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ASSESSMENT OF THE SURFACE MICROHARDNESS OF ENAMEL AND DENTIN EXPOSED TO VARIOUS SUBSTANCES OF HUMAN CONSUMPTION

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ABSTRACT The physicochemical integrity of tooth enamel in the oral environment depends entirely on the composition and chemical behavior of the liquids around it. Aim: To determine the microhardness of the enamel and dentin on the dental surface and the erosive potential of six beverages for human consumption. Materials and Methods: 32 recently extracted healthy first premolars were selected (two to define the points where the diamond tip for microdurometer was placed) and five samples for each of the six groups. A vestibular-palatine cut to was obtain 2 fragments (vestibular and palatine) and four points of identification were made in the crown (cervical, medium, cusp and groove), a total of 16 samples per surface before and 16 after the exposition to one of six solutions with a different acid pH for 45 minutes. In order to evaluate the superficial hardness of the enamel and dentin, the Vickers hardness microdurometer was used. The statistical analysis was obtained with the Kolmogorov Smirnov, Student's T test, Wilcoxon, Kruskal-Wallis tests and Dunn's Post-Hoc analysis. In this way, the erosion capacity of each of the solutions could be evaluated. Results: Microhardness showed significant statistical difference among all beverages used in the study and also comparing their difference before and after the exposition ($p<0.0001$). Conclusions: A significant loss of surface microhardness of enamel and dentin was found to be exposing samples to different types of solutions that are commonly consumed. A higher pH of the solution, a higher degree of demineralization of the enamel and dentin.

KEYWORDS : Enamel, Dentin, Microhardness, Vickers test.

INTRODUCTION

Dental enamel is the most mineralized tissue of the human body. It consists of 96% of inorganic material, 4% organic material and water. In dentin is constituted by 70% of inorganic material and 30% of organic compounds. The inorganic portion is composed by calcium phosphate disposed by hexagonal hydroxyapatite crystals. Its chemical formula is $Ca10\ (PO4)6\ 2(OH)$, but also contains other materials such as Na, Cl, and Mg.[1] Even when enamel is the hardest and most resistant tissue and protects dental tissues against external factors, it can be destroyed or irreversibly damaged. Acid pH is one of the main causes that can destroy enamel and it can be directly produced by the final products of carbohydrate metabolism performed by oral bacteria.[2]

Literature describes extensively the different forms of chronic destructive processes that affect teeth, in addition to tooth decay, producing irreversible loss of the tooth structure, being among them: abrasion, attrition, abfraction, reabsorption and erosion.[4]

The physicochemical integrity of tooth enamel in the oral environment depends entirely on the composition and chemical behavior of the fluids around it. The main factors governing the stability of enamel hydroxyapatite in contact with saliva are pH and calcium, phosphate and fluoride concentrations in solution.[3]

In order to a better understanding of the process by which a drink is more descaling it is necessary to understand the terms of superficial tension and capillarity; superficial tension (a manifestation of intermolecular forces in liquids), together with the forces that occur between liquid and solid surfaces that be in contact with them, gives rise to capillary. As an effect, the energy of surface can elevate or depresses the contact area with a solid. Therefore, certain liquids have greater capillary with the dentin lumps and with the surface of the enamel allowing a greater decalcification than other liquids used as beverage.[5,6]

Chronic exposure to extrinsic and/or intrinsic acids with a low pH leads to dental erosion. The erosion of the enamel is characterized by a centripetal dissolution that leaves a demineralized area behind. A significant number of mineral ions can be removed from hydroxyapatite prisms without destroying its structural integrity.[7]

The term "erosion" derives from the Latin verb erodere, erosi, erosum (to corrode), describes the process of gradual demineralization of the surface of a substance, usually by electrolytic or chemical methods. The clinical term "dental erosion" or erosium dentium is used to describe the physical result of chronic, localized and painless pathological loss of hard dental tissue by chemical action and/or chelation of an acid without bacterial intervention.[8]

As a progressive destructive process, it usually goes unnoticed by the patient and the dentist, until it causes sensitivity or presents an aesthetic commitment.[9] The main etiological factor of exogenous erosions are the acidic components of the diet and beverages, mainly those that are industrialized, since presenting them as a quick and effective alternative to meet certain personal needs, have become a common component in the population diet, and a significant increase in this injury can be observed, mainly in children and adolescents.[10]

Initially, the most common clinical feature of erosion by intrinsic factors, is the loss of enamel brightness, showing an U- shaped injury with no sharp angles. When compromising dentin, it causes sensitivity to cold, heat and osmotic pressure. When it is presented in restored teeth, restorations become prominent, projecting on top of the dental surface. [11]

