










# Effectiveness of Chewing Gum Containing CPP-ACP for Remineralisation of Demineralised Enamel around Orthodontic Brackets: An *in Situ* Study

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## ABSTRACT

**Objective:** To evaluate the effectiveness of chewing gums containing CPP-ACP in remineralisation of demineralised enamel around orthodontic brackets. **Material and Methods:** Enamel blocks from 120 bovine incisors were used. The blocks were flattened and polished, followed by the development of incipient subsurface caries. The blocks have been subjected to a baseline microhardness analysis. Orthodontic brackets were bonded to the exposed area of the blocks, reserving an area for hardness analysis. An *in situ* crossover (three-way crossover phases of 21 days with an interval of one week between them), controlled, randomised and blind experimental model was used, with the participation of 12 volunteers divided into groups: G1 – negative control group, without chewing gum; G2 – conventional sugar-free chewing gum, without CPP-ACP (placebo); and G3 – sugar-free chewing gum with CPP-ACP. The following parameters were analysed: superficial linear (Ra), volumetric roughness (Sa), and superficial structural loss, which was indicated by Gap measurement (difference between the healthy and treated area). Statistical tests used were the Friedman, Wilcoxon and Shapiro-Wilk, Kruskal Wallis' nonparametric tests and ANOVA. **Results:** There was a significant reduction in enamel microhardness after demineralisation in all the groups ( $p < 0.05$ ). This deleterious effect was attenuated in the three groups after the final evaluation, although there were no statistical differences ( $p < 0.05$ ); however, the final values did not return to the baseline values ( $p < 0.05$ ). **Conclusion:** Chewing gum containing CPP-ACP did not promote *in situ* remineralisation of demineralised enamel around orthodontic brackets.

**Keywords:** Chewing Gum; Dental Enamel; Orthodontic Brackets.

## Introduction

With the insertion of a fixed orthodontic appliance, it becomes difficult to perform oral hygiene, thus favouring greater bacterial accumulation [1]. Accumulation of bacterial biofilm, consumption of sucrose, associated with poor hygiene around orthodontic devices, leads to the reduction of local pH and an increase in the tooth enamel demineralisation process, culminating in the appearance of active white spot lesions on the enamel surface [2,3]. After orthodontic treatment begins, if this process is not interrupted, active white spot lesions can progress to caries lesions with cavitation of the tooth surface [4,5].

To prevent and intercept the appearance of dental caries lesions, a protein derived from milk, casein phosphopeptide – amorphous calcium phosphate (CPP-ACP), has been included in various commercially available products, such as toothpaste, mouthwash and chewing gum [6,7].

According to the literature [8,9], when in contact with bacterial biofilm, CPP-ACP releases calcium and phosphate ions, which raise the demineralisation threshold of hydroxyapatite and promote dental remineralisation. At present, chewing gums containing CPP-ACP are widely used by the population and have received international recognition [10,11]. In the presence of an acid, the ACP becomes dissociated, releasing calcium and phosphate ions, increasing the degree of saturation relative to demineralisation of the hydroxyapatite present in tooth enamel, and promoting its remineralization [12].

However, the effects of chewing gum containing APP-ACP on the tooth enamel around fixed orthodontic devices has not yet been evaluated in the literature. Therefore, the present study aimed to evaluate the remineralising capacity of chewing gums containing CPP-ACP in tooth enamel around orthodontic brackets, testing the hypothesis that chewing gums containing CPP-ACP remineralise demineralised enamel around orthodontic brackets.

## Material and Methods

### Experimental Design

The study was conducted using a blind, controlled and randomised protocol of three-way crossover phases of 21 days with an interval of one week between them. The factors under evaluation were treatment at three levels: G1–negative control group, without chewing gum; G2–conventional sugar-free chewing gum, without CPP-ACP (placebo); and G3–sugar-free chewing gum with CPP-ACP.

Blocks of enamel with demineralised surface ( $n = 108$ ) and presence of orthodontic accessories, in which the microhardness had been evaluated, were randomly divided into three groups of volunteers ( $n = 12$ , 2 enamel blocks in each appliance). The volunteers maintained the enamel blocks fixed to acrylic plates for 21 days for each stage of the experiment, with a rest of seven days between each period. The participants were instructed to use two tablets of chewing gum for 15 minutes, three times per day.

