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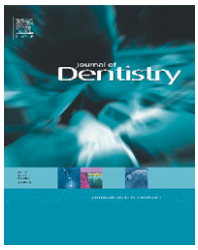

In vitro evaluation of the erosive potential of orange juice modified by food additives in enamel and dentine

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ABSTRACT

Objectives: To evaluate the erosive potential of orange juice modified by food additives in enamel and dentine.

Methods: Calcium lactate pentahydrate (CLP), xanthan gum (XG), sodium linear polyphosphate (LPP), sodium pyrophosphate tetrabasic (PP), sodium tripolyphosphate (STP) and some of their combinations were added to an orange juice. Pure orange juice and a calcium-modified juice were used as negative (C−) and positive (C+) controls, respectively. In phase 1, 15 modified orange juices were tested for erosive potential using pH-stat analysis. In phase 2, the additives alone and the combination with good results in phase 1 and in previous studies (CLP + LPP) were tested in an erosion–remineralization cycling model. In phase 3, the erosion and remineralization episodes were studied independently. Enamel was analysed by surface microhardness (SMH) and profilometry, whilst dentine by profilometry.

Results: In phase 1, reduction of the erosive potential was observed for all additives and their combinations, except XG alone. In phase 2, no detectable enamel loss was observed when CLP, LPP and CLP + LPP were added to the juice. XG, STP and PP had enamel loss similar to C− ($p > 0.05$). Amongst additives, the combination CLP + LPP showed the highest SMH values followed by CLP ($p < 0.05$). All the other groups presented SMH values similar to C− ($p > 0.05$). For dentine, only CLP + LPP lead to surface loss values lower than C− ($p < 0.05$). In phase 3, CLP, LPP and CLP + LPP seemed to protect against erosion; whilst none of the tested compounds seemed to interfere with the remineralization process.

Conclusions: CLP and LPP reduced erosion on enamel and this effect was enhanced by their combination. For dentine, only the combination CLP + LPP reduced erosion.

1. Introduction

The prevalence and incidence of dental erosion has increased over the last few decades,[1,2] and studies have related this fact to the increase of acidic soft drinks consumption worldwide.[3] Some important chemical aspects can modulate their potential to cause dental erosion, including pH,[4] titratable acidity,[5] type of acid,[6] buffer capacity,[7] chelating properties,[5] and concentration of calcium, phosphates and fluoride.[7] It is

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known that specific modifications on these parameters may lead to a reduction on the erosive potential of a given acidic beverage.[8]

A commonly investigated modification has been the use of additives, mostly salts containing calcium and/or phosphate ions.[7,9–12] They act based on the common ion effect, where the driving force for dental surface dissolution can be decreased by the saturated state of the drink with respect to the calcium and phosphate ions.[8] However, the addition of phosphates alone does not seem to be as effective as calcium.[13,14] The addition of food polymers has also been investigated and they have shown ability to reduce erosion due to their possible adsorption to the dental surfaces, leading to the formation of an acid-protective layer. This layer could reduce the exchange of H^+ and of calcium and phosphate ions between the hydroxyapatite and the solution.[15] The negative side of using food polymers could be that they also have mineralization-inhibiting properties, interfering with possible remineralization of the eroded dental substrate.[16]

In this study we aimed to investigate the modification of the erosive potential of an orange juice by the addition of salts of calcium and phosphate as well as of food polymers, either alone or in combination. Orange juice was chosen due to its acidic nature, well documented erosive potential[17–19] and widespread and worldwide consumption. The study hypothesis was that the additives, combined or alone, would be able to reduce dental erosion development, by either preventing the demineralization or enhancing the remineralization.

2. Materials and methods

2.1. *Experimental design*

This study was carried out in 3 phases. In the first, five substances and their combinations (total of 15 formulations) were added to a commercially available orange juice and the erosive potential of these solutions was compared with the pH-stat as a screening method, tested in triplicate. In the second phase, six solutions were tested, comprising the 5 additives alone and the combination that showed the best protective action in phase 1, as well as positive and negative controls. In this phase both human enamel and root dentine specimens ($n = 10$) were tested, using an erosion–remineralization cycling model. In the third phase, we further investigated the mechanism of action of the additives by breaking down the cycling model in two independent tests: demineralization only and remineralization. Bovine enamel was the substrate tested ($n = 5$). A single factor, completely randomized experimental design was used for all the tests. The response variable for phase 1 was the volume (ml) of the titrant (0.1 N HCl). For phases 2 and 3, the response variables were surface loss (μm) measured by optical profilometry, and/or surface microhardness (SMH) determined by the Knoop hardness number.

