Micro Energy-Dispersive X-Ray Fluorescence Mapping of Enamel and Dental Materials after Chemical Erosion

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Abstract: Energy-dispersive X-ray fluorescence was employed to test the hypothesis that beverage consumption or mouthwash utilization will change the chemical properties of dental materials and enamel mineral content. Bovine enamel samples (n = 45) each received two cavity preparations (n = 90), each pair filled with one of three dental materials (R: nanofilled composite resin; GIC: glass-ionomer cement; RMGIC: resin-modified GIC). Furthermore, they were treated with three different solutions (S: saliva; E: erosion/Pepsi Twist®; or EM: erosion + mouthwash/Colgate Plax®). It was found that mineral loss in enamel was greater in GICE samples than in REM > RMGICE > RMGICEM > REM > GICEM. An increased percentage of Zr was found in REM indicating organic matrix degradation. Dental materials tested (R, GIC, and RMGIC) were not able to protect adjacent enamel from acid erosion by the soft drink tested. The use of mouthwash promoted protection of enamel after erosion by the soft drink. To avoid chemical dissolution by mouthwashes, protection by resin composites with surface sealants is recommended.

Key words: dental materials, teeth, mouthwashes, carbonated beverages, X-ray spectrometry

INTRODUCTION

Dental erosion is defined as loss of tooth substance by chemical processes, not involving bacteria, caused by a variety of extrinsic and intrinsic factors (Heurich et al., 2010). It is a well-recognized dental problem that has increased among the younger population in the last few decades (Francisconi et al., 2008).

An additional concern is the global increase in consumption of erosive beverages like soft drinks, fruit juices, and sport drinks (Hooper et al., 2007). Soft drinks frequently contain citric or phosphoric acids and are considered extrinsic factors that cause dental erosion (De Carvalho Filho et al., 2011). Thus, teeth are daily exposed to acid environments, and understanding dental erosion can help the clinical treatment of tooth wear and assist in development of new dental restorative materials (Zheng et al., 2009).

Although restorative materials are less susceptible to erosion compared to enamel, the erosive attack can induce, at least to some extent, degradation of the matrix and fillers of restorative materials (Francisconi et al., 2008) with morphological changes and increased surface roughness (Soares et al., 2012).

Fluoride products are capable of impeding enamel erosion by beverages (De Carvalho Filho et al., 2011). The regular use of mouthwashes has become quite widespread among dental clinic patients. Although these solutions may be effective for controlling and reducing plaque and gingivitis (Gurdal et al., 2002), their influence on esthetic restorative materials is questionable (Colucci et al., 2009).

Additionally, mouthwashes may contain a high percentage of alcohol and other ingredients such as detergents, emulsifiers, and organic acids (Settembrini et al., 1995). These components can cause degradation and softening of dental materials (Mante et al., 1993).

Previous studies using scanning electron microscopy (SEM) reported that traditional glass-ionomer cement (GIC) materials are vulnerable to severe damage in patients experiencing strong citric or gastric acid induced erosion (Wan Bakar & McIntyre, 2008). Francisconi et al. (2008) showed through microhardness that amalgam, GIC, resin-modified glass-ionomer, and resin composite restorative materials tested were not able to protect adjacent enamel from erosion by cola drinks. Despite information provided in previous studies, there is still a lack of knowledge about the influence of beverage consumption and hygiene habits in the properties of restorative materials, such as fluoride release, on tooth structure adjacent to these restorations.

Thus, this in vitro study intended to evaluate, by micro-energy-dispersive X-ray fluorescence (µ-EDXRF), the effects of an erosive challenge on three restorative materials (R: nanofilled composite resin; GIC: glass-ionomer cement; RMGIC: resin-modified GIC) and surrounding enamel filled with these materials, as well as their ability to protect the
Preparation of the Specimens

This study was approved by the Ethics Committee of the Universidade do Vale do Paraíba (CEP n°A43/CEP/2008). Forty-five anterior bovine teeth were selected; (B) roots were removed; (C) polish of enamel surface; (D) two cavity preparations were executed per enamel slab; (E) 90 cavity preparations were randomly allocated to be filled with one of the three dental materials: R, nanofilled composite resin; GIC, glass-ionomer cement; and RMGIC, resin-modified glass-ionomer cement; (F) cavities were filled; (G) restorations were polished and surface protection executed; (H) 45 enamel slabs were sectioned resulting in 90 small enamel slabs; (I) the 90 enamel slabs were randomly distributed into nine groups with three types of dental materials and three different storage treatments: S, artificial saliva; E, erosion/Pepsi Twist®; EM, erosion Pepsi Twist® + remineralization in mouth rinse/Colgate Plax®.