After this period, the enamel blocks were removed from the plates, and after this, the final surface microhardness of the treated area and non-contact 3D profilometry of the protected and treated areas were evaluated.

### Enamel Sample Preparation

Initially, 120 healthy bovine mandibular incisors, free from scratches, hypoplasia, cracks, stains, abrasions, or any alterations visible microscopically and/or under stereoscopic loupe examination, were used.

The teeth were stored at ambient temperature in an aqueous solution of 2% formol at pH 7.0 until use. After this, the roots of these teeth were removed by means of a diamond disc. Later, the crowns were fixed using sticky wax onto acrylic plates, which were coupled to a cutter (Isomet, Buehler Ltd., Lake Bluff, IL, USA). Finally, using a dual faced diamond disk, four cuts were made in the central region of the vestibular surface of each crown to obtain enamel blocks measuring  $9 \times 9 \text{ mm}^2$ .

The blocks were then fixed to polypropylene devices, with the enamel facing upwards, and taken to a metallographic polishing machine for enamel wear and polishing, using abrasive papers (600 and 1200 grit, respectively). Between each polishing stage, the tooth/disc set was immersed in distilled and deionised (dd) water (dd), taken to an ultrasound device for 3 minutes. At the end of the final polishing stage, the specimens were immersed in dd water for 10 minutes.

Blocks selected for use in the study were subjected to initial enamel microhardness evaluation. For this purpose, a microdurometer (Buehler, Minneapolis, USA) with a pyramidal Knoop type diamond penetrator was used, with a load of 50 g applied for 5 seconds. Five indentations (laterally) were made in each test specimen, disposed in a column in the upper region of each specimen, with 100- $\mu\text{m}$  spacing between the indentations. A mark of origin was made with a 100-g load before the other indentations to serve as guidance for the subsequent analyses. Blocks presenting a microhardness value 10% above or below the mean of the test specimen values were discarded. After initial selection (mean value  $332.19 \pm 33.2 \text{ kg/mm}^2$ ), got 108 enamel blocks.

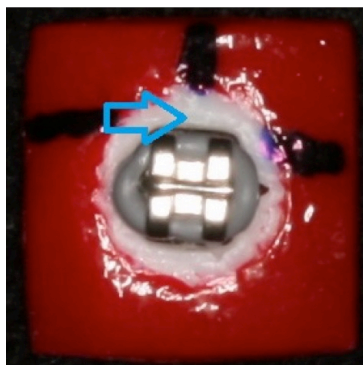
#### Demineralisation of Samples

To obtain demineralised lesions in enamel, the samples underwent artificial caries induction using 0.05 M acetate buffer solution, containing 1.28 mM calcium, 0.74 mM phosphate and 0.03  $\mu\text{g}$  fluoride/mL (pH 5.0). Thus, on each block, a circular area was demarcated for caries induction and the remainder of the surface was sealed with a protective varnish. The specimens were individually immersed in solution (2 mL/ $\text{mm}^2$  at pH 5.0) and were then placed in an oven at 37 °C for 16 hours.

After demineralisation had been induced, a new surface microhardness evaluation was conducted. This evaluation followed the same criteria used for the initial test. The blocks were selected based on the initial demineralised surface microhardness (SMH)  $207.53 \pm 28.35$  (No blocks were discarded). On conclusion of fabrication and standardisation of the test samples, these were subjected to a sterilisation process using ethylene oxide.

#### Bracket Bonding

The brackets (Edgewise Slim, Dental Morelli Ltda., Sorocaba, SP, Brazil) were bonded to the central region of the dental block. However, the SMH area was reserved so that it remained unchanged for later evaluations. Before bonding, the enamel surface was etched with a self-etching agent (TPSEP; 3M Unitek, Monrovia, CA, USA). After this, an orthodontic composite (Transbond XT; 3M Unitek, Monrovia, CA, USA) was applied at the base of the brackets, which received a pressure of 300 g/f to extravasate the excess composite. Next, the excess material was removed with an exploratory probe and light polymerisation was performed for 40 seconds. Finally, each orthodontic bracket received a small orthodontic wire segment fixed with orthodontic elastic to simulate the oral conditions (Figure 1).