2.2. *Phase 1*

In this phase, five food-approved substances were added alone or in combination to a commercial available orange juice (Minute Maid Original®, The Coca-Cola Company, Atlanta, GA, USA), creating the experimental groups showed in Table 1. The additives chosen for this study were: calcium lactate pentahydrate (CLP) (Fisher Scientific Pittsburgh, PA, USA); sodium polyphosphate with an average chain length of 25 phosphate units, linear structure (LPP) (Calgon 696, Thermos Inc., Cheshire, UK), which will be referred as 'sodium polyphosphate' during the paper; sodium tripolyphosphate (STP) (Sigma–Aldrich Co., St. Louis, MO, USA); sodium pyrophosphate tetrabasic (PP) (Sigma Aldrich Co., USA) and xanthan gum (XG) (Keltrol R; CP Kelco UK, Leatherhead, UK). The amounts used were based on previous publications.[10–12,15,20] The juice without additives was the negative control (C−) and a commercially available calcium-modified juice (Minute Maid Calcium®, The Coca-Cola Company, Atlanta, GA, USA), which has approximately 40 mmol/l of calcium[21] as calcium lactate,

Table 1 – Experimental groups and their codes, additives, concentrations used, means (SD) of the pH, volume of titrant (in ml) needed in the pH-stat method and hydroxyapatite dissolution (in mg).

Group code	Additives (g/l)					pH	Titrant volume	Hydroxyapatite
	CLP	XG	LPP	STP	PP	Mean (SD)	Mean (SD)	Dissolution mean (SD)
C−						3.83 (0.02)	1.23 (0.08)	15.50 (0.001)
CLP	3.1					3.83 (0.01)	0.46 (0.03)	5.80 (0.000)
XG		0.2				3.82 (0.02)	1.39 (0.01)	17.41 (0.000)
LPP			0.2			3.83 (0.00)	0.20 (0.01)	2.45 (0.000)
STP				0.2		3.82 (0.01)	0.73 (0.04)	9.15 (0.000)
PP					0.2	3.81 (0.01)	0.75 (0.08)	9.47 (0.001)
CLP + XG	3.1	0.2				3.82 (0.01)	0.63 (0.06)	7.87 (0.001)
CLP + LPP	3.1		0.2			3.83 (0.01)	0.03 (0.03)	0.43 (0.000)
CLP + STP	3.1			0.2		3.83 (0.00)	0.13 (0.10)	1.57 (0.001)
CLP + PP	3.1				0.2	3.82 (0.02)	0.16 (0.13)	2.03 (0.002)
XG + LPP		0.2	0.2			3.81 (0.00)	0.27 (0.02)	3.40 (0.000)
XG + STP		0.2		0.2		3.83 (0.01)	0.82 (0.01)	10.27 (0.000)
XG + PP		0.2			0.2	3.83 (0.01)	0.77 (0.01)	9.63 (0.000)
CLP + XG + LPP	3.1	0.2	0.2			3.83 (0.01)	0.04 (0.07)	0.52 (0.001)
CLP + XG + STP	3.1	0.2		0.2		3.82 (0.02)	0.07 (0.06)	0.88 (0.001)
CLP + XG + PP	3.1	0.2			0.2	3.83 (0.00)	0.01 (0.01)	0.09 (0.000)
C+						4.11 (0.01)	0.00 (0.00)	0.00 (0.000)

monocalcium phosphate and tricalcium phosphate was the positive control (C+). After the addition of the substances, the juices that presented an alteration in their pH were adjusted to the baseline values (3.8) with either NaOH or HCl. The pH values were determined using a calibrated pH electrode (Accumet 13-620-530; Fisher Scientific, Pittsburgh, PA, USA). Then, all the juices had their erosive potential tested with the pH-stat method.

