Universidad Europea

GRADUATION PROJECT

Degree in Dentistry

Evaluation of Enamel Surface Changes Induced by Acidic Beverages Using Stereomicroscopy and Scanning Electron Microscopy

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RESUMEN

Introducción: El consumo de bebidas ácidas y carbonatadas ha aumentado significativamente en las últimas décadas y se ha asociado con un mayor riesgo de erosión dental. La exposición repetida a ácidos puede provocar desmineralización del esmalte y alteraciones en su estructura superficial. Comprender los cambios micromorfológicos inducidos por estas bebidas es importante para evaluar su potencial erosivo y promover estrategias preventivas**. Objetivos:** Evaluar y comparar las alteraciones micromorfológicas de la superficie del esmalte después de la exposición a diferentes bebidas ácidas utilizando estereomicroscopía y microscopía electrónica de barrido (SEM). **Metodología:** Se realizó un estudio in vitro utilizando veinte incisivos humanos permanentes extraídos. Los dientes fueron distribuidos aleatoriamente en cuatro grupos (n = 5): Coca-Cola, Red Bull, vinagre de vino blanco y agua destilada (control). Las muestras fueron sumergidas en las bebidas durante 10 minutos, tres veces al día, durante 14 días. Tras el periodo de exposición, las superficies fueron teñidas con azul de metileno y analizadas mediante estereomicroscopía y SEM para evaluar las alteraciones del esmalte utilizando el Enamel Surface Alteration Score (ESAS) y una escala de intensidad de tinción. **Resultados:** Se observaron diferencias claras entre los grupos experimentales. El grupo control mantuvo una morfología del esmalte intacta, mientras que las bebidas ácidas produjeron diferentes grados de alteración superficial. El vinagre mostró los cambios estructurales más severos, mientras que Coca-Cola y Red Bull produjeron alteraciones moderadas. El análisis estadístico mediante la prueba de Kruskal-Wallis mostró diferencias significativas entre los grupos ($p < 0.05$). Los hallazgos microscópicos coincidieron con los patrones de tinción observados. **Conclusiones:** La exposición repetida a bebidas ácidas altera significativamente la estructura superficial del esmalte, como se demuestra mediante análisis con estereomicroscopía y microscopía electrónica de barrido (SEM). La gravedad de la erosión varía en función de la composición química y la acidez de las bebidas, lo que da lugar a diferencias estadísticamente significativas entre los grupos. Estos hallazgos destacan la importancia de limitar el consumo frecuente de bebidas ácidas para preservar la integridad del esmalte y prevenir la erosión dental.

PALABRAS CLAVE

Odontología, erosión dental, esmalte dental, bebidas ácidas, microscopía electrónica de barrido.

ABSTRACT

Introduction: The consumption of acidic and carbonated beverages has increased considerably over recent decades and has been associated with a higher risk of dental erosion. Repeated exposure to acidic solutions may lead to enamel demineralization and alterations of the enamel surface structure. Understanding the micromorphological changes induced by these beverages is important for evaluating their erosive potential and promoting preventive strategies. **Objectives:** To evaluate and compare the micromorphological alterations of enamel surfaces after exposure to different acidic beverages using stereomicroscopy and scanning electron microscopy (SEM). **Methodology:** An in vitro study was conducted using twenty extracted human permanent incisors. The teeth were randomly distributed into four groups (n = 5): Coca-Cola, Red Bull, white wine vinegar, and distilled water (control). Specimens were immersed in the assigned beverage for 10 minutes, three times per day, for 14 consecutive days. After the exposure period, enamel surfaces were stained with methylene blue and analyzed using stereomicroscopy and SEM. Surface alterations were assessed using the Enamel Surface Alteration Score (ESAS) and a staining intensity scale. **Results:** Clear differences in enamel surface alterations were observed among the experimental groups. Control specimens maintained intact enamel morphology, whereas acidic beverages produced varying degrees of surface damage. Vinegar caused the most pronounced alterations, while Coca-Cola and Red Bull produced moderate changes. Statistical analysis using the Kruskal-Wallis test revealed significant differences between groups ($p < 0.05$). Microscopic findings were consistent with the staining patterns observed. **Conclusions:** Repeated exposure to acidic beverages significantly alters enamel surface structure, as demonstrated by both stereomicroscopic and SEM analyses. The severity of erosion varies according to the chemical composition and acidity of the beverages, leading to statistically significant differences between groups. These findings highlight the importance of limiting frequent consumption of acidic drinks to preserve enamel integrity and prevent dental erosion.

KEYWORDS

Dentistry, dental erosion, enamel surface, acidic beverages, scanning electron microscopy

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1. INTRODUCTION

The consumption of carbonated and acidic beverages has increased significantly over the past decades (1,2).
It is well established that these drinks contain acids that can negatively affect oral health by exerting erosive effects on the teeth.
Dental erosion is defined as the irreversible loss of dental hard tissues caused by prolonged and repeated acid exposure, without bacterial involvement (3).

Based on the origin of the acids, dental erosion can be classified into intrinsic and extrinsic types. Intrinsic erosion results from internal sources such as gastroesophageal reflux disease (GERD) or frequent vomiting associated with stress, pregnancy, or eating disorders like bulimia.
On the contrary, extrinsic erosion is primarily related to dietary habits, particularly the frequent consumption of acidic foods and beverages (4).

Following exposure to acidic beverages, the oral pH decreases rapidly from neutral levels (~7) to values approaching the critical pH for enamel dissolution (~5.5) (5).
Upon contact with acid, hydroxyapatite crystals in the enamel start to dissolve, releasing calcium and phosphate ions and gradually weakening the tooth structure (6).
Over time, this mineral loss can lead to tooth sensitivity, pain, and an increased risk of caries (3,7).