Dental erosions can also be classified according to the clinical severity of lesions in:
Class I: Surface injury with exclusively enamel commitment.
Class II: Localized lesions affecting less than 1/3 of the surface and compromising dentin.
Class III: Generalized lesions with more than 1/3 of the surface compromising dentin.[9]

The acids responsible for erosion are not produced by the intraoral flora but come from is intrinsic and/or extrinsic factors. [9]

Intrinsic factors.
Dental erosion due to intrinsic factors is caused by gastric acid that reaches the oral cavity as a result of gastroesophageal vomiting or reflux. Since the clinical manifestation of dental erosion does not occur until gastric acid has acted on chronic hard tissue regularly for a period of several years, dental erosion caused by intrinsic factors has been observed only in those diseases which are associated with chronic vomiting or persistent gastroesophageal reflux for a long period of time. [9,12]

Extrinsic factors. The extrinsic causes of dental erosion can be grouped under the categories: environmental, diet, medication, and lifestyle. Environmental factors mainly involve exposure to acid vapors by workers in some low-pH chlorinated factories and pools due to inadequate maintenance. [13]

Dietary factors have received the most attention and are those that affect a larger segment of the population. With regard to the acidic diet, a particular attention has been given to fruits and drinks, many of these have been evaluated for their erosive potential in the laboratory and in experiments with animals. [15]

It has long been known that acidic beverages and foods can soften hard dental tissues. The erosive activity of citric acid, malic, phosphoric and other acids contained in beverages and meals has been demonstrated in many in vitro, in situ and in vivo studies. In addition, a number of studies indicate that the erosive potential of an acidic drink does not depend entirely on its pH but depends to a large extent on its measurable acid content (buffer capacity) and the calcium-chelation properties of food and beverages, as they efficiently capture the released calcium. The lower pH of the drink, the longer it will take for saliva to neutralize the acid. [9,19]

Shaking carbonated beverages in the mouth, quickly moving the liquid from the front to the back of the mouth to reduce the gas and avoid the unpleasant sensation it produces in the throat, is the second leading cause of wear and tear caused by erosion. [15]

Thus, in the category of non-alcoholic beverages we can highlight sodas, hydrating beverages and energize teas, a situation that poses a danger to oral health, because of its acidizing components (citric acid, phosphoric, among others), which in addition to improving the profile of the taste and aroma of each drink, are erosive for dentary enamel, because with the pH they present, enough potential is generated to remove minerals from the adamantine surface (critical pH of 5.2 – 5.5 for hydroxyapatite and 4.5 for apafluorite) [16,17]

Surface micro-hardening is a physical method used to evaluate the erosive effect and can be defined as the surface resistance exerted by a material to be scratched or to suffer permanent deformations on its surface. The most commonly used tests to assess surface hardness are Brinell, Rockwell, Vickers and Knoop. [18]

Knoop and Vickers scales are the most common for measuring the surface microhardness of the enamel due to its similarity in the value scale. The Vickers hardness test uses a square-based diamond pyramid penetrator with an angle of 136° between opposite faces at the vertex, which is pressed within the surface of the part to be analyzed using a prescribed force. The time for the initial application of the force is 2 to 8 seconds, and the test force is maintained for 10 to 15 seconds. After the force has been removed, the diagonal lengths of the penetration are measured and the arithmetic mean is calculated. The hardness number of Vickers HV is given by: HV-Constant x test force /surface or indentation area. [19,20]

Therefore, the objective of this study is to determine the microhardness of the enamel and dentin on the dental surface and the erosive potential of six beverages which are commonly consumed in humans

MATERIAL AND METHODS

The present study is experimental. When enamel and dentin specimens (samples) are submitted to the action of different low pH substances (risk factors) for three days, with a contact period of the samples of 15 minutes per day, by evaluating the surface microhardness of the enamel.

Experimental in vitro study that included 32 healthy first upper premolars with complete root formation, free of tooth decay, restorations, cracks, fractures and fluorosis, stored under an isotonic solution, previously tooth brushed with distilled water. They were placed in ultrasonic cleaning to remove any remaining organic tissue. To carry out the experiment, 2 samples were taken, to define the indentation points in enamel and dentin and 5 samples for each of the six groups: Group 1: Physiological serum (Pisa plas), Group 2: Cranberry juice (From the Valley Antiox), Group 3: Natural Yogurt (Yoplait), Group 4: Natural orange juice , Group 5: Coca cola (Coca-cola company), Group 6: Natural lemon juice. (Table 1).