2.2.1. *pH-stat method*

The pH-stat test was performed using an automatic titrator (Titralab 856, Radiometer Analytical, Lyon, France). The baseline pH of the substance was recorded. 25 ml of the test solution was placed in the reaction vessel and kept under constant agitation (~100 rpm). Then, 25 mg of anhydrous hydroxyapatite crystals (Acros Organic, Geel, Belgium) were added to the solution, starting the reaction. Aliquots of the titrant (0.1 N HCl) were automatically added to the vessel, at 0.5 ml/min rate, in a negative feedback setting so that the baseline pH was kept constant for a total reaction time of 5 min. After this period, the volume of HCl needed to maintain the pH was recorded. Then, this volume was converted to amount of hydroxyapatite dissolved (in mg), in accordance to the stoichiometric relation between the number of mols of HCl (given by: volume * concentration in mol/l) and amount of dissolved hydroxyapatite. For this calculation was considered the following reaction for the total dissolution of HA:

$$Ca_{10}(PO_4)_6(OH)_2 + 8H^+ \leftrightarrow 10Ca^{2+} + 6HPO_4{}^{2-} + 2H_2O$$

where, 1 mol of hydroxyapatite is correspondent to 8 mol of H^+.

2.3. *Phase 2*

In this phase, six selected solutions: CLP, CLP + LPP, LPP, PP, STP and XG, plus C+ and C− were tested using an erosion–remineralization cycling model. Restriction in the number of experimental groups was necessary due to the more elaborate nature of the experiment. The selection of the solutions considered the interest in learning about the protective effects of the 5 additives when added alone and in combination. The combination CLP + LPP was chosen since it showed the best overall results in phase 1 as well as in previous investigations.[20]

2.3.1. *Specimen preparation*

Enamel and root dentine specimens (4 mm × 4 mm × 2 mm) were sectioned from the crowns and the roots of the human teeth, respectively, using a microtome. The specimens were embedded in acrylic resin (Varidur, Buehler, Lake Bluff, IL, USA). The blocks were ground flat with water-cooled abrasive discs (500-, 1200-, 2400- and 4000-grit Al_2O_3 papers; MD-Fuga, Struers Inc, Cleveland, OH, USA) and polished with polishing cloth and diamond suspension (1 μm; Struers Inc.). Three indentations were made in the central area of the enamel specimens using a Knoop diamond indenter (2100 B, Instron Corporation, Wilson Instruments, Norwood, MA, USA; 50 g load, for 15 s) with 100 μm distance between them. The mean of these three indentations were calculated, and eighty specimens with SMH values ranging from 317 to 380 were selected. Tapes were placed on the polished surface, leaving a central area of 4 mm × 1 mm exposed to subsequent testing.

2.3.2. *Erosive challenge*

Eighty enamel and 80 dentine specimens were randomly allocated into the 8 experimental groups ($n = 10$). Then, they were submitted to an erosion–remineralization cycling model. One complete cycle consisted of: 5 min (10 ml/specimen) in 10 ml of the test solutions, with no agitation and at room temperature; and 60 min in 10 ml of artificial saliva (0.213 g/l of $CaCl_2{\cdot}2H_2O$; 0.738 g/l of KH_2PO_4; 1.114 g/l of KCl; 0.381 g/l of NaCl; 12 g/l of Tris buffer and 2.2 g/l of porcine gastric mucin), under 150 rpm and at room temperature. This cycle was repeated for 6 times a day, over 5 days. After the demineralization and remineralization periods, the specimens were rinsed with distilled water and gently dried with paper towel. The specimens were stored in artificial saliva (150 rpm, at room temperature) during the overnight period.

2.3.3. *Erosion assessment*

After cycling, the tapes were removed from the specimens and the surface analysed. An area 2 mm long (X) × 1 mm wide (Y) was scanned with an optical profilometer (Proscan 2000, Scantron, Venture Way, Tauton, UK). The scan covered the treated area and protected reference surfaces on both sides. The step size was set at 0.01 mm and the number of steps at 200 in the X-axis; and at 0.05 mm and 20, respectively, in the Y-axis. The depth of the treated area was calculated based on the subtraction of the average height of the test area from the average height of the two reference surfaces by using the dedicated software (Proscan Application software v. 2.0.17).

In addition, enamel specimens had the microhardness measurement performed with 3 indentations in the lesion area in order to determine the final surface microhardness. The same parameters described above were used.

2.4. *Phase 3*

The same solutions tested in phase 2 were tested.

2.4.1. *Specimen preparation*

Bovine enamel specimens (5 mm × 5 mm × 2 mm) were used for this test. The preparation of the specimens followed the same procedures described in phase 2. Their initial surface microhardness measurement was performed for the selection of eighty specimens with microhardness values ranging from 313 to 376. These specimens had tapes placed on, leaving a central area of 5 mm × 1 mm exposed. This was the testing surface area.

