Introduction

Dental erosion is a pathologic, non-bacterial hard dental tissue loss induced by extrinsic or intrinsic acids. This in vitro study aimed to evaluate and compare the morphology and surface roughness of dental enamel after erosive challenge in some extrinsic and intrinsic acidic substances, Coca-Cola, orange and gastric juice.

Enamel samples (n = 48), obtained by preparation of surgical extracted human third molars, were subjected to the erosive challenge of the artificial gastric juice and commercially available Coca-Cola and orange juice by immersion in 50 ml of erosive solutions for 15 min, three times daily, for 10 days. Between immersions, the samples were kept in filtered saliva. Twenty-four samples were prepared for the surface morphology analysis using scanning-electron microscope, and 24 for the analysis of Ra-surface roughness parameter (using a diamond-stylus-profilometer), including the 12 control samples (which did not undergo the erosion procedure). Results of the surface roughness were analyzed by one-way ANOVA Student-Newman-Keuls post hoc test.

Ultrastructural analysis of enamel surface after immersion in Coca-Cola and gastric juice showed type 1 etching pattern with the typical honeycomb appearance. After the erosive challenge with orange juice, a nonspecific morphological model was established. Profilometric parameter Ra was significantly increased for samples immersed in gastric juice compared to samples immersed in Coca-Cola and orange juice, as well as, in samples with Coca-Cola-erosion compared with orange juice-erosion. Gastric juice had higher erosive potential in relation to Coca-Cola and orange juice, with the most intense morphological changes and the highest roughness on the enamel surface.

Key words: Enamel erosion, soft drinks, gastric juice, SEM, surface roughness

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that fruit juices may also be potentially erosive, due to their high content of titratable acid (2, 4, 12).

On the other hand, acidic stomach contents refluxed into the oral cavity can dissolve tooth structures and cause erosive tooth wear (14, 15) because contact between the hydrochloric acid from the stomach (with pH from 1.5 to 3.5) and the oral cavity occurs for a few seconds, several times a day (16).

The aims of the present in vitro study were twofold: (1) to analyze the experimental models of enamel erosion after exposure to Coca-Cola, orange juice and artificial gastric juice at the ultrastructural level, and (2) to evaluate enamel surface roughness after erosive challenge in the same acidic solutions.

**Material and methods**

The material for this research included 12 human impacted mandibular third molars (from patients aged 18-25 years) disinfected in 1% thymol solution and kept in 1% sodium hypochlorite for 24 h. Organic debris was removed by carefully using a dentist’s set of instruments (17).

After the removal of the roots, at least 2 mm below cementoenamel junction, the crowns were cut (using a diamond saw under water irrigation) from the distal, mesial, buccal, and lingual side. Out of the total of 48 samples, 24 were used for SEM analysis and 24 were used for the analysis of enamel surface roughness (Table 1).

**Table 1. Distribution of samples used in experimental protocols.**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Number of samples for SEM analysis</th>
<th>Number of samples for surface roughness analysis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>6 (3 measurements)</td>
<td>12</td>
</tr>
<tr>
<td>Immersed in Coca-Cola</td>
<td>6</td>
<td>6 (3 measurements)</td>
<td>12</td>
</tr>
<tr>
<td>Immersed in orange juice</td>
<td>6</td>
<td>6 (3 measurements)</td>
<td>12</td>
</tr>
<tr>
<td>Immersed in artificial gastric juice</td>
<td>6</td>
<td>6 (3 measurements)</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>24</td>
<td>48</td>
</tr>
</tbody>
</table>

**Erosion solutions and human saliva**

The erosion models caused by soft drinks were obtained by immersing the samples in Coca-Cola (HBC - Serbia A.D. Zemun) and orange juice (NECTAR’ D.O.O. Backa Palanka, Serbia).

In the previous study it was established that Coca-Cola had pH 2.67 ± 0.06 and TA 1.87 ± 0.09, whereas orange juice had pH 3.73 ± 0.03, requiring 5.70 ml of NaOH to reach pH 7.0 (4).

The model of enamel erosion with GERD was created using artificial gastric juice according to the methodology of Stefaniak et al. (18) and it was modified in accordance with the established goals of the research. Its initial pH was 2.1 (Table 2.).

