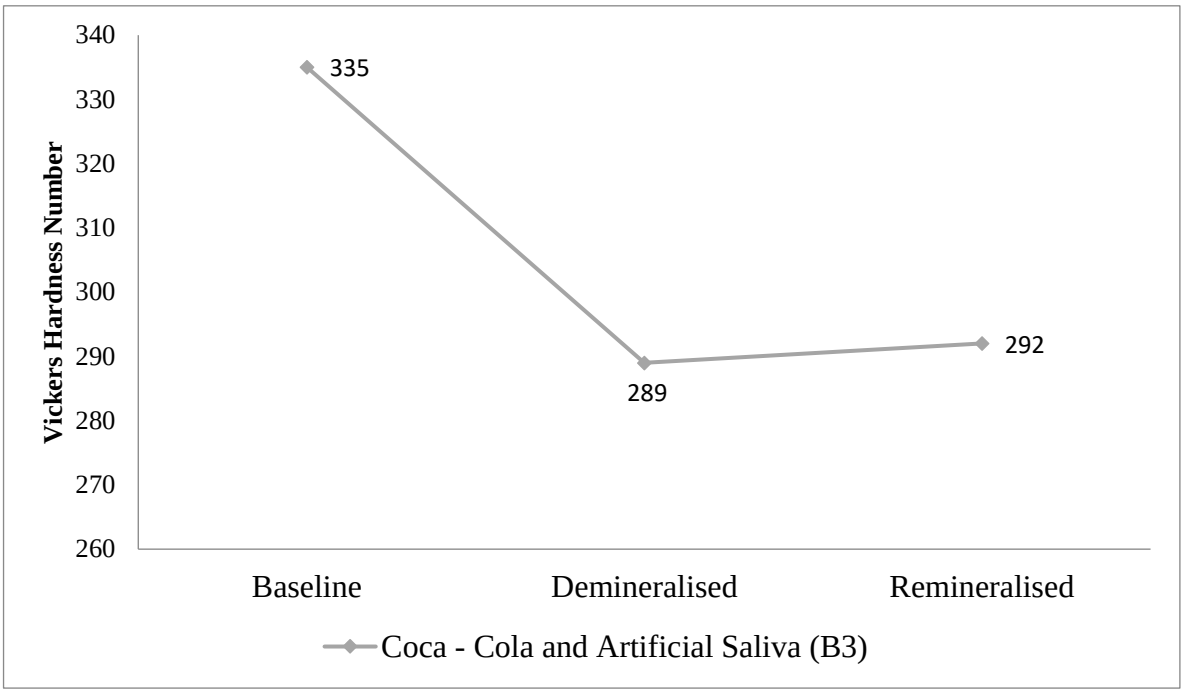


Figure 8: Enamel microhardness (Vicker's Hardness Measurement) at baseline, after demineralisation and after remineralisation for Coca-cola and Artificial Saliva group (Group B_3)



DISCUSSION:

Within the past decade, dental erosion has been recognized as an increasing problem by the dental experts. Introduction of carbonated beverages into the dietary retropire have a significant role in ushering in this phenomenon. Many investigators have focused on the strategies for prevention and reversal of tooth erosion. Recently, casein phosphopeptide amorphous calcium phosphate derived from cow's milk has been reported to reduce the demineralising effects of erosive agents and enhance remineralisation.

The demineralization protocol followed in our study is similar to studies by Muratha Panich et al and Wongkhatee et al, in which cyclic specimen immersions in beverage and artificial saliva were performed to simulate the washing effect of saliva in a person drinking a 325ml can of the beverage. The erosion process was done three times at six hourly intervals to represent the three meal times. This was followed by a single application of the remineralising agent reflecting the situation where the erosive challenge is followed by an application of the remineralising aid.

Mean microhardness reduction in the beverage group was 45.1 VHN. This is similar to the study by Muratha Panich et al (48.2 VHN) and lower than the study by Wongkhatee et al (99.8 VHN). This difference may be because Wongkhatee et al utilized cut enamel which may be more permeable to the erosive agent, thus potentiating the erosive ability of the beverage. Furthermore, in the aforementioned study, the teeth specimens were polished before treatment. Polishing removes the hypermineralised outer layer of the enamel, rendering it more susceptible to softening.

It was found that after immersion in artificial saliva, microhardness of the teeth increased. This is in accordance with several studies that have shown that artificial saliva can reharden acid softened enamel. For the remineralistion procedure in this study, casein phosphate and artificial saliva were used in combination as it more representative of the oral environment and studies have shown that this combination is more effective in remineralising teeth.

After remineralising procedures, the increase in enamel microhardness for the B_1 and B_2 groups were 14.3 and 13.2 respectively. This finding is consistent with many studies that demonstrate the remineralising effect of casein phosphate delivered in various forms such as mouthrinse, yogurt, milk, chewing gum, sugar confections and as a paste . This varied usage is possible as it is tasteless and non toxic, thus compatible to be incorporated into various modes of delivery.

However, the amount of remineralisation obtained in terms of Vickers Hardness Number is lower than in the study by Muratha Panich et al (37.8 VHN). This may be due to difference in

the composition of artificial saliva used in these studies. This can be substantiated by the fact that the increase in hardness by artificial saliva alone in the study by Muratha Panich et al and in the present study were 7.3 and nil respectively.

In this study the difference in remineralising capacity of CPP-ACP and CPP-ACFP did not show statistically significant difference. This is in agreement with a study by S.K Rao et al to evaluate the efficacy of CPP dentrifrices and fluoride tooth paste containing 1,190mg/kg of sodium monofluoro phosphate among school children in Bangalore. The authors concluded that 2% CPP paste was as effective as the fluoride paste.

The difference in quality of remineralisation between CPP-ACP and CPP-ACFP is microvolumetric. Those studies that indicate the synergistic effect of casein phosphate and fluoride have used techniques such as laser fluorescence and electron probe microanalyser to detect this micro volumetric difference. The present study protocol using Vicker's Hardness Tester is not ideally suited for this purpose.

Though it can be concluded that casein phosphate may enhance remineralisation of normal and eroded enamel surfaces, further studies are needed to ascertain this effect in vivo. Furthermore, the stability of the mineral deposit may be determined by imposing an acid challenge post remineralisation. The potential formation of calculus resulting from the supersaturated calcium phosphate state in plaque is another avenue that warrants future research.

CONCLUSION:

Through this in vitro study, it can be concluded that carbonated beverages decrease the microhardness of tooth enamel. CPP-ACP and CPP-ACFP increase the microhardness of enamel, irrespective of whether the tooth has been exposed to or unexposed to the carbonated beverage. Artificial saliva increases the hardness of enamel, but to a lesser extent than CPP-ACP and CPP-ACFP. No difference was found between the remineralising capacity of CPP-ACP and CPP-ACFP.

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