ORIGINAL RESEARCH

Remineralizing Effect of Casein Phosphopeptide–Amorphous Calcium Phosphate and Sodium Fluoride on Artificial Tooth Enamel Erosion: An *In Vitro* Study

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Abstract

Aim: To compare *in vitro* the remineralizing effect of toothpastes with casein phosphopeptide–amorphous calcium phosphate (FPC–FCA) and sodium fluoride on the artificial erosion of tooth enamel.

Materials and methods: For the first phase of artificial erosion, group I was considered as the control group without treatment or erosion. For groups II and IV, the drink was used (Coca-Cola®), for groups III and V, the drink (Inca-Kola®), and the four groups were demineralized four times a day (every 3 hours for 2 minutes) for 5 days. In the treatment phase in groups II and III, brushing was performed with sodium fluoride paste (Colgate®), groups IV and V received brushing with FPC–FCA complex (MiPaste®), and for all groups, the same procedure was performed four times a day (every 3 hours for 5 minutes) for 90 days. At the end, the microroughness of the surfaces of all the groups was evaluated by means of a Rugosimeter (Mitutoyo).

Results: It was evidenced that the group of sodium fluoride presented a microroughness of 2.79 µm being the group of least remineralization, but the FPC–FCA complex showed a microroughness of 1.96 µm; however, the control group presented a microroughness of 3.20 µm, and the groups sodium fluoride, FPC–FCA compared to the control group proved to be statistically significant with a $p < 0.05$.

Conclusion: The remineralizing effect of FPC–FCA (MiPaste®) complex proved to be greater than sodium fluoride paste (Colgate®) under artificial enamel erosive conditions.

Clinical importance: The results of this research serve as a basis for industries to generate products that have the potential for remineralization against various erosive beverages that are consumed daily.

Keywords: Casein phosphopeptide–amorphous calcium phosphate, Dental erosion, Microroughness, Remineralization, Sodium fluoride.

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Introduction

In recent years, there has been an increase in the consumption of acidic beverages by the population, thus being considered as the main erosive agent of tooth enamel.[1] Compounds for the inhibition of erosion have been investigated, such as casein phosphopeptide with complex of amorphous calcium phosphate (FPC–FCA); this complex can increase the level of calcium ions and inorganic phosphate in the surface of the tooth, allowing in this way the immediate remineralization of the enamel.[2]

Because dental erosion consists of the dissolution or superficial demineralization of hard dental tissues due to chemical processes that do not involve bacteria.[3] This demineralization process occurs when the calcium and phosphate ions are lost from the surface of the enamel, giving as an upcoming result to the formation of a caries lesion; in this first stage, the caries lesion is reversible through a process of remineralization that involves the diffusion of calcium and phosphate ions on the tooth surface to restore the structure lost. The solutions of FPC–FCA can remineralize the enamel surface at rates of $1.5–3.9 \times 10^8$ mol of hydroxyapatite (HA).[4]

Caseins are a heterogeneous family of proteins dominated by α 1, 2 and β caseins. FPC phosphorylates casein-derived peptides produced by tryptic digestion of casein. This protein from nanotechnology combines specific bovine milk phosphoproteins with the formation of FCA nanoparticles. The precise ratio is 144 calcium ions, 96 phosphate ions, and six peptides of FPC.[5]

The FPC bound to the FCA acts as a reservoir of calcium and phosphate ions including calcium acid orthophosphate ($CaHPO_4$).

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In acidic conditions, the FPC bound to the amorphous calcium phosphate (FCA) would buffer the pH in the plate and in doing so dissociate the calcium phosphate ions including the $CaHPO_4$. The acid is generated by the bacteria in the dental plaque or during the formation of HA in the enamel lesion during remineralization.[6] The application of sodium fluoride has been well-known for years and is mainly attributed to the reduction. The solubility of enamel, which is due to the incorporation of fluoride in the apatite network, can in turn create a favorable environment to protect teeth from attacks of acidogenic substances.[7]

Otherwise, sodium fluoride influences the balance between de-remineralization of teeth in two ways. First, the fluoride ions within the biofilm increase the critical pH for the dissolution of Ca and PO_4. Second, fluorides form chemically stable fluorapatite crystals in the enamel structure, thus reducing the solubility of the acid.[8]

Therefore, the purpose of this research was to compare *in vitro* the remineralizing effect of toothpastes with FPC–FCA and sodium fluoride on the artificial erosion of tooth enamel.