The teeth were cut, first circumferentially at the cervical level and vestibule-palatine to obtain 2 faces of the specimen with a diamond disc NTI (Primecut SL) diameter 190, thickness 20 microns (Newtechnology Instruments) NTI –Kahla in Camisch 3 -07768 (Kahia), mounted on a micro motor (Fosteralloy Grinder, Ray Foster Dental Equipment, USA).

Subsequently, the study population was placed randomly on two-sided self-polypolirizable acrylic cylindrical discs, 20 mm in diameter by 0.3 mm thick (Nic Tone MDC Dental LLC 17800 S. Main SL.201, Gardena CA USA), which were integrated into each of the groups. They were placed in physiological serum and stored, in

STATISTICAL ANALYSIS

The results of Vickers hardness measurements are expressed in mean, standard deviation and range and the distribution of the data was determined by the Kolmogorov Smirnov test. For comparison of measurements before and after exposure to commonly used beverages, the paired Student T-test was used for parametric data and the Wilcoxon test for nonparametric data. As for the comparison between the initial hardness of tooth enamel with that of dentin, Student's T-test was used and for comparison of initial measurements between the various beverages as well as the difference in Vickers hardness before or after exposure the Kruskal-Wallis test and Dunn's post-hoc analysis was applied.

Table 1. ***Group distribution***

Group	Substance	Ph
Group 1	Physiological serum (Pisa plas)	5.5
Group 2	Cranberry juice (From antiox valley)	2.6

Group 3	Natural yogurt (Yoplait)	4.5
Group 4	Orange juice	3.2
Group 5	Coke (Coca cola company)	2.8
Group 6	Lemon juice	2.3

Reyes, A., Ojeda, F, (2019)

RESULTS

The results of this study measuring Vickers Hardness in tooth enamel (Table 2), showed an initial average of 326. Subsequently, the specimens were exposed to the different common beverages included in the study and it was found that all beverages except orange juice produced a statistically significant decrease in Vickers hardness in tooth enamel ($p<0.0001$), obtaining the greatest difference when testing cranberry juice with a pH of 2.6 (130.3), followed by yogurt (89.89) and lemon juice (86.3), both with a pH of 4.5 and 2.3 respectively. Orange juice showed the lowest difference (3.4), and no significant statistical difference was presented (p-0.7689). It is worth mentioning that a significant or plateau change was detected between the final measurements of all the beverages used and also comparing their difference before and after ($p<0.0001$).

On the other hand, the results obtained for the Vickers hardness in dentin (Table 3), showed an initial average of 67.7, which is lower than the initial average obtained in tooth enamel ($p<0.0001$). Subsequently, as it was already mentioned, the samples were in contact with the different beverages and it was found that they all produced a statistically significant decrease in Vickers hardness in dentin ($p<0.0001$). The liquid in which a major change was observed was Lemon Juice (39.8) followed by cranberry juice (21) and orange juice (20.8) (p-0.7689). The cola soda got the smallest difference (3.2). Similar to enamel, significant statistical difference was detected between the final measurements of all beverages used and their difference before and after exposure ($p<0.0001$).

Table 2. *Comparison of vickers hardness of tooth enamel. Martinez, R. (2019)*

Drink	Initial	End	Difference	Q
	Mean (Range)			
Saline	460.9 x 308.3 (75.6 – 1232)	344.3 .134.8 (101.6-675.4)	116.7 .224.5 (-95.846.7)	0.0060**
Cranberry Juice	362.6 - 186.2 (194.4-1051.6)	232.6 -46.9 (95.7-308)	130.3 .184.7 (-79.2-833.5)	<0.0001 **
Yogurt	299.7 .62.4 (49.7-454.1)	209.9 -49.7 (37.5-291.3)	89.8 x 70 (-120.3-249.9)	<0.0001 **
Orange Juice	281.7-51.7 (75.2-417.6)	278.3 x 53.5 (204.2-406.4)	3.4 .73.7 (-176.6-213.4)	0.7689*
Cola Refreshment	293.3 x 68.5 (84.8-431.7)	230.1 x 40.3 (118.2-340.8)	63.2 .71.6 (-89.1-184.5)	<0.0001 *
Lemon juice	257.7 .78.5 (58-383.2)	171.4 x 39.5 (60-252.9)	86.3 .86.5 (-187.6-233.4)	<0.0001 **
DE: Standard deviation. *T of Paired Student, **Wilcoxon Test, <0.0001, Kruskal-Wallis-Dunn Test				