**Figure 1. Enamel block with orthodontic bracket bonded and wire segment fixed with orthodontic elastic.**

#### Volunteers and *in Situ* Phase

This study involved the direct participation of human beings and, therefore, was conducted in compliance with the Research Ethics Committee (Protocol number 37143414.0.0000.0055). The participants were invited by means of signing the Term of Free and Informed Consent (TFIC).

Twelve volunteers with a mean age of 23.4 years (range 20–25 years), comprising six men and six women, made up the sample. The volunteers selected presented a stimulated salivary flow higher than or equal to 1.6 mL/min and an unstimulated salivary flow higher than or equal to 0.4 mL/min. In addition, the volunteers were required to meet the following inclusion criteria: healthy individuals who were not using medications that would significantly change the microflora (e.g., antibiotics) or salivary flow (e.g., antidepressive agents, narcotics, diuretics or antihistamines); absence of periodontal disease or active caries lesions; non-smokers; absence of antibiotic use in the two months before the research began; absence of systemic diseases, such as autoimmune diseases, xerostomia, menopause, diabetes Type 1, malnutrition, gastro-esophageal problems, regulation disturbances and vomiting; and absence of a fixed orthodontic appliance.

A sample size of 10 volunteers was estimated based on 1- $\alpha$ -error of 5%, power of 20%, as estimated standard deviation and 15.0 as the minimum detectable difference in means. Twelve volunteers were selected, allowing for possible losses inherent to *in situ* studies.

As this was an *in situ* study, these volunteers served as hosts for the tooth enamel samples. Using impressions of the maxillary arch of these individuals, palatine plates were made of self-polymerising acrylic resin, with 04 retention clips made with 0.7 mm orthodontic wire. In each plate, two cavities (10 × 10 × 3) were made, which served as sites to receive the enamel blocks fixed with sticky wax. The blocks were randomly distributed among the study participants.

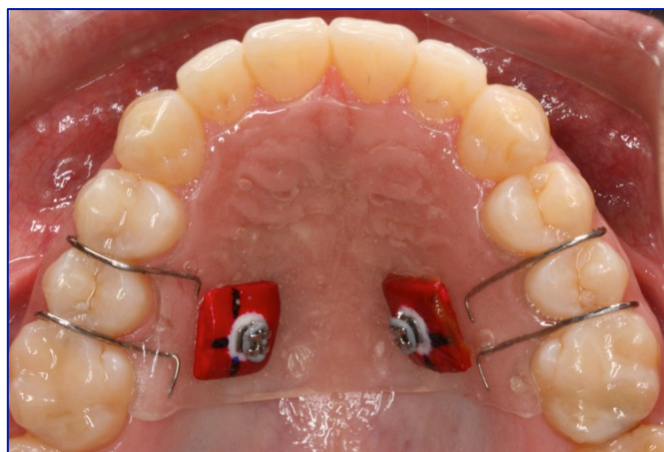
As previously described, a crossover study was conducted among the research participants, which was divided into three experimental phases. The choice of the participants who will compose each group was made by drawing lots. In the first phase, the volunteers used mint-flavored placebo chewing gum without sugar (Cadbury Adams Brasil Ind. e Com. de Produtos Alimentícios Ltda., Bauru, SP, Brazil). In the second phase, no chewing gum was used and this group served as the control group. Finally, in the third phase, the volunteers used mint-flavored Trident Total Recaldent® chewing gum (Cadbury Adams Brasil Ind. e Com. de Produtos Alimentícios Ltda., Bauru, SP, Brazil). All chewing gums had the same appearance, taste and smell.

Before insertion of the plates, the volunteers participated in the projection of an expository presentation of slides as a didactic resource for guidance. Immediately afterwards, each individual received a guide containing instructions, a chart for recording the exposures (use of chewing gum), a personalised

palatine intraoral plate, a plastic box, two tubes of fluoridated dentifrice (Colgate Total 12, 1100 ppm, Clean Mint 90 g; Colgate Palmolive Industrial Ltda., São Bernardo do Campo, SP, Brazil), an Oral-B Indicator Plus Soft 40 toothbrush (Gillette do Brasil Ltda., Manaus, AM, Brazil) and one reel of dental floss (Colgate Total, 50 m; Colgate Palmolive Industrial Ltda., São Bernardo do Campo, SP, Brazil).