Materials and Methods

The present *in vitro* research was carried out in the Multifunctional Science Laboratory of the Faculty of Health Sciences, Universidad Privada San Juan Bautista, Lima, Peru.

Ethics Statement

This research was approved with code CEPB-FCS 0013 by the Ethics Committee of the Faculty of Health Sciences of the Universidad Privada San Juan Bautista, where the justification for the *in vitro* study of sodium fluoride toothpastes and the FPC–FCA complex, because it did not generate any adverse effect since it was purely experimental.

Preparation of the Sample

The samples were polished with pumice stone and water with the help of a micromotor. Then, two enamel samples (4 × 4 × 2 mm) were obtained from the buccal and lingual or palatal surfaces of each tooth using a low-speed diamond saw cooled by water (Fig. 1). The surfaces were then rinsed with deionized water and placed in a solution of ethylenediaminetetraacetic acid (EDTA) at 17% for 1 minute to remove the smear layer (Fig. 2). They were then randomized into five groups. For the highest precision and convenience for the treatment steps, five containers (transparent) were selected for the samples (4 for treatment and 1 for the control group); for the erosion phase, another four containers were selected, drawn with indelible down by the outside at a height of 5 cm and covered with adhesive tape so that it is not erased, and this served to pour the same amount of drink in the four groups.[1,2]

A simple random probabilistic sampling was carried out, considering the groups of pastes to be used and the erosive agents. The unit of analysis was a dental piece previously extracted for orthodontic reasons or impaction, and the sample was formed by human dental pieces, which met the inclusion and exclusion criteria: for the sample size, the test was used to compare means with an α of 0.05 and a β of 0.8, determined ($n = 40$).

Group I: free of toothpastes and erosions, control group
Group II: with sodium fluoride paste + Coca-Cola®
Group III: with sodium fluoride paste + Inca-Kola®
Group IV: with FPC–FCA complex + Coca-Cola®
Group V: with FPC–FCA complex + Inca-Kola®

Artificial Dental Erosion

Transparent containers labeled 5 cm were used to pour the same amount of acidic beverages. For group I (control group without erosion), and for groups II and IV, the Coca-Cola® drink was used (the Coca-Cola Company, Lindley Corporation SA Lima25, Peru) four times a day (every 3 hours for 2 minutes) for 90 days. In addition, for groups III and V, the Peruvian Inca-Kola® drink was used (Corporation Lindley S.A. Lima25, Peru) four times a day (every 3 hours for 2 minutes). At the end of each erosion phase, the samples were rinsed with deionized water and stored in physiological saline until the next test.[1]

Remineralization of Enamel

It was done after each erosion phase. Before each application of the pastes, the samples were rinsed with deionized water. Group I was considered as the control group without treatment. Groups II and III were brushed with sodium fluoride paste (Colgate® Total 12, Colgate-Palmolive Company, Peru) four times a day (every 3 hours for 5 minutes) for 5 days. Groups IV and V were brushed with FPC–FCA complex (MiPaste®, Recaldent™, Peru) four times a day (every 3 hours for 5 minutes) for 90 days. The four groups were brushed with a microbrush for 10 seconds and left in contact for 5 minutes, then they were irrigated with deionized water to remove all traces of toothpaste and finally stored in physiological saline[1,2] (Figs 3 and 4).

Microroughness Test

The microroughness analysis was determined by a Rugosimeter (Mitutoyo SJ-201, High Technology Laboratory Certificate SAC). Once the erosion and treatment phase was completed, the surfaces of all the groups were evaluated: the first analysis was on the surfaces of the control group and the second measurement was made on the surfaces of the groups that underwent the erosion