2.4.2. *Demineralization model*

Forty bovine enamel specimens were randomly divided into the same eight experimental groups ($n = 5$) of phase 2. Then, they were immersed in 10 ml of the test solutions for a total of 150 min, without agitation, at room temperature. Specimens were kept in the solution for 30, 90 and 150 min, and then

evaluated regarding surface loss and SMH, using the same parameters as described in phase 2.

2.4.3. *Remineralization model*

Forty bovine enamel specimens were randomly divided into the same eight experimental groups ($n = 5$) of phase 2. They were immersed in 10 ml of the test solutions for 30 min and had the SMH measurement performed. In the sequence, they were immersed in artificial saliva for 24 h before the final SMH evaluation.

2.5. *Data analysis*

Means of the triplicates were calculated for the pH-stat test. For phases 2 and 3, homoscedasticity and normal distribution of the data was checked by the Hartley and Shapiro–Wilks tests. Once these assumptions were satisfied, one-way ANOVA and Tukey tests were carried out for comparisons amongst groups. The software SigmaPlot 11.0 (Systat Software Inc., Chicago Illinois, USA) was used for the calculations, with significance level of 5%.

3. Results

3.1. *Phase 1*

Table 1 shows the averages of hydroxyapatite dissolution obtained in the pH-stat method. It can be observed that the addition of 3.1 g/l of calcium lactate pentahydrate (approximately 10 mmol/l of calcium) to the orange juice was able to reduce the hydroxyapatite dissolution in 63% in comparison to the negative control (C–), whilst the C+ did not dissolve any amount of hydroxyapatite. All the phosphate polymers were able to reduce hydroxyapatite dissolution; however, LPP was most effective. Further reduction was observed when the phosphate polymers were combined with calcium. Xanthan gum alone did not show any positive effect.

3.2. *Phase 2*

The surface loss results of phase 2 are showed in Table 2. For enamel, the groups C+, CLP + LPP, CLP and LPP presented surface loss values below the detection limit of the method used for this methodology, approximately 0.3 μm; therefore, they were not considered in the statistical analysis. The surface loss values of the groups XG, PP and STP were not significantly different from C–. For dentine, the group C+ presented significantly less surface loss, followed by CLP + LPP. The surface loss values of the groups CLP, STP, LPP and XG were not significantly different from C–. Regarding enamel SMH, the results are also showed in Table 2. C+ presented significantly the highest final values of SMH followed by CLP + LPP and CLP. The SMH values for all the other groups were not significantly different from C–.

Table 2 – Results of the phase 2. Means (SD) of surface loss (SL) for enamel and dentine, in micrometres, and surface microhardness (SMH) for enamel.

Groups	SL enamel[a]	SL dentine	SMH enamel
C–	−0.49 (0.37)a,b	−5.92 (0.85)c,d	115.04 (11.34)d
CLP	−0.04 (0.36)	−5.02 (0.64)b,c	184.17 (25.46)c
XG	−0.97 (0.77)a	−6.82 (0.90)d	110.25 (11.07)d
LPP	−0.11 (0.22)	−6.72 (0.91)d	105.55 (15.75)d
STP	−0.33 (0.26)b	−6.09 (0.77)d	107.42 (17.94)d
PP	−0.33 (0.24)b	−6.05 (0.94)c,d	124.78 (14.40)d
CLP + LPP	0.15 (0.10)	−4.47 (0.55)b	266.70 (33.43)b
C+	0.11 (0.19)	−0.87 (0.24)a	321.63 (7.65)a

Different letters indicate significant difference ($p < 0.05$), in columns.

[a] SL values lower than 0.3 μm were considered below the detection limit of the method and, therefore not included in the statistical analysis.

3.3. *Phase 3*

Fig. 1 shows the means and standard deviations (SD) of the profilometry analysis for each experimental time of the demineralization model. After the first 30 min of acid exposure, the surface loss of all groups was very low and below the detection limit of the method, with exception of the group XG. This same finding was found for the groups C+, CLP + LPP and CLP in all experimental times. After 90 min, groups C–, STP and PP started to present a detectable SL. After 150 min, C–, PP, STP and XG almost doubled their SL values and the group LPP started to show detectable SL. The results of the microhardness analysis for this model are presented in Fig. 2. C+ presented significant higher values of SMH in relation to all the groups during all experimental times, with exception of the group CLP + LPP at 30 min. For the 90 and 150 min experimental times, the SMH of the CLP + LPP treatment were also significant higher in comparison to the C–. The CLP treatment group showed significant higher values of SMH in relation to the control for the 90 min experimental time, but not for 150 min.