Materials and Methods

Preparation of the Specimens

This study was approved by the Ethics Committee of the Universidade do Vale do Paraíba (CEP n°A43/CEP/2008). Forty-five anterior bovine teeth were obtained from bovine jaws (Fig. 1A). After extraction, the remaining soft tissue was removed from the tooth surface with a dental scaler (7/8; Duflex®, Rio de Janeiro, RJ, Brazil). The teeth were polished with a paste of pumice (S. S. White®, Rio de Janeiro, RJ, Brazil) and water, using a Robinson brush (Viking, KG Sorensen®, Barueri, SP, Brazil) in a low-speed hand-piece (KaVo® do Brasil SA, Joinville, SC, Brazil). Then they were cleaned and stored in a 0.1% aqueous thymol solution (Soares et al., 2009).

After the cleaning procedure, the roots of the bovine incisors were sectioned from crowns through the cemento-enamel junction buccolingually, using a low speed water-cooled diamond saw (Isomet 1000®, Buehler, Lake Bluff, IL, USA) (Fig. 1B). The enamel square blocks obtained from the crowns (4 mm long, 4 mm wide, 3 mm thick) were ground and polished with wet 600- and 1200-grit silicon carbide paper (Arotec®, Cotia, SP, Brazil) at 150 rpm (Fig. 1C) and sonicated for 10 min in deionized water.

Two standardized circle-shaped cavities (2.0 mm in width and 2.0 mm in depth) were prepared for each enamel slab, maintaining a 3 mm distance between them, which resulted in 90 prepared cavities (Fig. 1D). A spherical diamond bur (#1013, KG Sorensen®, Barueri, SP, Brazil) in a high-speed handpiece under watercooling (ExtraTorque 605C KaVo® do Brasil SA, Joinville, SC, Brazil) was used for these preparations. The cavity walls were standardized (length and depth) using the entire active point of the bur to ensure the same dimensions in all enamel blocks. The burs were replaced after every ten preparations. The 90 cavity preparations were randomly allocated to be filled with one of the three different dental materials (Fig. 1E).

Restorative and Polishing Procedures

The Z350® composite resin, Ketac Molar® glass-ionomer cement, and Vitremer® resin-modified glass-ionomer materials (Table 1) were handled as outlined by the manufacturers. After placement of the material in the prepared cavity (Fig. 1F), the surface of the restorative materials was covered with a polyester strip, and a glass slab under pressure was used to expel excess material from cavity. On the light cured materials, the polymerization procedure was carried out through the polyester strip for the manufacturers’ recommended exposure time, using a light-curing device (LEDition®, Ivoclar Vivadent, Australia). The specimens were restored with composite resin were individually stored in 1,000 μL of artificial saliva at 37 ± 1°C for 24 h.

Remnants of restorative materials were removed from the surface of the specimens by polishing (Fig. 1G) with wet 600- and 1200-grit silicon carbide paper (Arotec®, Cotia, SP, Brazil) at 150 rpm. After polishing, to avoid syneresis and imbition processes, GIC specimens were protected with a layer of Single Bond® Adhesive (3M ESPE) and resin-modified glass ionomer (RMGI) were protected with finishing gloss. Then specimens were stored in 1,000 μL of artificial saliva at 37 ± 1°C for 24 h. After that, the enamel slabs with restorations were sectioned at the middle (Fig. 1H) (resulting in two samples per slab) and randomly distributed into nine groups (n = 90) (Table 2, Fig. 1I).

Exposure to Soft Drink and Mouthwash

The specimens were submitted to demineralization–remineralization regimes and consecutively cycled through this regimen six times (De Carvalho Filho et al., 2011).

The cycling treatments were performed one immediately after the other as follows (S: saliva; E: erosion/Pepsi Twist®; or EM: erosion + mouthwash/Colgate Plax®):

Groups RS, GICS, and RMGICS (controls): storage in artificial saliva (37°C, 6 h) in a culture kiln (ORION 502, PANEM®, São Paulo, SP, Brazil) (samples were washed with distilled water, 30 s and saliva was changed every 1 h).