Fig. 1: Teeth cut before being submerged in experimental groups

Fig. 2: Polished specimens with pumice stone

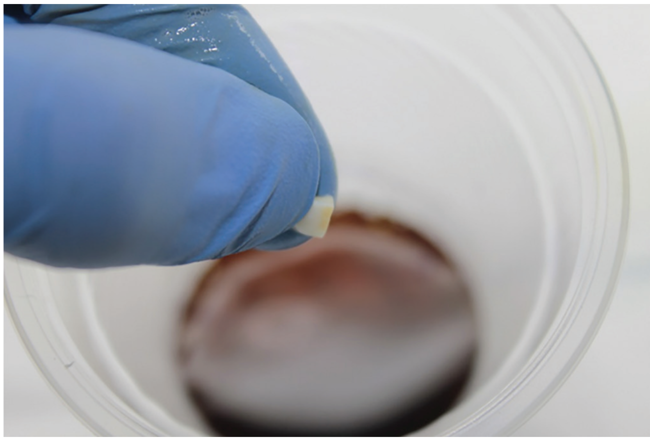

Fig. 3: Immersion of the teeth in the Coca-Cola® group

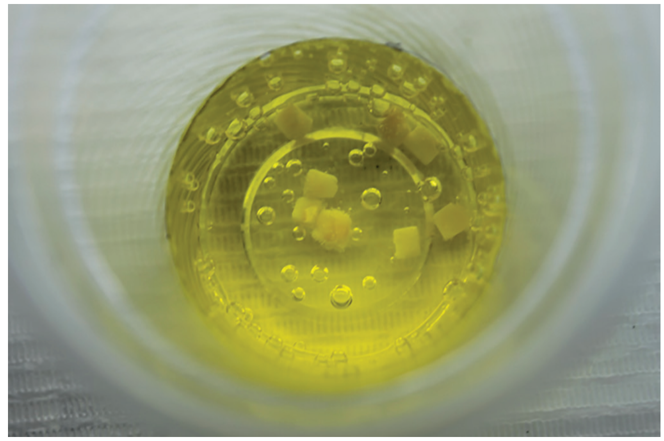

Fig. 4: Immersion of the teeth in the Inca-Kola® group

Fig. 5: Measurement of the microroughness of the dental surface

process previously treated with sodium fluoride toothpaste and FPC–FCA complex[1] (Fig. 5).

Statistical Analysis

For the univariate analysis, the mean, standard deviation, and minimum and maximum values of the variable remineralizing effect were obtained, according to the established groups (FPC–FCA and sodium fluoride) and the covariates of the study (Inca-Kola® and Coca-Cola®). In addition, it was determined that the sample presented normal distribution through the Shapiro–Wilk test. For the bivariate analysis, the ANOVA statistical test was performed for related data comparing the remineralizing effect of the different study groups according to the Inca-Kola® and Coca-Cola® beverages, and after presenting high statistical significance, the test was carried out. Bonferroni and the group that determined the largest statistically significant difference between the study groups was identified. The database was evaluated using statistical packages Stata® version 12.0, establishing a level of significance of $p < 0.05$.

Results

When comparing the microroughness means of the dental enamel of the different groups of Coca-Cola®, Inca-Kola® and control, for the group with Coca-Cola®, a microroughness of 2.57 µm ± 0.8 with a minimum of 1.54 µm was evidenced and a maximum of 4.08 µm being the group with the highest erosion; however, the Inca-Kola® group presented a microroughness of 2.38 µm ± 0.6 with a minimum of 1.37 µm and a maximum of 3.77 µm, being the lowest demineralization beverage presented dental enamel, and in the same way the control group demonstrated a microroughness of 3.20 µm ± 0.4 with a minimum of 2.44 µm and a maximum of 3.67 µm. When performing the Shapiro–Wilk test, it was found that all the groups present normal distribution with $p > 0.05$. On the contrary, when performing the ANOVA statistical test, it was evident that all the groups Coca-Cola®, Inca-Kola®, and control group obtained statistically significant differences with a $p < 0.05$ (Table 1 and Fig. 6).

After determining the existence of differences between the groups Coca-Cola®, Inca-Kola®, and control with the ANOVA test, we proceeded to identify the groups that determine this difference with the Bonferroni test, where it was determined that among the groups of Coca-Cola® and the control group did not present significant differences with a $p = 0.127$ in comparison between the Inca-Kola® group and the control group, where statistically significant differences were found with $p = 0.028$ being the groups that determined this difference among all (Table 1 and Fig. 6).