**Table 2. The contents of artificial gastric juice (primary electrolytes and ionic compounds)**

<table>
<thead>
<tr>
<th>Contents</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium chloride dihydrate (CaCl₂×2H₂O)</td>
<td>0.264 g/L</td>
</tr>
<tr>
<td>Magnesium chloride hexahydrate hexahydrat (MgCl₂×6H₂O)</td>
<td>0.152 g/L</td>
</tr>
<tr>
<td>Potassium chloride (KCl)</td>
<td>0.864 g/L</td>
</tr>
<tr>
<td>Sodium chloride (NaCl)</td>
<td>2.855 g/L</td>
</tr>
<tr>
<td>Hydrochloric acid (HCl)</td>
<td>1.426 (3.38 ml; 36.2%)</td>
</tr>
</tbody>
</table>

Human saliva was collected from healthy volunteers in the morning, 2 hours after fasting. Volunteers rinsed their mouths twice with distilled water before saliva collection (19). Filtrates were obtained with Whatman filter papers grade 1: 11 μm (Sigma-Aldrich, USA).
Erosive Challenge

This study was approved by the institution’s Ethics Committee. Tooth samples planned for analysis using SEM, immediately after cutting, rinsing and drying, were distributed into one of three erosive challenges, while the samples planned for analysis using profilometer, before exposure to acidic solutions, were prepared as follows: circular molds of 16 mm in diameter and 3 mm deep were filled by self-cured resin. Each sample was embedded in resin, with labial (oral) surfaces uppermost, and was cleaned with nonfluoridated pumice, rinsed with water and dried with oil-free compressed air.

All of the enamel samples were exposed to acidic solution according to the following protocol (10): immersion in 50 ml acidic solution (Coca-Cola/orange juice/gastric juice) for 15 minutes with occasional shaking, rinsing with distilled water, and immersion in human saliva. The cycle was repeated three times a day for 10 days. During the night, the samples were placed in human saliva, including the 12 control samples (which did not undergo the demineralization procedure). The experiment was conducted at room temperature.

Preparation of samples for SEM analysis

At the end of the experimental period the samples were dried, fixed to aluminum stubs with a fixing agent (Dotite paint xc 12 Carbon JEOL, Tokyo, Japan), sputter-coated with old/palladium (in the unit JFC 1100E Ion Sputter JEOL), and examined by scanning electron microscopy (SEM) (JEOL-JSM-5300).

Preparation of samples for surface roughness analysis

Surface roughness of the enamel samples was measured using a profilometer (Mitutoyo SurfTest SJ-301) (20).

Although four parameters of roughness are registered with the stylus of the Mitutoyo type profilometer, the statistical analysis took into account only one, the most frequently used parameter, Ra, which is defined as the average distance from the profile to the mean line over the length of assessment. A detailed description of the measurement method using a diamond stylus profilometer has already been published in our recent study (4). Statistical analysis was carried out using one-way ANOVA Student-Newman-Keuls post hoc test.

On enamel surfaces not exposed to the erosive challenge by Coca-Cola, orange and gastric juice (control group), the typical structures of sound enamel (grooves and perichimata lines) were apparent. Additionally, small depressions or ditches or grinding marks were observed and they were found to be indicative of the cumulative mechanical effects the teeth have experienced. (Figure 1)

![Control samples: the surface of untreated enamel with perikymata.](image)

Figure 1.

Results

SEM results are shown in micrographs 1 to 4 and the measurements of enamel surface roughness are shown in Table 3.

<table>
<thead>
<tr>
<th>Roughness parameter</th>
<th>Exposure (min)</th>
<th>Control</th>
<th>I Artificial gastric juice</th>
<th>II Coca-Cola</th>
<th>III Orange juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ra</td>
<td>15</td>
<td>0.67 ± 0.02</td>
<td>1.63 ± 0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.49 ± 0.08&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.27 ± 0.01&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>p < 0.05 vs control; <sup>b</sup>p < 0.05 vs. artificial gastric juice; <sup>c</sup>p < 0.05 vs. Coca-Cola;
The enamel surface of teeth exposed to the acidic solutions clearly demonstrated deep changes in enamel structure: scanning micrographs of enamel samples eroded by Coca-Cola and gastric juice exhibited a distinct pattern, showing hollowing of prism centers with relatively intact peripheral regions, reflecting honeycomb appearance (Figures 2, 4).

Figure 2. Erosive changes on enamel caused by Coca Cola: type 1 erosion, central parts of the prisms are affected and the peripheral parts are relatively preserved.

Figure 3. Erosive changes in enamel surface caused by orange juice with irregular erosion type.

Figure 4. Erosive changes in enamel surface caused by gastric juice with significant type 1 erosion, honeycomb appearance.

There was a statistically significant difference among all of the tested roughness parameter values.

Discussion

Literature data point to an increase in dental erosions in the modern society and they represent a challenge for the researchers. Considering that there is a decreasing tendency in caries instances, erosive tooth wear is becoming a more significant element in planning a long-term model of dental health (2). An increase in the prevalence of various gastrointestinal diseases and eating disorders leads to more frequent contacts between the teeth and gastric acid. Together with increasing use of acidic beverages, these conditions are considered significant risk factors for teeth demineralization.