Fig. 3 shows the results of the remineralization model. Under the experimental conditions adopted, none of the

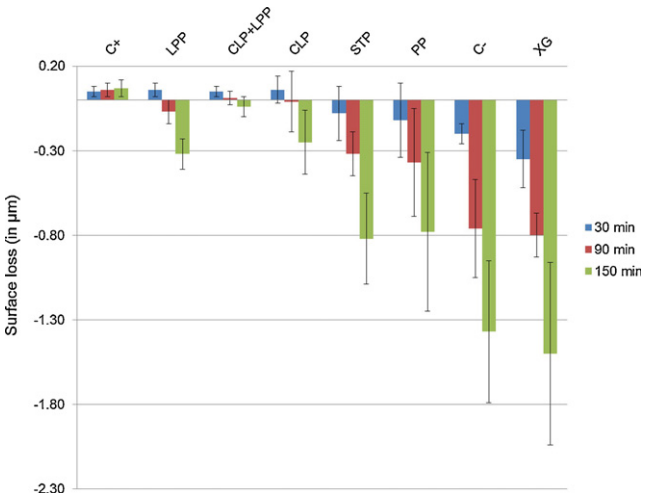

Fig. 1 – Enamel surface loss (in μm) of the demineralization model (phase 3) in all experimental times.

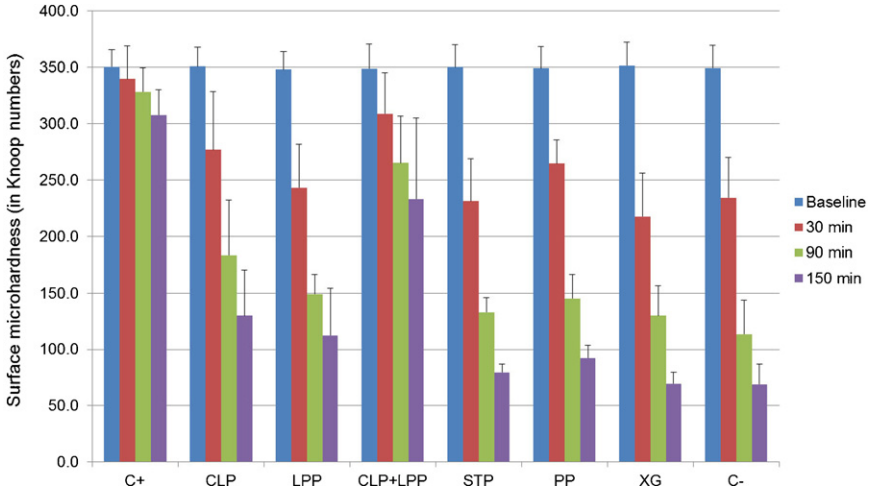

Fig. 2 – Enamel surface microhardness values of the demineralization model (phase 3) in all experimental times.

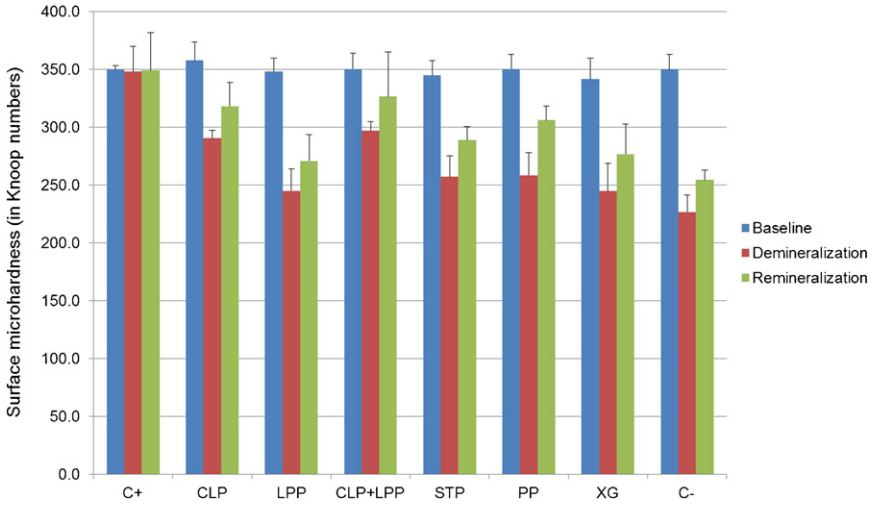

Fig. 3 – Enamel surface microhardness values of the remineralization model in all experimental times (phase 3).

additives interfered with the remineralization of softened subsurface lesions.