When comparing the microroughness, means of dental enamel of the different groups treated with toothpaste such as FPC–FCA complex, sodium fluoride, and control group. It was evidenced that the sodium fluoride group presents a microroughness of 2.79 µm ± 0.6 with a minimum of 1.96 µm and a maximum of 4.08 µm being the group of least remineralization, likewise the FPC–FCA complex presents a microroughness of 1.96 µm ± 0.3 with a minimum of 1.37 µm and a maximum of 3.24 µm being the group with the highest remineralization presented; however, the control group found a microroughness of 3.20 µm + 0.4 with a minimum of 2.44 µm and a maximum of 3.67 µm. When performing the Shapiro–Wilk test, it was found that all the groups present normal distribution with $p > 0.05$. Therefore, the ANOVA statistical test was performed, which showed that all the FPC–FCA, sodium fluoride, and the control group groups obtained statistically significant differences with $p < 0.05$ (Table 2 and Fig. 7).

After determining the differences between the FPC–FCA complex, the sodium fluoride, and the control group with the ANOVA test, we proceeded to identify the groups that determine this difference with the Bonferroni test, where it was determined that between the groups of sodium fluoride and the control group did not present significant differences with a $p = 0.629$ in comparison between the groups of FPC–FCA complex and the control group where a statistically significant difference was

Table 1: *In vitro* comparison of tooth enamel erosion and Coca-Cola® and Inca-Kola® beverages

Erosion of the dental enamel							
Beverages	*Mean ± SD*	*Median*	*Min*	*Max*	*p**	*p***	*p****
Coca-Cola®	2.57 ± 0.8	2.29	1.54	4.08	0.119		0.127
Inca-Kola®	2.38 ± 0.6	2.32	1.37	3.77	0.302	0.031	0.028
Control	3.20 ± 0.4	3.35	2.44	3.67	0.320		

All units of measurements were expressed in microns (μm)
*Shapiro–Wilk test
**ANOVA test
***Bonferroni test
Significance level $p < 0.05$

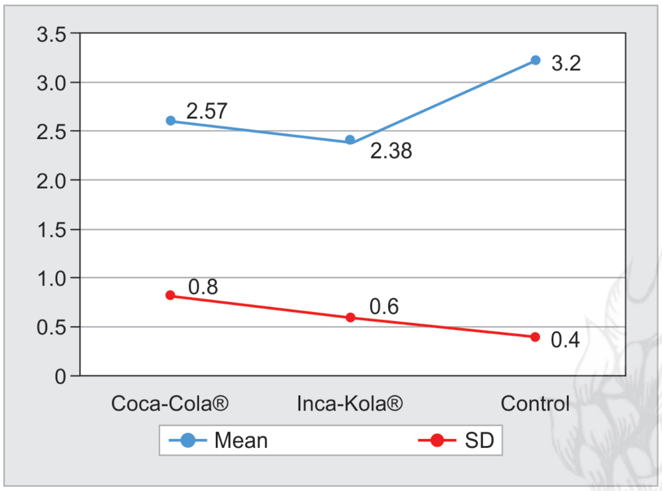

Fig. 6: *In vitro* enamel erosion

found with a $p < 0.001$ being the groups that determine difference between all (Table 2 and Fig. 7).

Discussion

Dental enamel is essentially composed of HA crystals and an organic matrix fraction that contains proteins, lipids, and water. Ion exchange occurs on hydrated surfaces containing calcium ions and phosphate in a state of supersaturation related to apatite crystals at a neutral pH. Enamel dissolution occurs when hydrogen ions derived from acids are bound to calcium, which leads to calcium-deficient carbonate HA and the release of calcium and phosphate ions.[9] Over the years, there is a high consumption of carbonated beverages by the population, thus being considered the main erosive or demineralizing agent of tooth enamel.[1] Several investigations[2,10,11] have demonstrated compounds for the inhibition of erosion, such as FPC–FCA complex. This complex can increase the level of calcium ions and inorganic phosphate in the surface of the tooth, allowing in this way the immediate remineralization of the enamel.