The goal of our research was to create the ultrastructural experimental models of enamel erosion caused by Coca-Cola, orange juice and artificial gastric acid and to determine differences and possible similarities between the erosions caused by external and internal factors on experimental model. To some extent, the findings could point to the significance of prevention of both internal and external causes of erosive tooth wear.

In order to simulate clinical conditions, the present research used gastric juice formula which, apart from HCl with 2.1 pH, contained only the primary electrolytes and ionic compounds, without organic and amino acids, carbohydrates and pepsin. Calcium, magnesium and sodium salts should act as buffer components which could probably control the erosive potential of gastric juice, similar to in vivo conditions. On the other hand, the majority of the results from laboratory studies regarding the enamel surface changes in reflux disease were obtained based on the use of pure HCl (16, 21, 22, 23, 24). Nevertheless, in studies by Barlet at al. and Braga at al. (9, 10), gastric juice which was aspirated from patients undergoing endoscopy for symptoms of reflux disease was used.

In the present study, the immersion cycles (3 times for 15 minutes) could imitate GERD symptoms
for a shorter period of time (10 days). Similarly, the
time of immersion of samples into Coca-Cola and
orange juice can imitate the frequency of consump-
tion of soft drinks. On the samples immersed in the
Coca-Cola and gastric juice, the following was ob-
erved: diffuse demineralization involved the rod
core, with decomposition of morphology of prisms:
they were severely affected, and a greater prism-
core dissolution compared with that in the interpris-
matic areas gave the enamel a “honeycomb pattern”
of etching, similar to the results published by Colo-
ombo et al. (25) and Braga et al. (10). Also, Arnold et
al. (22) showed that exposure to pure HCl results in
four different enamel etching patterns, and the
depth of the surface layer was dependent upon the
etching time. Our research showed that the degree
of destruction of central prism parts varied depend-
on location, whereby the most prominent changes
were observed in the vicinity of cement-enamel
junctions. In the present study, samples immersed
in orange juice showed atypical etching, which is
referred to as type 4 in the literature. (26).

In the current study, surface roughness was
measured using a stylus profilometer that overhangs
across the surface of the object, registering all of the
unevenness at a certain measuring length (17, 27,
28, 29, 30). According to some literature data stylus
profilometry shows some disadvantages (the risk of
the diamond tip causing damage to the specimens,
inability to detect valleys which are narrower than
the stylus tip) but nevertheless this technique has a
high degree of precision (31). Moreover, the current
national standards on measuring surface texture are
defined using stylus profilometry (11, 31, 32).

In a number of studies (28, 29), a difference
in the surface roughness of the samples examined
on various erosive challenges was determined only
on the basis of the Ra parameter, where valid con-
clusions were drawn. The present study showed that
this parameter was statistically significantly different
among all the tested samples. Likewise, all samples
of the experimental groups were also different from
the control samples according to the SEM analysis
and after analysis of the Ra parameter.

According to the latest literature data, the Ra
parameter provides no information on the characte-
ristics of surface irregularities, whereby both maxi-
mal and minimal irregularities may show the same
Ra values (31, 34). Therefore, this research also in-
cluded an ultramicroscopic analysis in order to ob-
tain more precise results.

Braga et al. (10) showed that the enamel sur-
face after orange juice had a generalized surface
roughening with no apparent evidence of a prism
pattern, and the surface was not completely etched.
The same authors used atomic emission and FT
Raman spectroscopy to analyze the mineral content
of enamel after exposure to gastric and orange
juice, and they determined that gastric juice has a
higher erosive potential than orange juice. Our re-
search analyzed surface roughness of enamel using
stylus profilometry following the exposure to the
same acidic solutions, as well as to Coca-Cola. It has
been determined that gastric juice has a higher ero-
sive effect on the enamel, which is in accordance to
the results of the mentioned authors. Roughness
parameter showed that, after exposure to gastric
juice, the enamel surface had prominent uneven-
ness of the surface which was statistically significant
compared to the surface texture of samples exposed
to Coca-Cola and orange juice.

According to information from the manufactu-
Rers, Coca-Cola contains phosphoric acid, compared
to citric acid, phosphoric acid is stronger (33). The
effect of phosphoric acid results in a superficial etch-
ed zone which might be permanently lost from the
tooth surface (26). On the other hand, citric acid
may act as a chelator capable of binding the calcium
from enamel or dentine, thus increasing the degree
of undersaturation and favoring demineralization
(35, 36).