Table 3. *Comparison of Vickers hardness of dentin before and after exposure to human drinks.*
Martinez, R. (2019)

Drink	Inicial	Final¶	Difference¶	P
	Mean ±SD (Range)			
Saline solution	69.6 ±47.7 (46.6-347.7)	57.4 ±6.4 (44.6-70.1)	12.2 ±47 (-7.9-287.6)	0.0004**
Cranberry juice	64.6 ±28.2 (42.1-187.3)	43.6 ±4.1 (36-54.5)	21 ±3 (-0.9-141.5)	<0.0001**
Yogurth	49.1 ±3.5 (42.1-57.3)	34 ±5.4 (16.6-44.7)	15.1 ±6.1 (1.7-34.8)	<0.0001*
Orange juice	58.9 ±7.5 (47.3-74.9)	38 ±7.1 (24.2-55.7)	20.8 ±10.4 (4.5-45.2)	<0.0001*

DISCUSSION

Dental erosion is the progressive and irreversible loss of dental hard tissue by a chemical process that does not involve the action of bacteria. [22]

At the end of this study, it was found that there was a significant loss of surface hardness of enamel and dentin by exposing the samples to different types of solutions of frequent consumption in humans.

There is a report from an in vitro study in which the erosive effect of three carbonated beverages on the surface of tooth enamel was evaluated. Comparison between study groups showed that carbonated beverages have an erosive effect measured by varying surface microhardness. The Kola Real® first-way drink had a similar erosive effect as the carbonated Coca Cola drink®, while Inca Kola®, compared to the previous ones had the least erosive effect and this difference was statistically significant. [6]

Our results were similar to those made by other authors, which mention that the largest groups of beverages with erosive potential are carbonated non-alcoholic beverages commonly known as soda or soft drinks such as fruit refresher fruit juices. Any drink or food with a critical pH value of less than 5.5 can act as an erosive agent and demineralize the teeth, this may vary depending on the concentrations of calcium and phosphate ions in saliva. In people with low salivary concentrations of calcium and phosphate, the critical pH may be 6.5. The time that saliva needs to neutralize and/or remove acids from dental surfaces is approximately five minutes, but varies by individual, amount and composition of saliva. [24]

In other study, they concluded that carbonated beverages contain carbonic acid formed by carbon dioxide in solution, when carbon dioxide disappears from the beverage, the pH remains acidic. This indicated that carbonated beverages have their inherent acidity due to other acids that are added to stimulate the taste and counteract the sweetness. [14]

As well as other authors, they observed that a carbonated tail drink demineralized the teeth between 3 and 336 hours of exposure. [25]

Another research found that when assessing the erosive potential of some fruit juices and water drinks by the amount of calcium released by the enamel; cola drinks and orange juice caused the greatest demineralization. [26]

In our study, our results are also far from those obtained by various authors in terms of carbonated beverages, perhaps from the times exposed of the samples in the solutions.

The lower erosive effect was produced by yogurt pH 4.04, this result differs from that found by Rytomaa et al., who concluded that pH drinks over 4 does not caused erosion in vitro. Differences in study results can be attributed to the type of test used, analyzed the surface profile, while this study evaluated surface micro-strength. [27]

In enamel, the average value on the Vicker`s scale is 250 to 360 VHN. In dentin, the average value is 50 to 60 H VHN. However, deviations from the standard value show significant variations in enamel, but less pronounced in dentin. For example, Craig and Peyton reported that the hardness of the enamel has a range of 344 +/- 49 to 418 +/- 60 VHN; Collys et al, 369 +/- 25 to 431 +/- 35 VHN; Wilson and Love from 263 +/- 26 to 327 +/- 40 VHN. The micro-strength of the enamel in the occlusal area varies from 359 to 424 HVV and in the cervical area varies from 227 to 342 HV. These variations can be produced due to their chemical and histological composition, the preparation of the dentary organ or an error in penetration reading.

CONCLUSIONS

All solutions showed a decrease in hardness in dentin and enamel, lemon juice showed the biggest difference, followed by cranberry juice and orange juice, while the cola soda got the least statistically significant difference.

Tooth enamel has a hardness four times greater than dentin, in this study a statistically significant variation was detected between the final measurements of all the beverages used and their difference before and after exposure. With these results it was concluded that the enamel also suffers erosion when coming into contact for a certain period of time with the types of solutions of frequent consumption in humans.

