OVIDIUS University

DOOCTORAL SCHOOL OF MEDICINE

## Doctoral Dissertation

ABSTRACT

# Modifications of the Hard Tissues of the Oral Cavity Due to Internal and External Chemical Exposure

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# TABLE OF CONTENTS

## Introduction

Teeth do not have the ability to function as separate organs. Instead, they work together as a collective unit, known as dentition, which is unique to each species. Scientists have recently achieved a thorough understanding of the mechanisms involved in the production and development of teeth.

Currently, investigations are underway to clarify how genetic variables influence tooth development.

The purpose of this study is to emphasize the morphophysiological disruptions of the hard dental tissues, specifically the enamel, caused by different chemical aggressors known as demineralizing agents.I desire the study to possess originality in both the chosen theme and the employed methodology.

Additionally, I hope that the study will serve as a wake-up call to minimize the detrimental effects of welding fumes, ultimately leading to the enhancement of oral cavity health.

This work comprises three in vitro experimental studies conducted on extracted teeth that were free from any conditions that could have affected the results. Each study had its own methodology and aimed to test the hardness of the enamel after exposure to different chemical factors, such as acids and welding fumes.

# CURRENT STATE OF KNOWLEDGE

## Chapter I - Introduction to the Principles of Embryology and Morphology of Dental Enamel

The morphological evidence of teeth emerges during the sixth week of intrauterine development, as epithelial bands derived from the ectoderm begin to build the future maxillae and other teeth. The initial emergence of deciduous teeth buds is succeeded by the development of permanent teeth buds.

Tooth creation entails the infiltration of mesenchymal tissue and the division of the dental lamina. The progressive growth of the dental lamina encompasses the commencement of temporary tooth creation, the initiation of successor permanent tooth formation, and the inception of permanent molar formation.

After the bud stage, the cup stage begins, during which the dental buds fold inward and the tooth-forming elements and supportive tissues undergo histodifferentiation. The enamel organ, dental papilla, and follicular sac undergo formation, subsequently giving rise to the structural components of the teeth. The bell stage signifies a higher level of histological development in teeth, characterized by intensive processes of histodifferentiation and morphodifferentiation.

At this stage, the enamel-dentin junction is established, marking the boundaries of the future tooth crown. Amelogenesis, the process of enamel production, starts after dentinogenesis and consists of two stages: the secretion of the enamel matrix and the maturation of the enamel.

Ameloblasts play a role in the secretion, arrangement, and uptake of the organic matrix. Their life cycle consists of many stages, such as secretion, maturation, and protection. After the enamel matrix is secreted, it undergoes mineralization. During the maturation stage, the enamel becomes completely mineralized and calcified. Ameloblasts undergo a polarity change to create the decreased enamel epithelium, which serves as a protective layer for the enamel.

Ultimately, the process of morphodifferentiation of teeth entails the interplay among ameloblasts, odontoblasts, and other cells implicated in tooth production, culminating in the growth and creation of fully functional teeth.

## Chapter II – Physical, Chemical, and Morphofunctional Characteristics of Dental Enamel

Dental enamel is a mineralized layer that rests on the underlying dentin and covers the dental crown. The thickness of the enamel can vary depending on the coronal area, reaching up to 2.6 mm at the level of the cusps. Enamel is essential for protecting the dentin and nerve endings from harmful stimuli and for the tooth's resistance to mechanical stress. At the level of the occlusal surface, the enamel features grooves that aid in the grinding process, but also promote the accumulation of food debris, leading to carious processes.

Dental enamel has the highest hardness of all the tissues in the body, rated between 5 and 8 on the MOHS scale. This can create difficulties in therapeutic interventions, requiring special tools for processing. The enamel has a variable color, ranging from off-white to blue-gray, due to different thicknesses and mineralization. At the junction between the crown and the root, in the area of the dental neck, the color of the enamel can vary from yellow to brown, due to the thinning of the enamel that allows the dentin to be seen. The chromatic nuances of enamel contribute to the individuality of each person's features and can be influenced by external factors.

The chemical composition of enamel is predominantly mineral, consisting of calcium phosphates and fluorapatite. Organic substances, such as amino acids and soluble proteins, make up only 1% of the composition.

The enamel does not have a uniform structure, containing a higher amount of water and organic substances in the deeper areas. The enamel prism represents the fundamental structural unit of enamel, with an oblique orientation to withstand masticatory pressures. The horizontal undulations of the prisms lead to the formation of Hunter-Schreger lines, contributing to the optical phenomenon of the tooth.In general, dental enamel is a good insulator against harmful stimuli and responds differently depending on their intensity and the area of application.