4. Discussion

We observed in all experiments that the commercially available calcium-modified juice (the positive control) was the formulation with the lowest erosive potential. However, it contains relatively high amounts of calcium (in order of 40 mmol/l), which can change the taste and the stability of the drink.[8] In addition, there are risks associated with exceeding the tolerable upper limit (level that may cause adverse health effects) of calcium (60 mmol/day), which includes increased risk of kidney stones and interference in the absorption of other minerals, including zinc, magnesium, and phosphorus.[22] The addition of lower amounts of calcium (10 mmol/l) to the orange juice (group CLP) was able to reduce dental erosion in the pH-stat and in the demineralization and erosion–remineralization models. In the pH-stat test, the addition of calcium lactate pentahydrate reduced the volume of titrant needed (and consequently the amount of dissolved hydroxyapatite) by approximately 63%; and in phases 2 and 3, it prevented occurrence of surface loss. In the erosion–remineralization model, SMH changes were observed for the calcium lactate pentahydrate-modified juice, but they were not as severe as that caused by the negative control. In phase 3, the results for SMH can be misleading since the calcium lactate pentahydrate group did not differ from the negative control. Although this may suggest lack of protective effect, it should be kept in mind that higher surface loss occurred for the negative control, which substantiates the overall protection of calcium lactate pentahydrate as mentioned above. These results corroborate the findings of Hughes et al.,[12] where the addition of similar amount of calcium to a blackcurrant juice drink could reduce its erosive potential. In that study, the pH of the modified drink was raised by 1 unit (10 fold decrease in H^+ concentration), which may affect the properties and characteristics of the drink, potentially making it more susceptible to

microbiological spoilage, shortening its shelf life.[15] In the present study, the pH value of the modified juices was adjusted to its original levels, in order to avoid those potential problems.

The anti-erosive effect of calcium lactate pentahydrate has been previously reported by Beiraghi et al.[9] and Magalhães et al.[23] It has been speculated that the lactate anion can contribute to its anti-erosive effects, since it forms stable complexes with calcium.[24] The association constant (Ko) for calcium lactate is approximately 10–20 $l\ mol^{-1}$, which is not as strong as for other compounds, such as citrate ($Ko = 6 \times 10^4\ l\ mol^{-1}$) or EDTA ($Ko = 5 \times 5^{10}\ l\ mol^{-1}$).[25] However, it appears to be just strong enough to protect the calcium ions from binding to other more stable complexing compounds present in the juice and thus allowing them to be available to interact with the tooth surface. In addition, calcium lactate pentahydrate is food-approved and extensively used as an acidity regulator, emulsifier, firming, stabilizing and thickening agent in a variety of processed foods and is known to be tasteless and nontoxic.[26] Thus, calcium lactate pentahydrate stands out as an interesting food additive option.

The phosphate polymers used were chosen since they are commonly used by the food-industry as meat preservatives or additives in non-alcoholic flavoured drinks.[15] In the current study, they also showed some erosion protective effect. In the pH-stat, sodium polyphosphate was the additive that most caused reduction (84%) on the erosive potential of the orange juice. Sodium tripolyphosphate and sodium pyrophosphate tetrabasic presented some reduction as well, but at much lower percentage (40%). Almost all the combinations that contained either calcium lactate pentahydrate or sodium polyphosphate were effective. These results are in agreement with the results achieved by Barbour et al.,[15] using a different pH-stat approach, which found 64% of reduction when supplementing a citric acid solution with sodium polyphosphate, 35% with sodium pyrophosphate and 46% with sodium tripolyphosphate.