Groups RE, GICE, and RMGICE: demineralization for 10 min in 1,000 μL of Pepsi Twist® (AMBEV, Jundiaí, São Paulo, Brazil) under agitation (Model 752A, FISATOM®, Fisatm Equipamentos Científicos, São Paulo, SP, Brazil), washing with distilled water (30 s), and remineralization in artificial saliva (1 h). This cycle was repeated five times (7 h in total).
Table 1. Chemical Composition of Materials and Solutions Tested in This Study.

<table>
<thead>
<tr>
<th>Materials and Manufacturer</th>
<th>Composition</th>
</tr>
</thead>
</table>
| Filtek Z350® (3M ESPE, St Paul, MN, USA) | - Filler: 59.5 vol% combination of aggregated zirconia/silica cluster ranging from 0.6 to 1.4 μm with primary particles size of 5–20 nm and nonagglomerated 20 nm silica filler  
- Polymeric matrix: Bis-GMA, Bis-EMA, UDMA, and TEGDMA |
| Vitremer® Kit (3M ESPE) | - Primer: HEMA, ethyl alcohol, Vitrebond copolymer  
- Powder: fluoro-aluminoisilicate glass, potassium persulfate, ascorbic acid  
- Liquid: polyacrylic acid copolymer, HEMA, water, carboxylic acid copolymer |
| Ketac® Molar Easymix (3M ESPE/AG) | - Powder: Al-Ca-La fluorosilicate glass, 5% copolymer acid (acrylic and maleic acid), and pigments  
- Liquid: acrylate, maleic acid copolymer, tartaric acid, benzoic acid, water |
| Artificial saliva (Nostra Fórmula Pharmaceutics, São José dos Campos, SP, Brazil) | - 1.5 mmol/L Ca(NO₃)₂; H₂O; 0.9 mmol/L Na₃HPO₄ 2H₂O; 150 mmol/L KCl; 0.1 mol/L H₂NC(CH₂OH)₆ (TRIS); 0.05 mg/mL NaF; pH 7.0 |
| Pepsi Twist® (PepsiCo., Inc., São Paulo, Brazil) | - Carbonated water, sugar, extract of cola nuts, concentrated lemon juice, caffeine, caramel color, sodium benzoate, sodium citrate, phosphoric and citric acid; 3.9 pH |
| Colgate Plax® (Colgate-Palmolive Co., São Bernardo do Campo, SP, Brazil) | - Triclosan 0.03%, sodium fluoride 0.025%, 7.1 pH, alcohol content: 8.0 (% v/v), aqua, sorbitol, glycerin, sodium lauril sulfate, sodium methyl cocoyl taurate, polyvinylmethyl ether (PVM) and maleic acid (MA) copolymer, aroma, disodium phosphate, sodium hydroxide, triclosan, sodium saccarin (Japan). |

Groups REM, GICEM, and RMGICEM: demineralization for 10 min in 1,000 μL of Pepsi Twist® under agitation, washing with distilled water (30 s), remineralization for 10 min in 1,000 μL of Colgate Plax® (Colgate-Palmolive Company, São Bernardo do Campo, SP, Brazil) under agitation, washing with distilled water (30 s), and remineralization in artificial saliva (1 h). This cycle was repeated five times (8 h in total). The pH of the artificial saliva, beverage, and mouthwash were measured with a pH meter (pH100, PHTEK®, Hexaysystems, São Paulo SP, Brazil). Composition of dental materials and solutions tested are shown on Table 1.

Table 2. Description of Group with Dental Materials Tested and Treatments Performed.

<table>
<thead>
<tr>
<th>Materials and Manufacturer</th>
<th>Treatments</th>
<th>Group*</th>
</tr>
</thead>
<tbody>
<tr>
<td>R—Z350® (3M ESPE)</td>
<td>Artificial saliva (control)</td>
<td>RS</td>
</tr>
<tr>
<td>Pepsi Twist®</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pepsi Twist® + Colgate Plax®</td>
<td>RE</td>
<td></td>
</tr>
<tr>
<td>GIC—Ketac Molar® (3M ESPE)</td>
<td>Artifical saliva (control)</td>
<td>GICS</td>
</tr>
<tr>
<td>Pepsi Twist®</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pepsi Twist® + Colgate Plax®</td>
<td>RMGICEM</td>
<td></td>
</tr>
<tr>
<td>RMGIC—Vitremer® (3M ESPE)</td>
<td>Artificial saliva (control)</td>
<td>RMGICS</td>
</tr>
<tr>
<td>Pepsi Twist®</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pepsi Twist® + Colgate Plax®</td>
<td>RMGICEM</td>
<td></td>
</tr>
</tbody>
</table>

*S: saliva; E: erosion; M: mouthwash; R: nanofilled composite resin; GIC: glass ionomer cement; RMGIC: resin modified glass ionomer cement.