Significant loss of surface microhardness of enamel and dentin was found in the samples exposed to different types of solutions of frequent human consumption, such as physiological serum, lemon juice, orange juice, cranberry juice, Coca-Cola, and yogurt. In more acid pH of the solution, a higher degree of demineralization of the enamel was found.

RECOMMENDATIONS

Due to its wide impact, the prevention of dental erosion should include a healthy food regimen, avoiding the consumption of acidic foods and beverages, the use of oral rinses with antacids such as baking soda, stimulate salivary flow and topical applications of fluoride, since the protective effect of fluoride has been evidenced, so it is advisable to use toothpastes that release of this element. In addition, in patients at high risk of erosion it is recommended to wait at least 15 to 20 minutes after consuming any drink or acidic fruit to begin tooth brushing. This is because there is a synergistic action of acid and tooth brushing which increases the potential for developing erosive lesions. A dietary modification is important in prevention. The Phosphopeptide casein phosphate and amorphous calcium phosphate complex is thought to maintain a concentration of calcium ions and phosphate high enough to promote enamel remineralization. Another method to improve the resistance of teeth to acid erosion is the application of adhesive agents for sealing of pits and fissures.

Order to control the moisture of the samples and prevent their drying, as this modifies the physical properties of enamel and dentin. Subsequently, the sample surfaces were polished with no water sandpaper. no. 600, 1200 and 2000 (FANDELI, Mexico), until achieving a surface as parallel as possible between the surface to be evaluated and the base of the dispositive to measure the micro-hardeness avoiding distortions in indentations. Four points of initial indentation in the crown (cervical, medium, cusp and groove) (Figure 1), by means of a micro durometer (Leitz Wetzlar Germany) in both enamel and dentin, under a load of 300 g for 15 seconds for a total of 16 per surface (enamel and dentin) and obtaining an average value of surface microhardness by measuring the diagonals that appear in the information in Vickers units (Table Buehler Tables for Knoop and Vickers Hardness Numbers) and obtaining the measure of microhardness in Kg/mm2.

Microscopic photography (OLYMPUS 100x scale) was subsequently used. (Figure 2 and 3), of the indentations to obtain a record of each of them, giving a total of 960 indentations, were encoded (Table 1) and stored in physiological solution, then submitted to one of 6 acid solutions with different pH and washed with physiological serum at intervals of 5 minutes in the morning, afternoon, and night giving a total of 15 minutes per day. This was done for 3 consecutive days, with a total of 45 minutes of exposure to the solutions for each sample, then the samples were indented again in the 16 points indicated per face (giving a total of 32 identifications per specimen), data were taken to compare the micro-strength of the enamel and dentin before and after being exposed to such solutions (Figure 4).

The waste of the samples used was deposited in a red container, which was collected by a company specialized in hazardous biological waste

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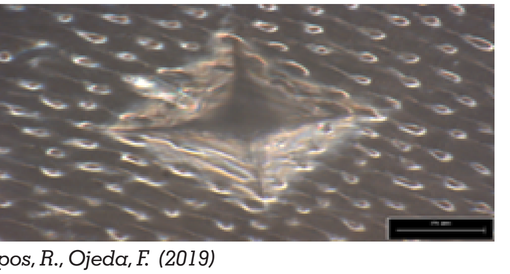
Figure 1. ***Representative scheme of the initial and final indentation points in the crown in enamel and dentin (cervical, medium, cusp and groove).***

Reyes, A., Ojeda, F. (2019)

Figure 2. ***Optical microscopy photography of the indentation produced by the enamel microdurometer, (sample2.4), magnification 100x, scale 25mm under a load of 300 g for 15 seconds.***

Campos R., Ojeda, F. (2019)

Figure 3. ***Optical microscopy photography of the indentation produced by the microdurometer in dentin, (sample5.1), magnification 100x, scale 25mm under a load of 300 g for 15 seconds.***



Campos, R., Ojeda, F. (2019)

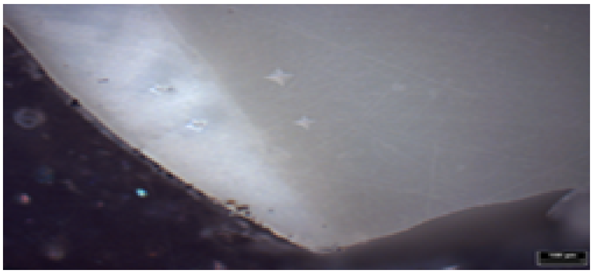


Figure 4. *Optical microscopy photography in which the indentation points in enamel and dentin in cervical area, magnification 10x., scale 100 m are observed*

Campos, R., Ojeda, F. (2019)

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