The research participants were instructed to perform oral hygiene and brushing the palatine intraoral plate after the main meals using only the toothbrush, toothpaste and dental floss supplied by the researcher. In addition, at the beginning of the first experimental phase, the participants received a masked pot containing placebo chewing gum, and in the third phase, they received another masked pot containing Trident Total Recaldent® chewing gum. Neither the volunteers nor the researchers knew which type of chewing gum was being used in each phase, as the pots were without identification or distinction.

The participants used the palatine plate continually for 21 days in each phase of the study, with the plate remaining in position for 24 hours per day (Figure 2). The plate was removed only to perform oral hygiene. The participants were instructed to use the chewing gum allocated to them three times per day, chewing two units for 15 minutes after each of the three main meals.



**Figure 2. Intraoral image of patient using the palatine plate with test specimens included.**

#### Final Microhardness Evaluation

The brackets were removed by a single researcher using orthodontic pliers. The hardness of the enamel blocks was evaluated again, making five indentations spaced at 100 µm from the baseline after demineralisation. The percentage of surface microhardness recovery (% SMR) from the specimens was calculated using the following equation:  $\% \text{ SMR} = \frac{\text{SMH demineralised} - \text{SMH after pH cycling}}{\text{SMH demineralised}} \times 100$ . All the surface microhardness evaluations were performed in a blind manner by the same examiner.

#### 3D Non-contact Profilometer Analysis

The surface topography of the specimens was evaluated in a blind manner by a single evaluator using a 3D non-contact profilometer (Nanovea PS50 Optical, NANOVEA Inc., USA). Firstly, six 3D images of the surface of each sample were captured. This image was captured by means of a white axial light confocal chromatic sensor (fiber optic). The scanning speed for each sample was 2 mm/s and a refractive index of 10,000. After this, measurements were taken from the images with the aid of the Nanovea Professional 3D software program (NANOVEA Inc., Irvine, CA, USA).

The parameters evaluated were linear surface roughness (Ra) and volumetric surface roughness (Sa), in addition to superficial structural loss. This latter parameter was used to evaluate the loss of hard tissue by means of Gap measurement (difference in height between the control and experimental areas). Comparison between the areas was possible after removal of the acid-resistant varnish that covered the healthy area (control).

To determine the Ra value, three horizontal linear measurements were made in each area (control and experimental) of the specimen. The mean of these three measurements was used as the final value for each area, with Ra1 being the mean of the control area and Ra2, the mean of the experimental area. Therefore, the value of Ra was calculated as follows:  $Ra = Ra1 - Ra2$ .

In the same way, three measurements were taken in each area (control and experimental) to calculate the value of Sa. Each measurement corresponded to a window measuring  $200 \mu\text{m} \times 200 \mu\text{m}$ . The mean was used as the final value for each area, with Sa1 being the mean of the control area and Sa2, the mean of the experimental area. The value of Sa was calculated as follows:  $Sa = Sa1 - Sa2$ .

The last parameter evaluated was the superficial structural loss, calculated as the difference in height between the control and experimental areas in each sample, denominated Gap. Three measurements were taken and the final value was also expressed as the mean of the three measurements.

### Statistical Analysis

The Friedman test was used to evaluate the differences in the enamel microhardness in the different time intervals (baseline, after demineralization and final), with the comparisons between the pairs being conducted using the Wilcoxon test. The Kruskal-Wallis test was used to test the differences in the mean hardness of the enamels between the groups (control, placebo and experimental) in each time interval. The level of significance adopted was 5% ( $\alpha = 0.05$ ). The data were tabulated and analysed using the software program IBM SPSS Statistics 21.0 for Windows (IBM Corp., Armonk, NY, USA). Concerning the results of profilometry, after evaluation of normality (Shapiro-Wilk test), the data were submitted to the paired t-test and ANOVA for repeated measures with Bonferroni correction ( $p < 0.05$ ).

## Results

### Microhardness Analyses

Based on intragroup comparisons, we observed that in all three of the groups there was a significant reduction in hardness of the enamel after after demineralization; this deleterious effect was attenuated in the three groups after the final evaluation, but the values did not return to baseline values (Table 1).