The composition of the materials was determined using an energy-dispersive micro-X-ray fluorescence spectrometer, model μ-EDX 1300 (Shimadzu®, Kyoto, Japan). Equipment calibration and chemical balance were performed as previously reported (Soares et al., 2009).

A total of 180 line-scanning measurements were performed (two per sample). The maps were scanned with a line of 1 × 30 μm, steps of 20 μm and a count rate of 50 s per point, with different energy parameters (enamel: 15 kV; restorative materials: 40 kV). The first line-scanning was performed at the center of the restoration and the second starting from the margins of restorations.

The Ca and P quantity lost after the treatments from each specimen considering each restorative material was estimated from the difference in mineral group content (MGC) and mineral artificial saliva content (MSC) for each group because their values were different. This was expressed as the percentage difference in mineral variation (MV) between the data collected after treatments as follows:

\[
MV(\%) = \frac{[(MGC - MSC)/MSC] \times 100.}
\]

Data mapping and Statistical Analysis

The mapping data of each element were processed by the equipment software (Shimadzu® microEDX MP ver. 1.03, Shimadzu Corp., Kyoto, Japan) after EDXRF mappings. The software provided average values for each component (enamel: Ca and P; nanofilled composite resin: Si and Zr; resin-modified glass-ionomer: Sr; glass-ionomer cement: Si, La). These elements were considered because they were the main content in the materials. Fluoride was not quantified in this study because its atomic weight is below the detection limit of the equipment.

Statistical analysis of the EDXRF results was performed with Kruskal-Wallis and Dunn’s multiple comparisons tests using the Instat® software (GraphPad Software Inc., San Diego, CA, USA).
The R and GIC groups showed that erosion (E) resulted in significant reduction of Ca and P on enamel when compared to the S and EM treatments. The S and EM treatments were not different relative to the enamel mineral content (p > 0.05) (Table 3). For the RMGIC groups, no significant mineral reduction of enamel was detected among the treatments performed (p > 0.05). The Ca/P ratio was not significantly affected in all of the experimental groups of R, GIC, and RMGIC materials (p > 0.05).

In the comparisons among dental materials considering the storage in saliva (S), significant less mineral content on enamel was found in the RMGICS group when compared to the RS and GICS groups (R: Ca and P, p < 0.001; GIC: Ca, p < 0.05). Among dental materials after erosion (E), significantly less mineral content was found near enamel adjacent to the GIC restoration when compared to RE (Ca and P, p < 0.01). No statistical difference in enamel mineral content was found between RE and RMGICES (p > 0.05). After erosion/mouthwash (EM) treatments, less mineral content was found on enamel near RMGICEM when compared to REM (Ca, p < 0.01 and P, p < 0.001) and to GICEM (Ca and P, p < 0.01).

The results of MV calculation (Fig. 2) indicated that REM specimens had lower mineral reduction (~1% of Ca and ~4% of P) than that in RE (~9% of Ca and ~8% of P).

**RESULTS**

For components of the restorative materials, our results are in agreement with previous studies, which demonstrated that Si is the main inorganic component in composite resins. Dos Santos et al. (2010) using SEM-EDX analysis GICEM showed an extremely evident reduction of Ca (~26%) and P (~27%) in enamel. However, in enamel of GICEM specimens, there was no Ca loss and an increase in P content (~1%). The specimens of RMGIC and RMGIEM had similar mineral reduction (RMGICEM: 5% of Ca and 7% of P; RMGIEM: 5% of Ca and P).

Regarding dental materials elements, significant increase in Zr content was found for REM when compared to RS and RE (RS × REM, p < 0.01 and RE × REM, p < 0.001) (Table 4).