Knowing the physical, chemical, and morphofunctional characteristics of enamel is essential for diagnosing and treating dental conditions, such as carious processes or structural defects. It is important to pay special attention to enamel care for maintaining oral health and preventing dental issues.

## Chapter III – Physiological Concepts of Enamel

Under the influence of a wide range of favorable factors (diet – consumption of acidic foods and beverages, chronic use of tobacco and alcohol, presence of tartar, orthodontic treatments) as well as determining factors (salivary pH, uncontrolled presence of bacterial plaque), the hard dental substances, especially enamel, due to the direct contact it has with the external environment of the oral cavity, constantly undergoes processes of demineralization and remineralization.

Knowing this fact, the imbalance between these two phenomena, with the scale tipping in favor of the demineralization process in its early stages, gives rise to the initial enamel lesions (the minimal early stage of possible carious processes) – "white spot" – without having such a major impact as to halt the remineralization phenomenon.

Although enamel lesions become visible macroscopically, the demineralization processes began long before this, at the ultrastructural level, through the dissolution of hydroxyapatite crystals.

Physiologically, our body reacts to aggressors that demineralize enamel and initiates various processes to remineralize the hard dental substance, with the help of salivary factors.

## Chapter IV – Etiopathogenesis of Dental Erosion

Dental erosion is defined as the chronic and localized process of loss of the hard dental surface, a loss resulting either from its dissolution caused by the acid imbalance in the oral cavity, but which does not involve bacterial factors (plaque / tartar / carious processes), or from chelating substances that come into contact with the hard dental surface.

Two types of dental erosion can be distinguished:
- *Of extrinsic cause* – a case in which erosive lesions occur due to acidity from beverages, food, chronic medication use, or the surrounding environment. (mediul de lucru).
- *From an intrinsic cause* – where the factor of dissolution comes from within the body, referring to hydrochloric acid in the stomach and duodenum.

Erosion lesions appear as a lack of hard substance (enamel) in a lenticular, oval-round shape, resulting in the thinning of the enamel layer over time, and potentially even the disappearance of the incisal edge (in the case of affected incisors). If they appear at the cervical level of the teeth, their deepening can lead to early exposure of the dentin or even the dental pulp, causing marked dental sensitivity and even inflammation of the pulp tissue, potentially leading to the loss of the tooth's vitality, in which case endodontic treatment becomes necessary.

If dental erosion affects the occlusal surface, over time it leads to the collapse of the occlusion, resulting in the emergence of occlusal disharmonies and impacting the condition of the temporomandibular joint and even the oro-facial musculature.

The localization of erosive lesions depends on the acidic source (exogenous/endogenous), while the depth and dynamics of their evolution depend on the presence of risk factors and the frequency and duration of exposure to acid attack.

Exogenous erosions are primarily located on the vestibular surfaces of the maxillary anterior dental group, while endogenous lesions mainly involve the palatal and occlusal surfaces of the maxilla, as well as the lingual and occlusal surfaces of the mandible.

**Therapeutic possibilities and recommendations in the treatment of lesions caused by dental erosion**

The easiest stage in the treatment of dental erosions, in the early phase, consists of eliminating the risk factors. For the therapy of lesions of endogenous origin, the underlying condition (e.g., reflux disease) must be properly treated in the initial phase. To minimize the development of exogenous erosions, it is necessary to discuss, analyze, and adapt the nutritional needs and habits of the patient, aiming to reduce the intake of acidic food or beverages.

It is preferable to consume drinks using a straw to eliminate the stagnation of acids on the surface of the teeth.Dental hygiene is not recommended to be performed immediately after consuming erosive products; instead, it is advised to wait for a period of 30 to 60 minutes after consumption before brushing your teeth. It is also recommended to avoid brushing with hard or abrasive devices or products and to use an appropriate brushing technique, without applying pressure or making harsh movements on the dental surface.

Cases of extensive hard substance loss, either on the surface or in depth, should be appropriately addressed in the dental office, with several therapeutic options available depending on the clinical situation presented, aiming at the functional and aesthetic restoration of the affected teeth.

For the most accurate choice of therapeutic method, it is advisable to conduct a thorough evaluation ("tooth-by-tooth") of the dental units and to indicate the individual restoration technique based on the structural integrity of the teeth and the necessity of removing affected structures. Additionally, the selection of restorative materials can be influenced by the presence of parafunctional habits. It is recommended to use combined materials, especially for total rehabilitations.

Another option would be to proceed with complete smoothing and fixed, partially mobile prosthetics of the dental arches, preserving the dental stumps and subsequently covering them to help maintain proprioception, reduce/slow down ridge resorption, ensuring the stability and support of the prosthesis in the prosthetic field, which can also be improved by applying special systems (slides, clips, magnets) to increase retention in the field.