Preserving enamel integrity is essential for both dental health and aesthetics. Erosion affects not only the strength of the enamel but also its surface texture, color, and brightness (7).
As enamel wears down, it becomes rougher and loses its natural shine, often appearing yellower as the underlying dentin becomes more visible.
In advanced stages, weakened enamel may chip or crack more easily (7,8).

From a public-health perspective, the growing consumption of soft drinks and energy beverages represents a major concern, especially among adolescents and young adults. A national study by the Fundación Española de la Nutrición (FEN) and the Instituto Nacional de Estadística (INE) shows a 721% increase in non-alcoholic beverage consumption since 1964, with a 38% rise between 2000 and 2014 alone (2). Energy-drink intake has also expanded dramatically in the

last two decades and has been linked to a 2.4-fold higher risk of dental erosion due to their low pH and high sugar content (1).
These trends reflect how current dietary habits promote the regular intake of acidic beverages that can affect the mineral structure of enamel. Understanding their effects is therefore essential to guide preventive strategies and raise awareness of healthier consumption patterns.

Microscopic techniques are commonly used to investigate the structural alterations associated with enamel erosion. Stereomicroscopy provides a practical method for the initial examination of enamel surfaces, allowing the visualization of macroscopic changes such as surface roughness, loss of gloss, and discoloration with minimal sample preparation (9). This technique is widely used for documenting surface alterations and evaluating the progression of erosive lesions in experimental studies. However, a more detailed evaluation of enamel microstructure requires higher-resolution imaging. Scanning electron microscopy (SEM) enables the observation of enamel surface morphology at high magnification, allowing detailed visualization of structural changes such as prism dissolution, interprismatic alterations, and surface porosities (10). The combined use of stereomicroscopy and SEM therefore provides complementary information, as stereomicroscopy allows initial surface assessment while SEM enables detailed analysis of enamel microstructural alterations caused by erosive processes (11).

Therefore, the aim of this study is to evaluate enamel surface alterations induced by common acidic beverages using both stereomicroscopy and SEM. By analyzing and comparing the effects of commonly consumed drinks, this research seeks to provide visual and measurable evidence of the erosive potential of these beverages.

The findings may help reinforce the importance of preventive oral care and dietary education, encouraging healthier consumption habits. In this way, the study not only contributes to a better understanding of enamel erosion mechanisms but also supports long-term oral health promotion and sustainability through evidence-based awareness of daily consumption habits.

Based on these considerations, the null hypothesis (H0) states that there is no significant difference in enamel surface alterations among teeth exposed to different acidic beverages and those exposed to distilled water.

The alternative hypothesis (H1) states that significant differences in enamel surface alterations exist between teeth exposed to acidic beverages and those exposed to distilled water.

2. OBJECTIVES

1. To evaluate and compare the micromorphological alterations of enamel surfaces after exposure to different acidic beverages using a stereomicroscope and SEM.
2. To compare the degree of enamel erosion among different beverage types.

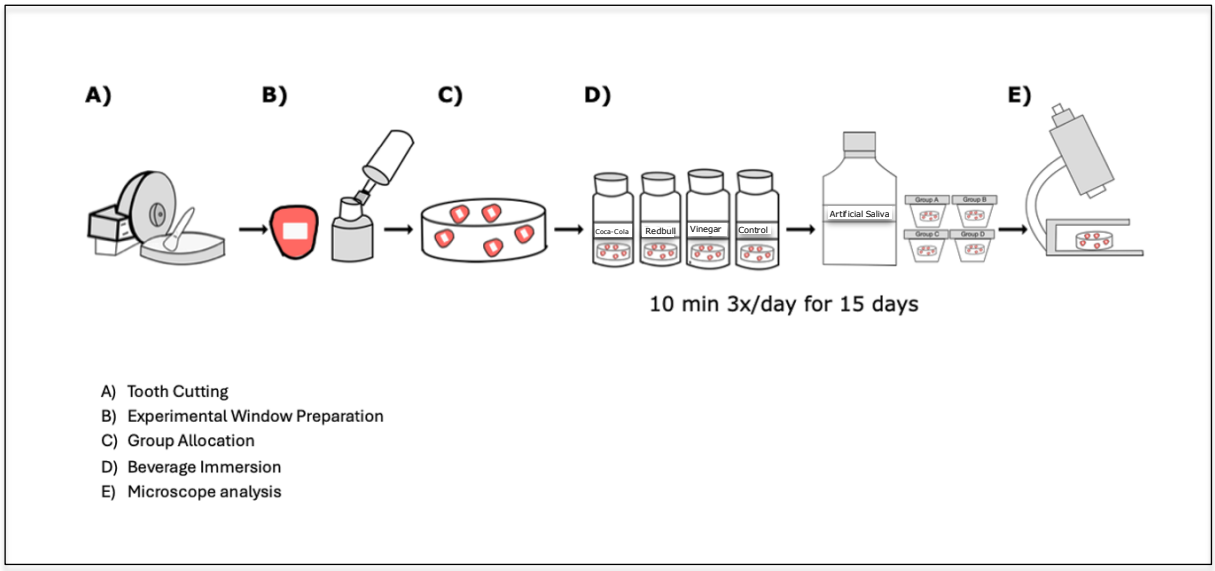
Hypotheses:

H0: There is no significant difference in enamel surface alterations among teeth exposed to different acidic beverages and those exposed to distilled water.

H1: There is a significant difference in enamel surface alterations among teeth exposed to different acidic beverages and those exposed to distilled water

3. MATERIALS AND METHODS

Figure 1. Experiment Representation



Schematic representation of the experimental workflow illustrating the main stages of the experimental procedure.

3.1 Sample Selection

A total of twenty (n=20) sound human permanent incisors extracted for periodontal reasons were collected for this in vitro study. After extraction, all specimens were stored in a 0.1% thymol solution until use. The teeth were randomly assigned into four experimental groups of five samples each.