In our previous study the erosive potential of
various soft drinks was examined by measuring ini-
tial pH and titratable acidity and enamel surface
roughness using different exposure times. It was
found that Coca-Cola had the highest erosive poten-
tial in the shortest time interval exposure (15 min),
although it had the lowest titratable acidity (4).
These results are in accordance with literature data
which show that cola-based drinks have a higher ero-
sive potential than orange juices immediately after
exposure (12). Profilometric parameters have demo-
nstrated that pure orange juice causes greater ena-
mel erosion during longer exposures. A statistically
significant lower degree of roughness compared to
Coca-Cola in shorter exposure can be explained by
higher initial pH in orange juice compared to Coca-
Cola (3.73 vs. 2.67) (4).

In the current study, gastric juice was signifi-
cantly more erosive to enamel than Coca-Cola and
orange juice, and Coca-Cola is more erosive than
orange juice. Other studies attest that gastric juice
(aspirated from patients undergoing endoscopy) has
a greater potential for erosion than orange juice (10)
and carbonated drinks (Bartlett and Coward, 2001).
Results by Bartlett and Coward reflect the lower pH
and titratable acidity of gastric juice compared with
the carbonated drink. If this result is extrapolated to
the clinical situation, it confirms the suspicion that
gastric juice has the potential to produce the severe
pattern of erosion found in patients with eating dis-
orders and reflux disease (9).

Conclusion

Despite the limitations characteristic of in vi-
tro studies, it can be concluded that experimental
erosion model of enamel surface exposed to Coca-
Cola and artificial gastric juice shows type 1 acidic
erosion (honeycomb appearance) by SEM analysis.
Degree of destruction of central prism parts varied
depending on location, whereby the most prominent
changes were observed in the vicinity of cement-
enamel junctions. Ultrastructural experimental mo-
del of enamel surface erosion after exposure to
orange juice demonstrates atypical etching with no
apparent evidence of a prism pattern. Profilometric
parameter Ra was significantly increased for sam-
pies immersed in gastric juice compared to samples
immersed in Coca-Cola and orange juice, as well as
in samples with Coca-Cola-erosion compared with
orange juice-erosion. The results of this study point to a higher erosive potential of gastric juice, compared to Coca-Cola and orange juice, with the most intense morphological changes and the highest roughness on the enamel surface.

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EVALUACIJA HRAPAVOSTI I MORFOLOŠKIH PROMENA NA GLEĐNOJ POVRŠINI POSLE IZLOŽENOSTI COCA-COLI, SOKU OD NARANDŽE I VEŠTAČKOM ŽELUDAČNOM SOKU: IN VITRO STUDIJA

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erozija zuba je patološki gubitak tvrdih zubnih struktura izazvan spoljašnjim i unutrašnjim kiselinama, bez učešća bakterija. Ova in vitro studija imala je za cilj da proceni i uporedi morfologiju i površinsku hrapavost gleđi nakon erozivnog izazova nekih eksternih i internih kiselih supstanci kao što su Coca-Cola, sok od narandže i veštački želudačni sok.

Uzorci gleđi (n = 48), dobijeni ekstrakcijom humanih trećih molara, podvrgnuti su erozivnom izazovu veštačkog želudačnog soka i komercijalno dostupnih bezalkoholnih pića (Coca-Cola i sok od narandže) uranjem u 50 ml kiselog rastvora u trajanju od 15 min, tri puta dnevno tokom 10 dana. Između potapanja, uzorci su držani u filtriranoj pljuvački. Primljena su dvadeset i četiri uzorka za analizu površinske morfologije korišćenjem skening-electronskog mikroskopa i 24 za analizu Ra-parametra hrapavosti (korišćenjem stylus profilometra sa dijamantnom iglom, uključujući i 12 kontrolnih uzoraka (koji nisu podvrgnuti proceduri erozije). Rezultati površinske hrapavosti analizirani su pomoću one-way ANOVA Student-Newman-Keuls post hoc testa.

Ultrastrukturna analiza površine gleđi posle potapanja u Coca-Colu i želudačni sok pokazala je tip 1 model nagrizanja sa tipičnom honeycomb strukturom. Nespesifican morfo-loški model ustanovljen je nakon erozivnog izazova sokom od narandže. Profilometrijski parametar Ra je značajno povećan kod uzoraka potopljenih u želudačni sok u poređenju sa uzorcima izloženim Coca-Coli i soku od narandže, kao i u uzorcima sa Coca-Cola-erozijom u poređenju sa erozijom izazvanom sokom od narandže. Želudačni sok je pokazao veći erozivni potencijal u odnosu na Coca-Colu i sok od narandže, sa najintenzivnijim morfo-loškim promenama i najvećom hrapavošću na površini gleđi.


Ključne reči: gledna erozija, bezalkoholna pića, želudačni sok, SEM, površinska hrapavost