**DISCUSSION**

Table 3. Mean and Standard Deviation (n = 10) of Calcium (Ca) and Phosphorus (P) Weight Percentages (wt%) and Ca/P Molar Ratios Obtained by X-Ray Fluorescence, after Sample pH Cycling.*

<table>
<thead>
<tr>
<th>Groups**</th>
<th>Ca</th>
<th>P</th>
<th>Ca/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS</td>
<td>37.23(1.72)Aa</td>
<td>19.29(0.57)Aa</td>
<td>1.51(0.06)</td>
</tr>
<tr>
<td>RE</td>
<td>33.97(1.99)Aa</td>
<td>17.69(0.75)Ba</td>
<td>1.50(0.00)</td>
</tr>
<tr>
<td>REM</td>
<td>36.94(3.70)Ab</td>
<td>18.64(1.22)Aa</td>
<td>1.53(0.07)</td>
</tr>
<tr>
<td>GICS</td>
<td>35.57(3.06)Aa</td>
<td>18.16(1.52)Aab</td>
<td>1.53(0.07)</td>
</tr>
<tr>
<td>GICE</td>
<td>27.12(6.08)Bb</td>
<td>13.61(3.84)Bb</td>
<td>1.61(0.26)</td>
</tr>
<tr>
<td>GICEM</td>
<td>35.49(1.20)Aa</td>
<td>18.24(0.40)Aa</td>
<td>1.51(0.03)</td>
</tr>
<tr>
<td>RMGICS</td>
<td>32.99(1.91)Ab</td>
<td>17.14(0.98)Ab</td>
<td>1.51(0.06)</td>
</tr>
<tr>
<td>RMGICE</td>
<td>31.42(4.06)Ab</td>
<td>15.90(2.35)Ab</td>
<td>1.56(0.12)</td>
</tr>
<tr>
<td>RMGICEM</td>
<td>31.18(2.68)Ab</td>
<td>16.30(1.34)Ab</td>
<td>1.50(0.08)</td>
</tr>
</tbody>
</table>

*Statistical comparisons were performed by the nonparametric Kruskal-Wallis test and by the Dunn multiple comparisons test considering: (1) the same dental material and comparing different treatments (different superscript capital letters show significance); (2) the same treatment between different dental materials (different superscript small letters show significance). Statistical comparisons for Ca/P molar ratio were not significant.

**S: saliva; E: erosion; M: mouthwash; R: nanofilled composite resin; GIC: glass ionomer cement; RMGIC: resin modified glass ionomer cement.

**Table 4. Mean and Standard Deviations (n = 10) of the Percentages of the Restorative Materials Elements (Si, Zr, Sr, La) Obtained by X-Ray Fluorescence after Sample pH Cycling.*

<table>
<thead>
<tr>
<th>Groups**</th>
<th>Si</th>
<th>Zr</th>
<th>Sr</th>
<th>La</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS</td>
<td>24.87(1.07)A</td>
<td>8.17(0.46)A</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>RE</td>
<td>23.63(0.90)B</td>
<td>8.13(0.57)A</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>REM</td>
<td>25.10(1.76)B</td>
<td>9.76(0.97)B</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>GICS</td>
<td>10.82(1.35)B</td>
<td>—</td>
<td>—</td>
<td>2.33(0.85)</td>
</tr>
<tr>
<td>GICE</td>
<td>9.78(1.43)B</td>
<td>—</td>
<td>—</td>
<td>2.98(1.30)</td>
</tr>
<tr>
<td>GICEM</td>
<td>10.86(0.58)B</td>
<td>—</td>
<td>—</td>
<td>2.97(1.11)</td>
</tr>
<tr>
<td>RMGICS</td>
<td>—</td>
<td>3.38(0.22)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>RMGICE</td>
<td>—</td>
<td>3.37(0.24)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>RMGICEM</td>
<td>—</td>
<td>3.32(0.30)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Comparisons were performed by Kruskal-Wallis and Dunn’s multiple comparisons test. Different small letters in the same column indicate significant differences.

**S: saliva; E: erosion; M: mouthwash; R: nanofilled composite resin; GIC: glass ionomer cement; RMGIC: resin modified glass ionomer cement.
found resin matrix degradation and loss of load particles after immersion of resin Supreme XT® (3M ESPE) in artificial saliva, Coke®, tea and coffee, and Coke® affected the material’s surface more than the other tested drinks.

The significant increase in Zr wt% of Z350 resin after EM treatment when compared to the other treatments (RS and RE) (Table 4) may be related to the type of particles and the resin composition associated with the influence of pH and components of the soft drink and mouthwash. Colgate Plax® mouthwash contains fluoride and Pepsi Twist® contains phosphoric acid, and those components probably influenced the resin matrix degradation. Increase in the weight percentage of Zr after EM treatment could be explained by the exposure of lower layers of resin, indicating a probable degradation of the organic matrix. This occurred because the fluoride ions are capable of causing depolymerization reactions at the filler-resin matrix interface and hydrolysis of the organosilicon ester group (Yeh et al., 2011).