Inclusion criteria

- Permanent incisors with intact enamel
- No caries
- No restorations
- No visible cracks or structural defects

Exclusion criteria

- Teeth with carious lesions
- Previously restored teeth
- Teeth with fractures or enamel defects
- Deciduous teeth

3.2 Experimental Groups

To minimize bias, the specimens were randomly assigned to four beverage groups, with five specimens per group, ensuring equal sample distribution across experimental conditions

- **Group A:** Coca-Cola
- **Group B:** White Wine Vinegar
- **Group C:** Red Bull
- **Group D:** Distilled water (control)

Table 1. Composition, acid type and pH of the beverages used in the experimental groups

Group	Beverage	Main Components	Primary Acid	Acid Category	pH
A	Coca-Cola	Carbonated water, sugar, caramel colour (E-150d), phosphoric acid, natural flavourings, caffeine	**Phosphoric acid (H_3PO_4)**	**Inorganic acid** (high erosive potential)	~2.7
B	Red Bull	Carbonated water, sugars, citric acid, taurine, sodium bicarbonate, caffeine, vitamins B3, B5, B6, B12	**Citric acid ($C_6H_8O_7$)**	**Organic acid** (chelating effect: binds Ca^{2+})	~3.3
C	White Wine Vinegar	Wine vinegar, antioxidant E-223 (sulfites)	**Acetic acid (CH_3COOH)**	**Organic acid** (preservative; strong demineralizer)	~2.4
D	Distilled water (Control)	H_2O	—	Neutral	7.0

3.3 Tooth Preparation

All teeth were maintained in 0.1% thymol, which served as both a preservative and antibacterial solution. The roots were subsequently sectioned at the cementoenamel junction using a laboratory model trimmer equipped with a silicon carbide disc (Mestra, Spain, Ref. 080080), allowing only the anatomical crowns to be retained.

After cleaning, a circular adhesive sticker (0.4 mm diameter) was placed on the cervical and middle thirds of the buccal surface.

Nail polish (Revlon, USA) was applied as an acid-resistant coating. Once dry, the sticker was removed, generating two clearly defined areas:

- one uncoated enamel window (exposed to the beverages),

- one protected area (covered with nail polish).

This procedure ensured precise delimitation of the working area for each specimen.

Following specimen preparation, the five teeth belonging to each experimental group were embedded collectively in an epoxy resin mold (FansArriche, Spain). Embedding them within a single circular block ensured uniform positioning and provided a stable support throughout the analytical procedures. This method enhanced standardization across groups, reduced handling variability, and facilitated subsequent microscopic examination.

3.4 Beverage Exposure Protocol

1. Teeth were rinsed and stored in 0.9% saline solution prior to immersion.
2. Each enamel window was immersed in its assigned beverage for 10 minutes, three times per day, for 14 consecutive days.
3. Between immersion cycles, samples were stored in distilled water.
4. After the 14 days exposure period, teeth were stained with methylene to enhance surface contrast.
5. Samples were examined under a stereomicroscope and a scanning electron microscope at x1000 magnification.
6. Surface alterations (roughness, loss of gloss, microcracks, staining intensity) were documented and scored using a standardized erosion scale.

3.5 Surface Staining with Methylene Blue

After completion of the immersion protocol, all specimens were rinsed with distilled water to remove residual beverage and then air-dried. The enamel surfaces were subsequently impregnated with methylene blue solution (Loeffler formulation; Panreac AppliChem, Spain) for 5 minutes, following the manufacturer's guidelines. After staining, the specimens were rinsed again with distilled water to eliminate excess dye. This procedure allowed visualization of surface porosity and facilitated the assessment of staining intensity associated with enamel demineralization.

3.6 Scoring System for Surface Alteration

Table 2. Enamel Surface Alteration Score (ESAS)

SCORE	CONDITION	DESCRIPTION
0	*Intact enamel*	Smooth surface, no visible changes, uniform reflection
	No staining	No visible dye uptake: enamel remains uniformly translucent
1	*Mild alteration*	Slight roughness, faint loss of luster, minimal dye uptake
	Light staining	Light dye absorption: faint coloration limited to surface layer
2	*Moderate alteration*	Surface irregularities, microcracks, increased dye uptake
	Moderate staining	Clear dye penetration into surface irregularities: localized deeper coloration
3	*Severe alteration*	Pronounced erosion, pits/grooves, deep staining, enamel thinning
	Intense staining	Strong, diffuse staining penetrating deeply into demineralized areas

3.7 Data Collection and Microscopic Analysis

3.7.1 Stereo Microscopy

Samples were examined using a stereomicroscope (Nikon SMZ445). Surface changes such as roughness, loss of gloss and staining intensity were documented and scored using the ESAS scale.

3.7.2 Scanning Electron Microscopy (SEM)

To complement stereomicroscopy and obtain higher resolution surface information, selected specimens representing each score level were examined under a SEM (JEOL 6400 JSM) at magnification x1000.

SEM analysis allowed detailed visualization of erosive patterns including enamel prism alteration, interprismatic dissolution and surface porosities.

SEM analysis was performed qualitatively to corroborate and further characterize the surface alterations observed under stereomicroscopy.

3.8 Statistical Analysis

The ESAS values will be analyzed using the Kruskal-Wallis test, a non-parametric statistical method appropriate for ordinal data and small sample sizes.
If significant differences are found, post-hoc pairwise comparisons will be performed to identify which groups differ.
A value of $p < 0.05$ will be considered statistically significant.

3.9 Ethical Considerations

- The study protocol was reviewed and approved by the Research Committee of Universidad Europea (Comisión de Investigación). Authorization for the development of this research was granted under CI code 2026-684 and Department code OD.004/2526 (Annex 1).
- Teeth were obtained from dental clinics with informed patient consent.
- No personal data were collected.
- The study complies with institutional and national ethical regulations for the use of human biological material.