**Table 1. Medians and interquartile deviations of hardness of enamel, according to groups and time intervals of measurement (n=12 per group).**

Groups	Hardness Measurement ( $\mu\text{m}$ )			p-value
	Baseline	ACC	Final	
G1 (Control)	328.75 <sup>a</sup> ± 33.37	213.26 <sup>b</sup> ± 48.82	256.75 <sup>c</sup> ± 33.32	<0.001
G2 (Placebo)	334.80 <sup>a</sup> ± 26.01	204.67 <sup>b</sup> ± 38.89	268.47 <sup>c</sup> ± 41.44	<0.001
G3 (Experimental)	343.96 <sup>a</sup> ± 35.95	201.53 <sup>b</sup> ± 50.71	284.82 <sup>c</sup> ± 48.18	<0.001
p-value	0.097	0.440	0.503	

ACC, after demineralization. Friedman's Test; <sup>a,b,c</sup>Values with different superscript letters indicate significant differences in the mean hardness of enamel, between the different time intervals (Wilcoxon test); Kruskal-Wallis - compare the different groups within the same time interval.

In terms of percentage, in Group 1 (control), there was a recovery of hardness to the order of 16.8%; in Group 2 (placebo), 23.8%, and in Group 3 (experimental), 29.2%. Intergroup comparisons using the Kruskal-Wallis test showed that the hardness of enamel did not differ significantly between any of the groups in the different time intervals evaluated, indicating the absence of the effect of the treatment.

### 3D Non-contact Profilometry Analysis

The results of non-contact 3D profilometry are presented in Table 2, with the initial and final values of Ra and Sa, and in Table 3, with the values of Ra, Sa and Gap for each of the groups. No group presented alterations in Ra, and Sa when compared before and after treatment ( $p > 0.05$ ). G1, G2 and G3 did not differ between with regard to Ra, Sa and % PRS ( $p > 0.05$ ). G3 presented the lowest Gap values ( $3.11 \mu\text{m} \pm 1.09$ ) when compared with G1 ( $3.88 \mu\text{m} \pm 1.05$ ) and G2 ( $5.96 \mu\text{m} \pm 0.05$ ) ( $p < 0.05$ ), and there was no significant difference between G1 and G2 ( $p > 0.05$ ) (Figure 3).

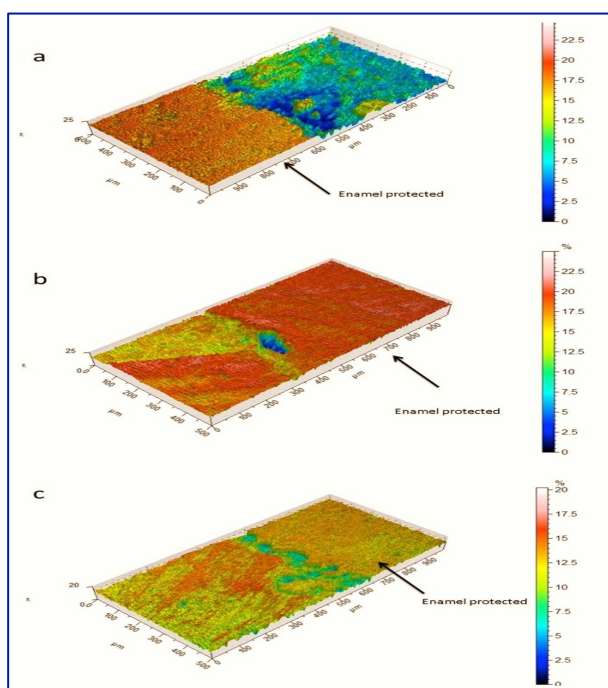
**Table 2. Mean ( $\pm$ SD) of initial and final Ra, Sa for groups evaluated (n=12 per group).**

Groups	3D Non-contact Profilometry Results			
	Ra initial	Ra final	Sa initial	Sa final
G1 (Control)	$0.35 \pm 0.17$	$0.36 \pm 0.17$	$0.40 \pm 0.18$	$0.45 \pm 0.24$
G2 (Placebo)	$0.32 \pm 0.14$	$0.35 \pm 0.13$	$0.39 \pm 0.15$	$0.48 \pm 0.20$
G3 (Experimental)	$0.27 \pm 0.09$	$0.29 \pm 0.12$	$0.31 \pm 0.09$	$0.34 \pm 0.10$