In the demineralization and in the cycling models, the addition of sodium polyphosphate to the orange juice led to final SMH values similar to the negative control. However, this compound was able to protect against erosion, since it avoided detectable surface loss, contrasting with the sodium tripolyphosphate, sodium pyrophosphate tetrabasic and xanthan gum groups. The sodium polyphosphate group only started to present a detectable SL in the experimental time of 150 min of the demineralization model, and this loss was more than four times lower than the negative control. Nevertheless, best results were achieved with the combination of calcium lactate pentahydrate and sodium polyphosphate, which confirms an additive effect between these two substances, as was previously suggested by Hooper et al.[20]

It was not clear why sodium pyrophosphate tetrabasic and tripolyphosphate reduced the erosive potential of the juice in the pH-stat test, but not in phases 2 and 3. It can be speculated that, in the pH-stat, the substrate used was hydroxyapatite in crystal form, which has more available surface to react with the phosphate polymers than the tooth surface and hydroxyapatite is also in a purer form. Other factor that might be taken into consideration is the length of the reaction. To better simulate the oral conditions during the ingestion of an acid drink, we set the time of the pH-stat reaction to 5 min.[27] However, in phases 2 and 3, the total contact time for the juices was much higher; therefore, we may suggest that sodium pyrophosphate tetrabasic and tripolyphosphate might not have a prolonged action such as sodium polyphosphate. Barbour et al.[15] hypothesized that the better performance of the sodium polyphosphate compared to the other phosphate polymers tested could be related to its longer chain length.

The additive xanthan gum was not able to reduce the erosive potential of the orange juice in any of the models tested. This is in contrast with the results found by Barbour et al.[15] where xanthan gum could protect hydroxyapatite from demineralization by approximately 30%. Probably, the protective effect of the gum is minimum and its role as an additive of acid drinks might be more related to the improvement in the acceptability of the calcium-modified drinks than as an anti-erosive agent.

In the phase 3 study of remineralization inhibition, it could be observed that none of the additives tested were able to interfere with the remineralization of the previously created surface softened enamel lesions, rejecting the hypothesis that was raised in previous investigations.[16,28]

For dentine, the only additive that presented some anti-erosive effect was the combination of calcium lactate pentahydrate and sodium polyphosphate. That might be explained by the different composition and morphology of this substrate in comparison to enamel, which may have interfered with its interaction with the phosphate polymers. According to some investigations,[15,16] the phosphate polymers have affinity to the hydroxyapatite surface and once they adsorb to that surface they reduce the demineralization process. However, since dentine has less mineral content than enamel,[24] it might be hypothesized that the adsorption of the phosphate polymers to dentine occurred to a lesser extent and some protective effect could only be observed when sodium polyphosphate was combined with calcium, corroborating the idea of their additive effect. This, however, deserves further investigation.

In phase 3 of the study, in order to further investigate the mechanism of action of the additives, the cycling model was broke down in two independent models: demineralization and remineralization. Since, this was a complementary test, it used bovine enamel specimens instead of human, due to the greater availability of bovine teeth, smaller variation[29] and also, to its relative similarity to human enamel, as showed in a previous erosion investigation.[30]

This study assessed dental erosion by optical profilometry and surface microhardness. According to Barbour and Rees,[31] hardness measurements are a simple and suitable method to observe the early stages of dental erosion, whilst profilometry is more adequate to measure more advances stages. The decision to use both methods were related to the use of substrates with different susceptibility to erosion (enamel and dentine) and to a difference in the erosive potential of the juices tested.

This in vitro study has been followed up by a more clinically relevant in situ investigation testing the same experimental groups (unpublished data). In such conditions, we did not observe similar significant protection for sodium polyphosphate against enamel erosion development although the

erosion protection by calcium could be reproduced. This can be possibly explained by the limitations of the in vitro conditions adopted in the present study that did not consider the salivary protective factors. It is known that some salivary proteins have also affinity to the hydroxyapatite surface[32] and could compete for binding sites with the food polymers.[15] Although the in situ results may question the relevance of sodium polyphosphate as an additive for erosion protection, it was important for the present study to help clarify its mechanism of action. This may guide research for this additive towards new potential applications in the future. In that sense, the elimination of influences of salivary proteins on the anti-erosive effect of sodium polyphosphate, as done with the in vitro approach, showed to be very useful. Extrapolating the findings of this in vitro study to the clinical application should be done with caution.

5. Conclusions

Considering the in vitro nature of this study, it can be concluded that, for enamel, calcium lactate pentahydrate and sodium polyphosphate provided the best results regarding erosion reduction and their association seemed to enhance their individual effects. On root dentine, some protection against surface loss was achieved only by the association of calcium lactate pentahydrate and sodium polyphosphate. None of formulations tested achieved the degree of erosion protection found with the commercially prepared orange juice with added calcium.

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