4. RESULTS

4.1 Macroscopic Observations

The results showed clear differences in enamel surface alterations between the experimental groups. Control specimens maintained intact enamel morphology, while the acidic beverages produced varying degrees of surface damage. Vinegar caused the most severe alterations, whereas Coca-Cola and Red Bull produced moderate changes. These findings were consistent across macroscopic observations and SEM analysis.

4.1.1 Baseline Appearance (Before Exposure)

Before immersion in the experimental solutions, all specimens presented a similar macroscopic appearance. The enamel surfaces were glossy, smooth, and translucent, with no visible discoloration or structural defects. No differences between groups were observed at baseline.

4.1.2 Appearance After Beverage Exposure

Clear visual differences were observed between groups following the 14-days immersion protocol.

A. Control Group

The control specimens maintained their original glossy and translucent appearance. No visible discoloration, opacity, or surface dullness was detected.

B. Coca-Cola Group

Teeth exposed to Coca-Cola developed a noticeable brownish discoloration localized primarily to the immersion window. The coloration began to be visible from the third day of exposure and progressively intensified throughout the experimental period. The color change was clearly visible to the naked eye, and the enamel appeared darker compared to baseline. Despite this pigmentation, the surface morphology remained macroscopically intact. The enamel retained its general gloss, although it appeared slightly less reflective than the control specimens.

C. Red Bull Group

Specimens exposed to Red Bull did not demonstrate evident macroscopic color change compared to baseline. The enamel maintained its general translucency and gloss. No clear discoloration or matte appearance was observed to the naked eye.

D. Vinegar Group

The vinegar group exhibited the most pronounced macroscopic surface change. The enamel lost its natural shine and appeared opaque and matte white. The surface presented a chalky appearance, clearly different from baseline and from the other experimental groups.

4.1.3 Appearance After Methylene Blue Staining

Following application of methylene blue, differences in dye uptake became clearly visible between groups. The staining scores obtained for each specimen and their corresponding summary values are presented in Tables 3 and 4.

Table 3. Staining score per specimen

Group	*Tooth 1*	*Tooth 2*	*Tooth 3*	*Tooth 4*	*Tooth 5*
Control	0	0	0	0	0
Coca-Cola	2	2	3	3	3
Red Bull	2	2	3	2	2
Vinegar	3	3	3	3	3

Table 4. Summary of staining scores

Group	*Median*	*IQR**
Control	0	0-0
Coca-Cola	3	2-3
Red Bull	2	2-2
Vinegar	3	3-3

* IQR: Interquartile Range

A. Control Group

Control specimens showed no dye uptake. The enamel surface remained uniformly translucent and unchanged compared to baseline. No localized deep staining or concentrated dye retention was observed.

Staining Intensity Level: 0 (No staining)

B. Coca-Cola Group

Specimens exposed to Coca-Cola exhibited a relatively uniform blue coloration within the exposed enamel window. The staining was clearly visible and distributed across most of the exposed enamel surface. However, the coloration was not completely opaque. Certain regions displayed lighter areas where the underlying enamel was still partially visible, creating a slightly translucent appearance within the stained field.

Staining Intensity Level: 2 (Moderate)

C. Red Bull Group

Red Bull specimens demonstrated a non-uniform staining pattern. The blue coloration was distributed irregularly across the exposed enamel window. Linear areas of dye accumulation were visible against regions that appeared relatively intact. The staining was less homogeneous compared to the Coca-Cola group and showed alternating stained and unstained areas.

Staining Intensity Level: 2 (Moderate)

D. Vinegar Group

The vinegar group exhibited the most intense and homogeneous staining pattern. The exposed enamel surfaces showed strong and diffuse blue coloration. The staining appeared uniform and more opaque compared to the other groups.

Staining Intensity Level: 3 (Intense)

4.2 Statistical Analysis of Staining Intensity

Staining intensity scores were compared among the four experimental groups using the Kruskal-Wallis test. The analysis revealed a statistically significant difference among groups ($H = 15.30$, $df = 3$, $p \approx 0.002$). Therefore, the null hypothesis was rejected.

Median staining intensity values were 0 for the control group, 2 for Red Bull, and 3 for both Coca-Cola and vinegar.

Post-hoc pairwise comparisons revealed significant differences between the control group and all acidic beverage groups. A significant difference was also observed between Red Bull and vinegar, as well as between Red Bull and Coca-Cola. No statistically significant difference was found between Coca-Cola and vinegar.

4.3 Microscopic Analysis (SEM)

SEM observations were evaluated using the Enamel Surface Alteration Score (ESAS) to standardize the assessment of surface alterations. The individual ESAS values and their corresponding summary statistics are presented in Tables 5 and 6.

Table 5. ESAS values per specimen

Group	*Tooth 1*	*Tooth 2*	*Tooth 3*	*Tooth 4*	*Tooth 5*
Control	0	0	0	0	0
Coca-Cola	2	2	3	3	3
Red Bull	2	2	3	2	2
Vinegar	3	3	3	3	3

Table 6. Summary of ESAS values

Group	*Median*	*IQR*
Control	0	0-0
Coca-Cola	3	2-3
Red Bull	2	2-2
Vinegar	3	3-3

A. Control Group

SEM micrograph of control enamel at ×1000 magnification showing a smooth and continuous surface (Figure 2). At this magnification, no exposed enamel rods or prism-related structures are visible. The enamel surface maintained structural continuity, with no evidence of porosity, prism exposure, or structural disorganization. The enamel architecture therefore remained intact, consistent with ESAS 0, corresponding to intact enamel (Table 2).