**Table 3. Mean ( $\pm$ SD) of Ra, Sa and Degree for groups evaluated.**

Treatments	3D Non-contact Profilometry Results		
	Ra	Sa	Gap
G1 (Control)	$-0.01 \pm 0.15^a$	$-0.05 \pm 0.16^a$	$5.96 \pm 2.30^a$
G2 (Placebo)	$-0.03 \pm 0.10^a$	$-0.09 \pm 0.16^a$	$3.88 \pm 1.05^a$
G3 (Experimental)	$-0.02 \pm 0.09^a$	$-0.02 \pm 0.10^a$	$3.11 \pm 1.09^b$

Means followed by distinct letters are statistically different ( $p < 0.05$ ); Paired-t test and ANOVA.



**Figure 3. Images obtained in profilometer: a) Group 1, b) Group 2, and c) Group 3.**

## Discussion

The use of fixed orthodontic devices, in association with deficient oral hygiene and consumption of sucrose, leads to an increase in bacterial biofilm, which, in turn, favours the development of carious lesions at the level of enamel. To reverse this process, that is to remineralise the active white spot lesions, the professional may use materials for this purpose [13]. At present, emphasis has been placed on products that contain CPP-ACP [7-9].

Chewing gums containing CPP-ACP in their formulation would be of great interest in orthodontics, given that a large proportion of patients under orthodontic treatment are at the adolescent stage, a period of life when they are most likely to consume these products [14]. Furthermore, according to Cai et al. [15], chewing gums have been shown to be an appropriate vehicle for the use of CPP-ACP for promoting the remineralisation of subsuperficial lesions in human tooth enamel.

Accompanying this tendency, the industry has incorporated this component into chewing gums and made them commercially available. With the aim of testing the effectiveness of this product, Prestes et al. [11] and de Alencar [10] developed studies of enamel erosion.

According to de Alencar et al. [10], remineralisation of erosive lesions using CPP-ACP involves mineral deposition in the porous zone. It is accepted that the mechanism by which CPP-ACP reduces caries in tooth enamel differs from that of dental erosion due to the different structural characteristics of the lesions. In the white spot lesions of dental caries, the superficial layer of the subsurface of the lesion is a relatively intact layer, rich in minerals. These pores present organic materials, which provide passage for solutions or external agents, enabling contact with the deeper tissues.

From an orthodontic perspective, a question arises: Would these materials be capable of remineralising enamel around orthodontic brackets? Based on this premise, we developed this *in situ* study. An *in situ* clinical study is characterised by carrying and maintaining the test samples in the mouth and, after conclusion of the experimental period, performing laboratory analysis of the samples [12,16].

Following this study methodology, in the proposed experiment, palatine intraoral plates were used. These plates served to maintain the enamel blocks in contact with the oral medium, allowing exposure to salivary dynamics and the variables presented by the environment (saliva, microbiota, change in pH, enzymes, pigments, food, and chemical hygiene products). In the present study, we used a crossover methodology in which the same individuals formed part of the three study groups, thereby preventing factors inherent to individuality from interfering in the results achieved. Furthermore, during analyses of the data, the evaluators were blinded, with no knowledge of which group was being evaluated.

The microhardness results revealed a percentage increase in surface hardness of 23.8% in the group that used the placebo chewing gum, 16.8% in the group in which no chewing gum was used, and 29.2% in the group that used the chewing gum containing CPP-ACP. These results may be attributed to the stimulation of salivation promoted by the act of chewing gum, especially the increase in parotid gland function, resulting in a larger quantity of calcium and phosphate ions being available for precipitation on the enamel [17]. The findings of the present study are in contrast with those reported by de Alencar et al. [10] when they evaluated the remineralising power of chewing gums containing CPP-ACP in eroded human enamel. These authors found a recovery of 50% for patients who used chewing gum containing CPP-ACP, and 30% for those who used conventional chewing gum. Whereas, Prestes et al. [11], in a similar study to that of de Alencar et al. [10] but using bovine teeth, reported a 29.6% recovery of surface hardness for the group that used CPP-ACP, and 19.1% and 10% for those using chewing gum without CPP-ACP and the control group, respectively.