A clear example is the study realized by West et al.[12] who evaluated the erosive effect of dietary beverages in relation to the exposure time, performing the erosive phase three times a day and collecting the data in 10-minute interval with a total of 30 minutes of exposure. He showed that if there was a statistically significant relationship between the erosive effect and the exposure time, however in this work, an erosive effect could be obtained in a period of 3 days with an exposure of 2 minutes for each erosive cycle.

Similar results to this research were described by Kitchens et al.[13] who determined the erosive potential of carbonated beverages such as Classic Coca-Cola, Dietary Cola, Gatorade, and Red Bull and non-carbonated beverages such as Starbucks Frappuccino Coffee, Dasani water (bottled), and tap water (control), which showed that all beverages, both carbonated and non-carbonated, had a level of erosion on the tooth enamel, but carbonated beverages proved to have a greater erosive power. This agrees with the application of the Coca-Cola and Inca-Kola beverages used in this study.

In relation to the time of demineralization, the study by Torres et al.[14] raised the same variable as West et al.[12] when evaluating the relationship of carbonated beverages and exposure time, but they demonstrated this by applying them three times a day for only 5 minutes for a period of 60 days. The data were obtained at 7, 15, 30, 45, and 60 days and in this way I can show the relationship between the erosive effect and the exposure time in days, which with respect to this work was obtained erosive results in an estimated time of only 3 days.

On the contrary, in relation to the pastes with FPC–FCA, the study of Claudio et al.[15] with the toothpaste Sensodyne Pronamel (GlaxoSmithKline, Brentford, Middlesex, United Kingdom) which is a derivative of Sensodyne toothpaste and is made of bioavailable sodium fluoride (1,450 ppm of fluoride) and which in turn contains potassium nitrate favors the prevention against tooth enamel erosion and at the same time mitigates the effects of dentine hypersensitivity; the next paste that he used is BioRepair Plus (Coswell SpA, Bologna, Italy), which is a fluoride-free toothpaste composed of HA nanocrystals, which have been introduced to the market due to their excellent biological properties. As a result, it showed that both toothpastes offer a degree of protection of the tooth enamel and in turn share the same effectiveness as the FPC–FCA complex used for this study. These were similar to the results of our study and with the research of Manton et al.[16] who evaluated FPC–FCA complex by adding them directly to acidic beverages to evaluate their direct relationship with toothpaste and effectively demonstrated to decrease the level of erosivity by adding 0.2% FPC–FCA in four acidic beverages with pH range (2.2–2.4), and its effect on dental enamel in a 50 mL solution was investigated during 30 minutes. When it comes to combating erosion, we would not only focus on performing toothbrushing with remineralizing agents, as in this research, but also consider what Maton proposed.

Coinciding finally, with what was described by Amaral et al.[8] who carried out a study to evaluate sodium fluoride pastes (Colgate 360° Sensitive, Colgate-Palmolive Ind. Com. Ltd, São Paulo, Brazil) and FPC–FCA with fluorine in a simulated oral environment, which

Table 2: *In vitro* comparison of the remineralizing effect of toothpastes with casein phosphopeptide–amorphous calcium phosphate and sodium fluoride on the erosion of tooth enamel

Erosion of the dental enamel								
Toothpastes		*Mean ± SD*	*Median*	*Min*	*Max*	*p**	*p***	*p****
Sodium fluoride	Coca-Cola®	2.95 ± 0.8	2.91	1.96	4.08	0.052	0.000	0.629
	Inca-Kola®	2.79 ± 0.6	2.54	2.03	3.77			
FPC–FCA	Coca-Cola®	2.19 ± 0.5	2.06	1.54	3.24	0.451		0.000
	Inca-Kola®	1.96 ± 0.3	1.93	1.37	2.55			
Control	Saline solution	3.20 ± 0.4	3.35	2.44	3.67	0.320		

FPC–FCA: casein phosphopeptide with amorphous calcium phosphate complex, the unit of measurement of the specimens were expressed in microns (μm)
*Shapiro–Wilk test
**ANOVA test
***Bonferroni test
Level of significance $p < 0.05$