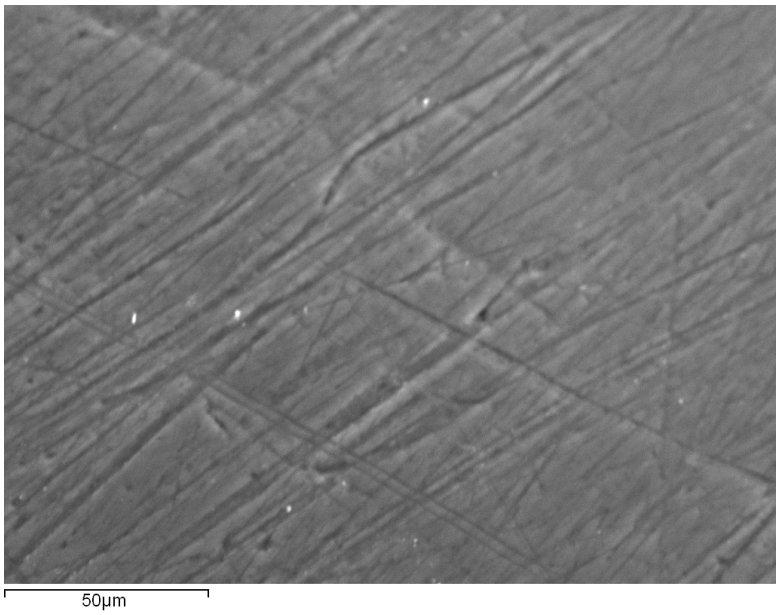


Figure 2. Control enamel (SEM, ×1000).

B. Coca-Cola Group

Specimens exposed to Coca-Cola demonstrated clear surface alterations compared with the control group. At magnification x1000, the enamel surface exhibited a porous granular texture characterized by numerous rounded depressions (Figure 3). The pattern was consistent with interprismatic dissolution, producing a honeycomb-like morphology indicative of localized mineral loss.

The morphology also appeared to suggest preferential dissolution of the prism cores, while the prism peripheries remained relatively preserved. The erosive pattern appeared relatively homogeneous across the observed field.

No evidence of dentin exposure or deep cracks through the enamel thickness was detected. Overall, the alterations correspond predominantly to ESAS 2 (moderate alteration), with some areas approaching ESAS 3 (severe alteration), according to criteria presented in Table 2.

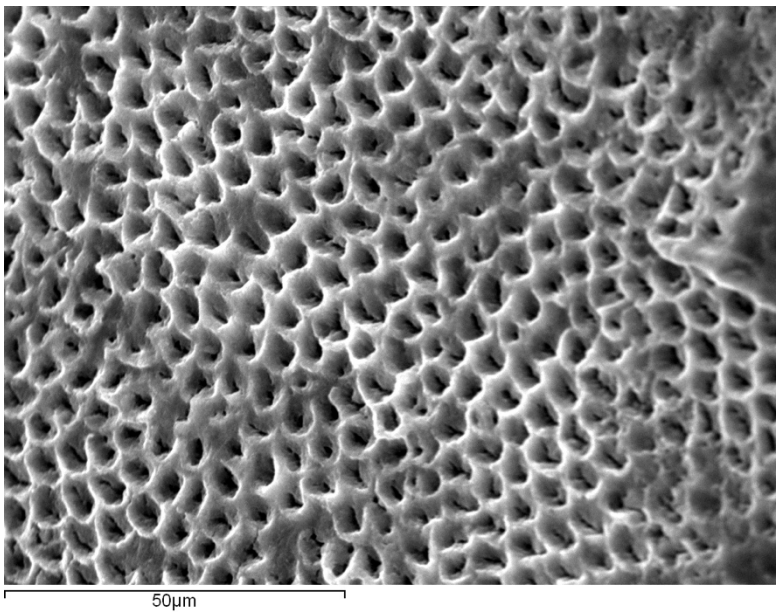


Figure 3. Coca-Cola group (SEM, ×1000).

C. Red Bull Group

SEM images demonstrated increased surface roughness and generalized surface irregularity compared with the control group. At ×1000 magnification, areas of porosity and alterations in the enamel prism pattern were evident (Figure 4). The enamel surface presented a diffuse granular appearance with numerous rounded depressions, consistent with interprismatic dissolution.

The observed morphology appeared to suggest preferential alteration of the prism peripheries, while the prism cores remained relatively preserved. Although the general prism architecture remained partially recognizable, the surface morphology was clearly compromised. No dentin exposure was observed. The alterations were consistent among specimens and correspond predominantly to ESAS 2 (moderate alteration), with several areas approaching ESAS 3 (severe alteration), as outlined in Table 2.

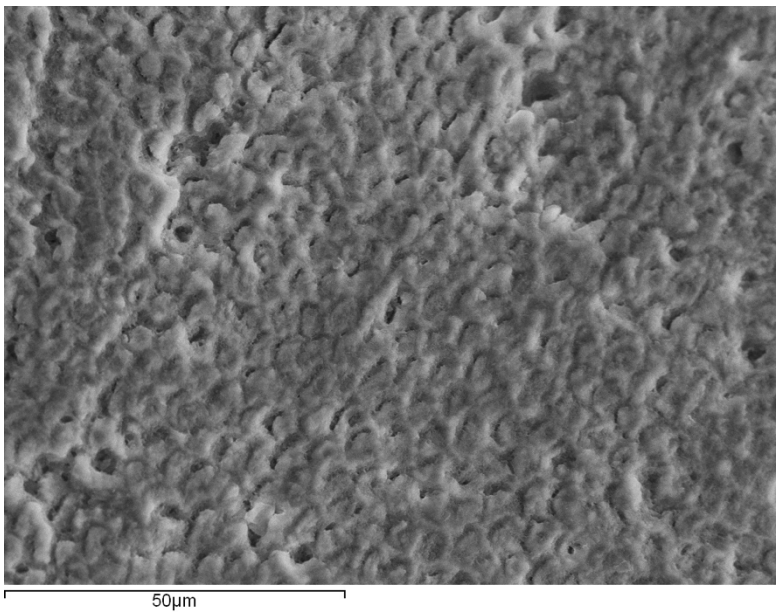


Figure 4. Red Bull group (SEM, ×1000).