Another possible explanation for the differing results was the chewing gum protocol used. The study of de Alencar et al. [10] involved using chewing gum for 30-minute periods, four times per day. In the present study, chewing gum was used three times a day, after the three main meals, with two units being chewed each time for 15 minutes; therefore, a shorter period was involved and a smaller quantity of product remained in contact with the specimens.

The period of 21 days used in the present study was based on this being the meantime between orthodontic maintenance consultations. At this consultation, the orthodontist will evaluate the patient's oral condition and, if necessary, indicate measures to minimise bacterial accumulation around the orthodontic brackets.

In their study, Shen et al. [18] demonstrated that the incorporation of CPP-ACP into chewing gum without sugar resulted in an increase in remineralisation of superficial lesions in the enamel, which was directly proportional to the dose. According to these authors, an increase of 9% occurred in remineralisation promoted by the chewing gum with 0.19 mg CPP-ACP, 63 % with 10.0 mg, 102 % with 18.8 mg, and 152% with 56.4 mg. By means of the results obtained, associated with a comparison with the data found by these authors, we could infer that the concentration of CPP-ACP in the Trident Total Recaldent® chewing gum evaluated may be low.

Morgan et al. [19] developed an *in situ* study in which they evaluated the remineralising power of three different chewing gums, of which one contained CPP-ACP. Using microradiographic analysis of the samples, it was demonstrated that the chewing gum containing CPP-ACP produced a significantly higher degree of remineralisation ( $18.4 \pm 0.9\%$ ) than the other two chewing gums ( $8.9 \pm 0.5\%$  and  $10.5 \pm 0.9\%$ ). The results obtained may be explained by the type of dental enamel used in the experiment, which was from human third molars.

However, in spite of the absence of a statistically significant difference in the treatment with chewing gums containing CPP-ACP, by means of analysis of the data, an effective increase in remineralisation of the enamel occurred, at a percentage level above that which positive results were found with regard to the maintenance of oral hygiene by tooth brushing. Effective brushing using only fluoridated dentifrice, associated with the use of dental floss, was shown to be sufficient to reverse white spot lesions at the level of enamel, leading to its remineralisation. Corroborating these data, Bröchner et al. [20] investigated the effect of topical applications of 10% CPP-ACP (Tooth Mousse, GC Europa) on white spot lesions after treatment with fixed orthodontic appliances. Topical treatment with an agent containing CPP-ACP resulted in a reduction in the lesions after four weeks; however, not superior to “natural” regression after the daily use of fluoridated dentifrice.










The efficacy of CPP-ACP in remineralisation of superficial lesions in enamel is shown to be well-founded in the literature [21]. Therefore, it is necessary for further research to be conducted using chewing gums containing different concentrations of CPP-ACP to evaluate whether the concentration of this component influences the remineralisation of white spot lesions around orthodontic brackets.

From analysis of the results of the present study, with others available in the literature, it may be supposed that the presence of brackets on the surface of the enamel blocks may have been a factor of influence on obtaining the results found, starting with the idea that the presence of the brackets favoured greater bacterial accumulation, making it difficult for the remineralising action of the chewing gum containing CPP-ACP to occur.

## Conclusion

Chewing gum containing CPP-ACP did not promote remineralisation of demineralised enamel around orthodontic brackets. However, chewing gum containing CPP-ACP led to an increase of 30% in the surface hardness of the demineralised enamel and, in addition, was capable of preventing superficial structural loss of enamel. The hypothesis that chewing gums containing CPP-ACP remineralise enamel around orthodontic brackets was not confirmed.

### Authors' Contributions

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AMS		<a href="https://orcid.org/0000-0001-9786-1752">https://orcid.org/0000-0001-9786-1752</a>	Resources and Funding Acquisition.
TKSF		<a href="https://orcid.org/0000-0003-1340-9967">https://orcid.org/0000-0003-1340-9967</a>	Formal Analysis, Investigation, Resources and Project Administration.
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LCM		<a href="https://orcid.org/0000-0003-1026-9401">https://orcid.org/0000-0003-1026-9401</a>	Conceptualization, Visualization, Supervision and Funding Acquisition.
All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.			

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None.

### Conflict of Interest

The authors declare no conflicts of interest.

### Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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