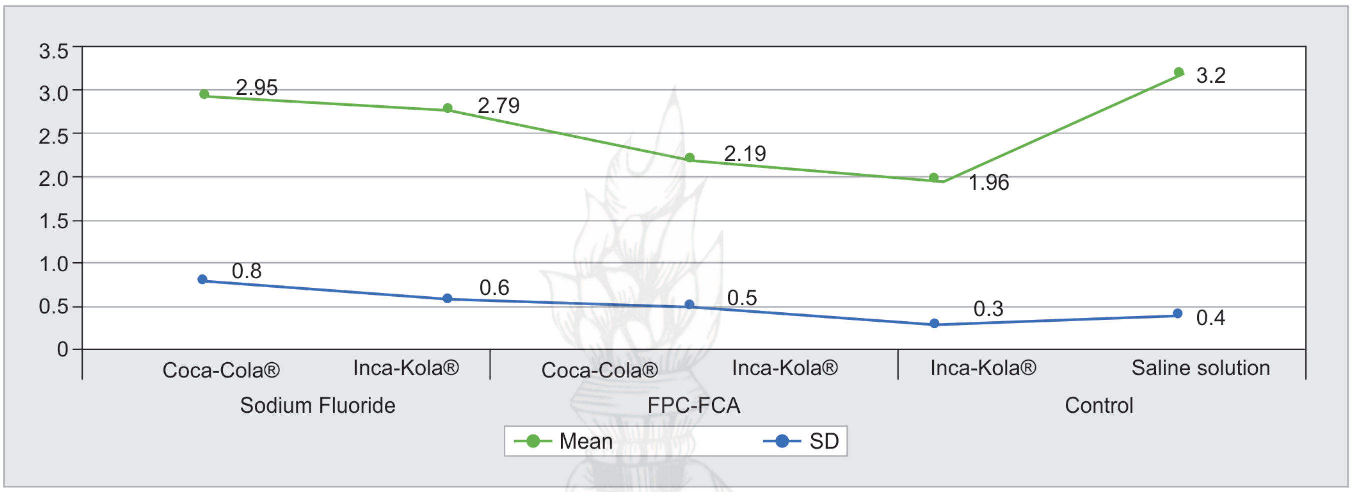

Fig. 7: Remineralizing effect of toothpastes

managed to do so with a drip of 0.4 mL/minute of artificial saliva, which in comparison with this study was used physiological saline as a means of preservation of the samples. For both studies, favorable results of the remineralizing agents on the eroded dental enamel could be verified.

Like our research, for this work, the main erosive agents were Coca-Cola and Inca-Kola, however Tocolini et al.[17] used as an erosive agent juices based on natural, industrial, and soy-based grapes to evaluate its level of erosivity on tooth enamel in primary teeth. It was evidenced that the juice of natural grape presented a greater erosive capacity in the primary dental enamel, also erosivity was evidenced by the other two drinks, but they were not significant among themselves.

The present study had some limitations during the execution, such as the calibration of the instrument in some of the dental enamel samples due to a slight curvature that prevented accurately determining the microroughness, also in the procedure of making the cuts, taking care not to perform microabrations. On the structure that was worked, however, keeping the drinks at room temperature was of vital importance since one of the factors that depends on the erosivity of the beverages is the high temperature conditions and for this reason it was important to control it in times of summer.

This research has a great impact because it revealed the ability to remineralize the tooth enamel; especially under artificial erosive conditions, toothpastes containing FPC–FCA complex, likewise, in the prevention of dental caries as in preventive treatment for patients with xerostomia and, on the contrary, the favorable results of the study can provide new lines of research and in-depth knowledge on the subject for the dentist and the general public, thus promoting its application in different commercial brands and ease of availability for all.

Conclusion

In conclusion, the remineralizing effect of the FPC–FCA (MiPaste®) complex proved to be greater than the sodium fluoride paste (Colgate®) on tooth enamel under erosive conditions. The effectiveness of remineralizing agents on dental enamel under erosive conditions was evidenced for both FPC–FCA (MiPaste®) complex and sodium fluoride paste (Colgate®). Finally, by comparing the microroughness of the tooth enamel eroded by carbonated beverages, it was demonstrated that the Inca-Kola® beverage caused a lower degree of demineralization (microroughness) of the dental enamel than the Coca-Cola® beverage.