D. Vinegar Group

The vinegar group exhibited the most pronounced enamel surface alterations. SEM analysis demonstrated extensive surface roughness, generalized porosity, and marked disruption of the normal enamel surface morphology (Figure 5). At ×1000 magnification, the surface displayed a well-defined honeycomb pattern consistent with extensive interprismatic dissolution. Although the prism architecture remained identifiable, it appeared clearly altered and irregular. The erosive pattern was diffuse and generalized rather than focal. Alterations were consistent across specimens and corresponded predominantly to ESAS 3 (severe alteration), as classified in Table 2.

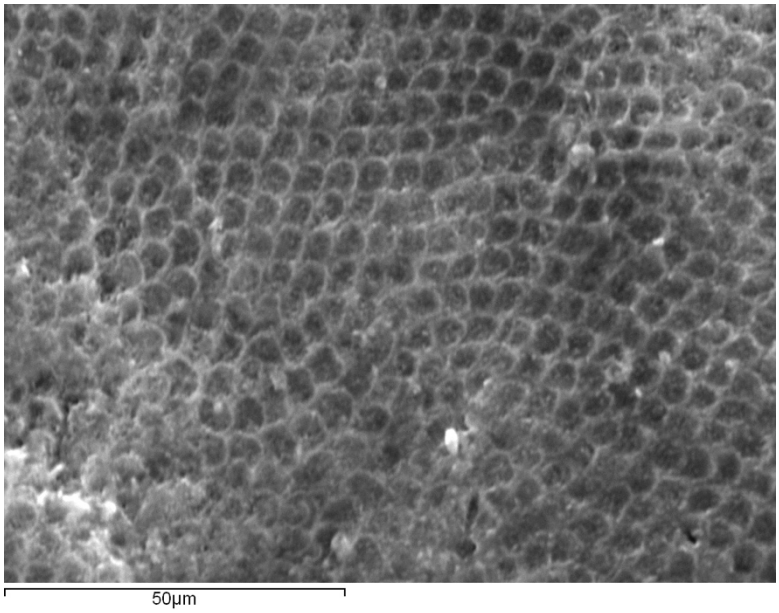


Figure 5. Vinegar group (SEM, ×1000).

4.3.1 Comparative Analysis Between Groups

Comparative SEM evaluation revealed a progressive increase in enamel surface alteration from the control group to the Coca-Cola and Red Bull groups, which both demonstrated moderate surface damage, while the most pronounced alterations were observed in the vinegar group. Control specimens maintained normal surface morphology and structural integrity, corresponding to ESAS 0 (no alteration). In contrast, the Coca-Cola group showed moderate surface alterations characterized by localized porosity and early disruption of the enamel surface, corresponding predominantly to ESAS 2 (moderate alteration). Specimens exposed to Red Bull exhibited more generalized surface irregularity and clearer alterations in prism architecture, also corresponding mainly to ESAS 2, with some areas approaching ESAS 3 (severe alteration). The vinegar group demonstrated the most severe changes, including extensive surface roughness, widespread porosity, and marked disruption of enamel morphology, consistent with ESAS 3 (severe alteration). No evidence of dentin exposure was observed in any of the groups.

Overall, the SEM observations indicate that exposure to acidic beverages produced varying degrees of enamel surface alteration, with the most severe structural changes observed in the vinegar group, while the Coca-Cola and Red Bull groups demonstrated moderate but clearly detectable enamel surface degradation compared with the control specimens.

4.4 Statistical Analysis of ESAS Values

ESAS values were compared among the four experimental groups using the Kruskal-Wallis test, as the data were ordinal and the sample size per group was limited (n = 5). The analysis revealed a statistically significant difference among groups ($H = 15.30$, $df = 3$, $p \approx 0.002$).

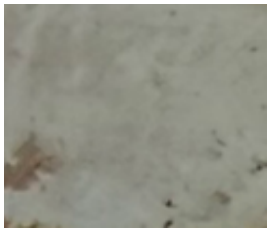
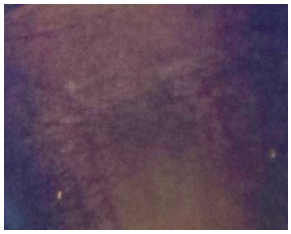
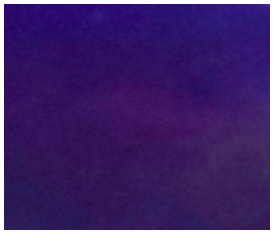
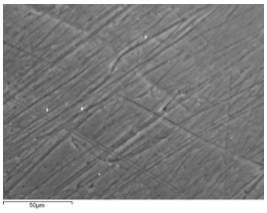
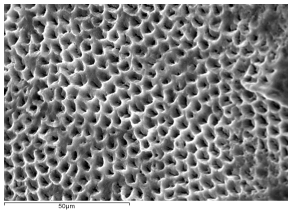
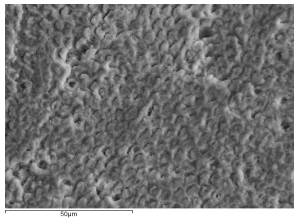
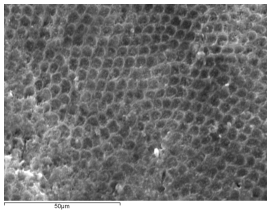
Median (IQR) ESAS values were 0 (0–0) for the control group, 2 (2–2) for Red Bull, and 3 (2–3) and 3 (3–3) for Coca-Cola and vinegar, respectively.

These results indicate a progressive increase in enamel surface alteration from the control group to the acidic beverage groups, with the highest severity observed in the vinegar group.

Staining intensity scores showed an identical distribution across the experimental groups and similarly demonstrated a statistically significant difference.