Alterations of composite resins soaked in organic acids are mainly due to the softening of bis phenol-A-glycidyl methacrylate-based polymers (Honório et al., 2008). The observed changes in filler content could be attributed to the sorption and solubility after soft drink and mouthwash treatments, which may serve as precursors to a variety of chemical and physical processes that not only create biological concerns but also produce deleterious effects on structure and function of the resin matrix (Sideridou et al., 2008). These effects include swelling, plasticization and softening, oxidation, and hydrolysis that may lead to a permanent alteration of resin matrix properties (Dos Santos et al., 2010).

For the GIC, our results are in agreement with previous reports where the main inorganic filler of GIC is Si (Yap et al., 2003). The ability of glass-ionomer cements to resist dissolution was found to vary with the composition of the medium and not to rely simply on its pH (McKenzie et al., 2003). McKenzie et al. (2004) found that GICs dissolved after 6 months of storage in fruit juices of pH 2.5–3.4. However, GICs conditioned in Coca-Cola®, also of pH 2.4, had comparable strength to specimens conditioned in neutral media after 6 months (McKenzie et al., 2003). The phosphate ions in Coca-Cola® may suppress dissolution of GICs at low pH. It has been reported that some components, for example calcium and phosphate in the oral environment, may increase surface hardness of a highly viscous GIC (Wang & Yap, 2010). Thus, in the present study, the ion exchange among enamel, GIC restorations, artificial saliva, Pepsi Twist®, and Colgate Plax® probably suppressed the material’s dissolution.

The low weight percentage of Ca and P found in enamel near the resin composite and in the GIC restorations for the E group (Table 3) indicates that mouthwash (group GICEM) provides protection against erosion after soft drink treatment. In this case the formation of fluoride products (CaF₂) in the enamel could act in the inhibition of demineralization and enhancement of remineralization by the use of mouthwashes against erosive processes (Moi et al., 2008).

Meyer-Lueckel and Tschoppe (2010) also found positive effects against demineralization with the use of fluoride containing mouthwash combined with artificial saliva substitutes. Within this context, applications of high concentrations of fluoride can increase the abrasion resistance and decrease the development of erosions in enamel and dentin (Amaechi & Higham, 2005).

Considering the GIC restorative material, the lower weight percentage of Ca and P found in enamel samples of GICE when compared to the other groups (GICS and GICEM) (p < 0.05) indicates that, due to the low concentration of fluoride content, GIC was not sufficient to prevent demineralization caused by soft drinks possibly due to high aggressiveness in the erosion process (Rios et al., 2008).

No significant change was observed in the enamel mineral content among the RMGIC groups. This effect may be due to the hybrid composition of the material and application of protective surface gloss to the final stage of restoration, according to the manufacturer’s protocol.

Mineral variation results (Fig. 2) showed that the groups which received the mouthwash application after soft drink treatment had less mineral decrease when compared to the groups without this protection. This data is more evident between GICE and GICEM where a positive mineral variation can be noted in the specimens of GICEM compared to negative variation found in GICE (~26% of Ca and ~27% of P). This indicates that the GIC restoration in GICEM samples was probably recharged with fluoride after mouthwash application and this fluoride was available for remineralization. Our results showed no significant chemical changes in enamel around the samples filled with RMGI.

In summary, the mouthwash treatment after the soft drink utilization resulted in protection of enamel as showed by the mineral concentration. However, the application of mouthwash resulted in significant changes in the composition of the resin composite material. On this basis, a remineralizing mouthwash should be administered immediately after an acidic challenge to teeth, especially in conditions of frequent acid attack seen in patients with eating disorders or with misuse of acidic beverages. Another possible recommendation is the application of surface protection materials in the surface of composite resin material to avoid degradation by the mouth rinse. In addition, a professional should periodically monitor the glass-ionomer and resin restorations, as they degrade over time as shown in our study.

**Conclusions**

Nanofilled composite resin, glass-ionomer cement, and resin-modified glass-ionomer cement restorations were not able to protect adjacent enamel from acid erosion of soft drink. We accept partially the initial hypothesis that the beverage consumption and mouthwash utilization would change chemical properties of dental materials because chemical changes occurred in the resin composite. Also enamel mineral content was reduced after erosion by soft drinks. The use of mouthwash containing fluoride promoted evident
protection of enamel after the erosion process by soft drinks. Micro energy-dispersive X-ray fluorescence analysis provided compositional insight into the alterations produced by demineralization-remineralization, largely not accessible by other current techniques, and highly relevant for understanding dental erosion.

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