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References

1. Moezizadeh M, Alimi A. The effect of casein phosphopeptide–amorphous calcium phosphate paste and sodium fluoride mouthwash on the prevention of dentine erosion: an *in vitro* study. J Conserv Dent 2014;17(3):244–249. DOI: 10.4103/0972-0707.131787.
2. Carvalho F, Brasil V, Silva Filho T, et al. Protective effect of calcium nanophosphate and CPP–ACP agents on enamel erosion. Braz Oral Res 2013;27(6):463–470. DOI: 10.1590/S1806-83242013000600004.
3. Wegehaupt F, Tauböck T, Stillhard A, et al. Influence of extra- and intra oral application of CPP–ACP and fluoride on re-hardening of eroded enamel. Acta Odontol Scand 2012;70(3):177–183. DOI: 10.3109/00016357.2011.600713.
4. Gupta R, Prakash V. CPP–ACP complex as a new adjunctive agent for remineralization: a review. Oral Health Prev Dent 2011;9(2):151–165.
5. Gupta N, Mohan C, Nagpal R, et al. A review of casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) and enamel remineralization. Compend Contin Educ Dent 2016;37(1): 36–39.
6. Gurunathan D, Somasundaram S, Kumar S. Casein phosphopeptide-amorphous calcium phosphate: a remineralizing agent of enamel. Aust Dent J 2012;57(4):404–408. DOI: 10.1111/adj.12006.
7. Ambarkova V, Goršeta K, Glavina D, et al. Učinak fluoridirane paste za zube na remineralizaciju cakline i mikrotvrdoću nakon demineralizacije *in vitro*. Acta Stomatol Croat 2011;45(3):159–165.
8. Amaral C, Miranda M, Correa D, et al. Sodium fluoride and casein phosphopeptide–amorphous calcium phosphate cream plus sodium fluoride efficacy in preventing enamel erosion in a simulated oral environment study model. Indian J Dent Res 2014;25(4):464–469. DOI: 10.4103/0970-9290.142536.
9. Agrawal N, Shashikiran N, Singla S, et al. Effect of remineralizing agents on surface microhardness of primary and permanent teeth after erosion. J Dent Child (Chic) 2014;81(3):117–121.
10. Ranjitkar S, Kaidonis J, Richards L, et al. The effect of CPP–ACP on enamel wear under severe erosive conditions. Arch Oral Biol 2009;54(6):527–532. DOI: 10.1016/j.archoralbio.2009.03.006.
11. Hegde M, Devadiga D, Jemsily P. Comparative evaluation of effect of acidic beverage on enamel surface pre-treated with various remineralizing agents: an *in vitro* study. J Conserv Dent 2012;15(4):351–356. DOI: 10.4103/0972-0707.101902.
12. West N, Hughes J, Addy M. Erosion of dentine and enamel *in vitro* by dietary acids: the effect of temperature, acid character, concentration and exposure time. J Oral Rehabil 2000;27(10):875–880. DOI: 10.1046/j.1365-2842.2000.00583.x.
13. Kitchens M, Owens B. Effect of carbonated beverages, coffee, sports and high energy drinks, and bottled water on the *in vitro* erosion characteristics of dental enamel. J Clin Pediatr Dent 2007;31(3): 153–159. DOI: 10.17796/jcpd.31.3.1157l653t8206100.
14. Torres C, Chinelatti M, Gomes J, et al. Surface and subsurface erosion of primary enamel by acid beverages over time. Braz Dent J 2010;21(4):337–345. DOI: 10.1590/S0103-64402010000400009.
15. Claudio P, Lombardini M, Marco C, et al. Impact of two toothpastes on repairing enamel erosion produced by a soft drink: an AFM *in vitro* study. J Dent 2010;38(20):868–874. DOI: 10.1016/j.jdent.2010.07.010.
16. Manton D, Cai F, Yuan Y, et al. Effect of casein phosphopeptide amorphous calcium phosphate added to acidic beverages on enamel erosion *in vitro*. Aust Dent J 2010;55(3):275–279. DOI: 10.1111/j.1834-7819.2010.01234.x.
17. Tocolini D, Dalledone M, Brancher J, et al. Evaluation of the erosive capacity of children's beverages on primary teeth enamel: an *in vitro* study. J Clin Exp Dent 2018;10(4):383–387. DOI: 10.4317/jced.54546.