Overall, these findings lead to rejection of the null hypothesis (H_o), confirming that exposure to acidic beverages resulted in significant enamel surface alterations compared with the control group

Table 7: Representative enamel surface images across experimental groups at different stages: before exposure, after exposure, after staining, and SEM (×1000).

	Control	Coca-Cola	Red Bull	Vinegar
Before Exposure				
After Exposure				
After Staining				
SEM (x1000)				

A representative specimen from each group was selected according to the median staining score and is illustrated through sequential macroscopic and SEM images to demonstrate the progression of surface changes.

5. DISCUSSION

The present study demonstrated that exposure to acidic beverages produced measurable enamel surface alterations that were detectable both macroscopically and microscopically. These findings provide insight into the micromorphological mechanisms underlying enamel erosion caused by commonly consumed beverages. The rejection of the null hypothesis further confirms that the observed differences in enamel surface alterations between groups are statistically significant. A clear severity gradient was observed among the experimental groups, with control specimens maintaining intact enamel structure, whereas the acidic beverages induced varying degrees of surface disruption. These findings directly address the objective of the present study, which was to evaluate the extent and pattern of enamel surface alterations induced by different acidic beverages. Vinegar produced the most pronounced and generalized structural changes, while Coca-Cola and Red Bull induced predominantly moderate alterations, with some areas approaching severe surface disruption. The consistency between SEM findings and staining intensity results suggests that increased structural disorganization of enamel is associated with greater surface permeability. These findings support the hypothesis that repeated exposure to commonly consumed acidic beverages may progressively compromise enamel structural integrity under controlled experimental conditions (3,5).

Considering the growing consumption of acidic beverages worldwide, particularly among adolescents and young adults (3), the present findings are clinically relevant. The enamel surface alterations observed in this study highlight the potential cumulative impact of repeated acid exposure in daily life.

Enamel is the most highly mineralized tissue in the human body and is composed primarily of inorganic material. Approximately 96% of enamel consists of hydroxyapatite crystals formed by calcium and phosphate ions (12). These crystals are organized into structural units known as enamel prisms, or rods, which extend from the dentino-enamel junction to the external surface of the tooth. Between these prisms lies interprismatic enamel, where the crystals are oriented differently. This structural organization contributes to the mechanical strength of enamel but also influences how the tissue responds to acidic challenges (13,14). In addition, an outer aprismatic layer may be present at the enamel surface, where crystal orientation is more uniform. The arrangement of prism cores and interprismatic regions plays an important role in determining preferential dissolution patterns during acid exposure (5).

Enamel demineralization occurs when acidic solutions reduce the surrounding pH below the critical threshold of approximately 5.5, leading to dissolution of hydroxyapatite crystals (5). The susceptibility of enamel to erosion depends not only on pH but also on several additional variables, including acid type, frequency of exposure, temperature, titratable acidity, and buffering capacity (15). Different acids interact with enamel in distinct ways depending on their chemical composition and physicochemical properties, which can influence the pattern and extent of prism dissolution observed under SEM (5). Frequency of exposure also plays an important role, as repeated acid challenges reduce the time available for remineralization (15). In addition, certain drinking habits may further increase the erosive potential of acidic beverages. Behaviors such as retaining an acidic drink in the mouth for prolonged periods, swishing it around the oral cavity, or passing the liquid slowly through the teeth can extend the duration of contact between the acid and the enamel surface, thereby enhancing the risk of erosion (16). In the present study, specimens were immersed three times daily for 10 minutes over 14 days, resulting in a cumulative exposure time of 420 minutes. According to previously proposed exposure models (17), this cumulative exposure could correspond to several decades of daily acidic contact, although such estimations should be interpreted cautiously within the limitations of an in vitro design.

Temperature is another factor that can influence the erosive effect of acidic beverages. In the present study, beverages were maintained at room temperature, ranging from approximately 20 °C to 26 °C, ensuring that temperature was not an additional variable contributing to enamel alteration. Drinks consumed at higher temperatures have been shown to cause greater enamel mineral loss compared with cold beverages. This effect can be explained by the increased solubility and diffusion of calcium and phosphate ions in solution as temperature rises, which may accelerate hydroxyapatite dissolution (5,15). It has been reported that raising the temperature from 5 °C to 60 °C significantly increases enamel and dentine tissue loss ($p < 0.05$) (15). Although higher temperatures may enhance the rate of mineral dissolution, the overall erosive potential of a beverage remains strongly influenced by its intrinsic chemical characteristics.

The SEM analysis revealed distinct patterns of enamel surface alteration depending on the acidic beverage tested. In the Coca-Cola group, SEM images showed a well-defined surface morphology suggesting preferential dissolution of prism cores while the prism peripheries

remained relatively preserved. This pattern indicates localized mineral loss affecting the central regions of enamel prisms. As indicated in Table 1, Coca-Cola contains phosphoric acid, which has been reported to contribute to the erosive potential of cola-based beverages due to its low pH and its interaction with hydroxyapatite crystals (5,15). Results in this study were consistent with previous in vitro studies, which have demonstrated that cola-based beverages can induce rapid surface mineral loss and increased surface roughness (6,16).

In contrast, the Red Bull group exhibited a more heterogeneous surface morphology characterized by irregular roughness, localized depressions, and partial alterations of the enamel prism architecture. According to Table 1, Red Bull contains citric acid, which not only lowers the pH of the solution but also acts as a calcium-chelating agent. Citric acid can prolong the demineralization process by binding calcium ions and maintaining mineral undersaturation in the surrounding environment (5,15,16). This chelating effect may explain the more irregular and patchy demineralization pattern observed under SEM in the present study, as calcium binding prolongs mineral loss and disrupts uniform dissolution processes (14).

The morphological features observed in the present study are comparable to the classical enamel etching patterns described by Silverstone (Table 8). In the present study, the honeycomb-like morphology observed in the Coca-Cola specimens resembles a Type I etching pattern, whereas the cobblestone-like appearance observed in the Red Bull group appears more consistent with a Type II-like dissolution pattern (18,19).

Table 8 : Acid etching pattern described by Silverstone

ETCHING PATTERN	SEM MORPHOLOGY	PREFERENTIAL DISSOLUTION
TYPE I	Honeycomb-like	Prism cores
TYPE II	Cobblestone-like	Prism peripheries

The vinegar group demonstrated the most severe and generalized structural disruption among the tested beverages. SEM analysis revealed extensive surface roughness, diffuse porosity, and widespread disruption of the normal enamel architecture. These findings are consistent with previous experimental studies reporting that vinegar exposure can produce pronounced mineral loss and significant enamel surface damage (7,8). As shown in Table 1, vinegar contains acetic acid, which creates a highly acidic environment capable of rapidly dissolving hydroxyapatite crystals once the environmental pH falls below the critical threshold for enamel

demineralization (7). The homogeneous structural disruption observed in this group suggests that repeated exposure to acetic acid can affect the overall prism architecture rather than producing localized dissolution patterns.

The macroscopic observations corresponded closely with the microscopic findings. The matte, chalky appearance of vinegar specimens may be explained by increased surface roughness and mineral loss, which alter light reflection and reduce enamel gloss (5,7). The brown discoloration observed in the Coca-Cola group is likely associated with the caramel coloring present in the beverage combined with increased surface porosity facilitating retention of staining pigments (7). In the Red Bull group, heterogeneous methylene blue uptake corresponded with the irregular prism alterations observed under SEM, suggesting localized increases in enamel permeability.

A similar severity gradient was observed when comparing ESAS values and staining intensity. Specimens presenting greater structural disorganization under SEM also demonstrated increased dye penetration, supporting the association between mineral loss and increased surface permeability. This phenomenon can be explained by the development of micro-porosities and interprismatic spaces during enamel demineralization, which facilitate the diffusion and retention of staining agents within the enamel surface (13,20).

Overall, the findings of this study reinforce the role of acidic beverage exposure as a significant factor contributing to enamel surface degradation, highlighting the importance of preventive strategies aimed at reducing erosive dietary habits.

However, these results should be interpreted in light of certain limitations inherent to the study design. The present investigation was conducted under in vitro conditions, which do not fully reproduce the complexity of the oral environment. In particular, natural saliva, which plays a key protective role through buffering capacity, mineral supply, and formation of the acquired pellicle, was not replicated, as specimens were stored in distilled water between immersion cycles (5,6). Consequently, the degree of enamel alteration observed may be greater than what would occur under clinical conditions, where continuous remineralization and protective mechanisms are present.

Additionally, real-life erosive processes are influenced by behavioral factors such as drinking habits, frequency of exposure, and contact time, which were standardized in this experimental model (4,5). Furthermore, enamel erosion is affected by multiple chemical variables beyond pH alone, including titratable acidity and buffering capacity. Therefore, the present findings should be interpreted as an estimation of erosive potential under controlled conditions rather than a direct reflection of clinical outcomes (15,20).

6. CONCLUSIONS

1. Within the limitations of this in vitro study, repeated exposure to acidic beverages produced significant micromorphological alterations of the enamel surface, detectable both macroscopically using stereomicroscopy and at higher resolution through SEM analysis. These findings confirm that enamel surface integrity is significantly affected by acidic exposure, supporting the rejection of the null hypothesis (H0) and the acceptance of the alternative hypothesis (H1).

2. The severity of enamel erosion differed among the beverages, confirming that enamel degradation depends not only on pH but also on acid composition and chemical behavior. Vinegar induced the most generalized and severe surface disruption, while Coca-Cola exhibited a pronounced prism-related etching pattern consistent with phosphoric acid activity. Red Bull produced more heterogeneous alterations, likely related to the chelating properties of citric acid. Overall, these findings highlight the clinical importance of limiting frequent acidic exposure to preserve enamel integrity over time.

7. SUSTAINABILITY

This study supports a sustainable approach to oral health in accordance with the United Nations Sustainable Development Goals SDG 3: Good Health and Well-Being (Salud y Bienestar) and SDG 12: Responsible Consumption and Production (Producción y Consumo Responsables) (21). By studying how acidic beverages affect the enamel surface, this work highlights the strong influence of daily consumption habits on dental health and long-term oral integrity. The results emphasise the importance of prevention and continuous education in reducing dental erosion caused by frequent acid exposure. Promoting healthier beverage choices and raising public awareness about the erosive potential of acidic drinks can help reduce the incidence of dental erosion, enamel damage and hence the need for restorative procedures.

This, in turn, limits the use of dental materials, decreases clinical waste generation, and optimises the use of resources in dental practice. Therefore, this study contributes to both social and environmental sustainability by promoting preventive care, patient education, and the responsible use of materials in everyday dental practice.

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9. ANNEXES

Annex 1. Ethical approval for the research project

Ethical approval for this study was granted by the Research Committee of Universidad Europea (Comisión de Investigación) under CI code 2026-684 and Department code OD.004/2526.

Universidad Europea

Comisión de Investigación

Villaviciosa de Odón, 9 de marzo de 2026

Estimado/a investigador/a,

La Comisión de Investigación de la Unidad de Investigación, una vez revisada la documentación e información, remitida por el investigador responsable con fecha 09/03/2026, relativa al proyecto abajo indicado, autoriza su desarrollo en la Universidad Europea.

Título del proyecto:	Micromorphological analysis of enamel surface alterations after exposure of acidic beverages: A light microscopy study on extracted human teeth.
Tipo de proyecto:	TFG
Investigador/a responsable:	JOVES MENDEZ- GERARDO
Código CI:	2026-684
Código OTRI:	Sin especificar
Código Departamento:	OD.004/2526
Dictamen:	APROBADO

Atentamente,

Ruth M. Risueño

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