## Chapter V. The Effect of Welding Gases on the Upper Respiratory Tract

Welding procedures generate contaminating factors that pose significant risks to the environment and represent a danger in the workplace. Metal particles and fumes generated during welding operations pose health risks for workers, as both short-term and long-term exposure to welding fumes has been linked to a variety of adverse effects on overall health according to epidemiological studies.

Recent scientific discoveries, whether in the form of epidemiological investigations, cross-sectional studies, or case reports, focus on the correlation between metals used in various

welding processes, health conditions, and emerging diseases, serving as a statement to advance preventive measures that influence and benefit the health of welders.

Among a variety of health risks affecting workers in this field, there is one that stands out from the others, namely, the impact of fumes generated during the welding process on the respiratory system, emphasizing the description of effects on the upper respiratory tract. (36)

**V.1 The Premises of Respiratory Impairment**

If the metal particles and residual gases resulting from the welding process (which are usually smaller than 1 micrometer) manage to penetrate the respiratory tract, some of them are capable of reaching the level of the pulmonary alveoli, at which point one of two situations can occur: either they are destroyed, phagocytized by the defense system of the respiratory apparatus and then dispersed throughout the body; or they stagnate at the pulmonary level and in the nearby lymph nodes, leading to the emergence of a wide variety of respiratory phenomena.

Inflammatory changes in the respiratory tract can be mild or moderate; however, chronic exposure to these toxins leads to the onset of respiratory symptoms, with the emergence of pulmonary dysfunction—difficulty in inhalation (restrictive issues), difficulty in exhalation (airway obstruction), and even the inability to dissociate oxygen from the inhaled air for transport by blood cells to the pulmonary capillaries.

**V.2. Conditions caused by welding fumes in the upper respiratory system**

Regardless of the body's and respiratory system's cleaning and defense mechanisms (mucus, mucociliary mechanism), nanoparticles appear to be capable of translocation from the lungs to various organs in the body, such as the liver, spleen, heart, and very likely others. These phenomena occur through the process of endocytosis, which is the responsibility of the alveolar epithelium.

Tables II-IV presented below briefly illustrate the toxic effects that welding fumes, gases, and organic vapors can have, along with their sources, on overall health, with those causing dysfunctions in the respiratory system highlighted in color.

**Table 1**
**Source and Health Effect of Welding Fumes**

| ***FUME TYPE*** | ***SOURCE*** | ***HEALTH EFFECT*** |
|---|---|---|
| Alluminium | Aluminum component of some alloys, e.g., Inconels, copper, zinc, steel, magnesium, brass and filler materials. | Respiratory irritant. |
| Beryllium | Hardening agent found in copper, magnesium, aluminum alloys and electrical contac | "Metal Fume Fever." A carcinogen. Other chronic effects include damage to the respiratory tract. |
| Cadmium Oxides | Stainless steel containing cadmium or plated materials, zinc alloy | Irritation of respiratory system, sore and dry throat, chest pain and breathing difficulty. Chronic effects include kidney damage and emphysema. Suspected carcinogen. |

| | | |
|---|---|---|
| Chromium | Most stainless-steel and high- alloy materials, welding rods. Also used as plating material. Converts to hexavalent chromium during welding. | Increased risk of lung cancer. Some individuals may develop skin irritation. Some forms are carcinogens (hexavalent chromium). |
| Copper | Alloys such as Monel, brass, bronze. Also some welding rods. | Acute effects include irritation of the eyes, nose and throat, nausea and "Metal Fume Fever." |
| Florides | Common electrode coating and flux material for both low- and high-alloy steels. | Acute effect is irritation of the eyes, nose and throat. Long-term exposures may result in bone and joint problems. Chronic effects also include excess fluid in the lungs. |
| Iron Oxides | The major contaminant in all iron or steel welding processes. | Siderosis – a benign form of lung disease caused by particles deposited in the lungs. Acute symptoms include irritation of the nose and lungs. Tends to clear up when exposure stops. |
| Lead | Solder, brass and bronze alloys, primer/coating on steels. | Chronic effects to nervous system, kidneys, digestive system and mental capacity. Can cause lead poisoning. |
| Magnease | Most welding processes, especially high-tensile steels. | “Metal Fume Fever.” Chronic effects may include central nervous system problems. |
| Mollybdenum | Steel alloys, iron, stainless steel, nickel alloys. | Acute effects are eye, nose and throat irritation, and shortness of breath. |
| Nickel | Stainless steel, Inconel, Monel, Hastelloy and other high-alloy materials, welding rods and plated steel. | Acute effect is irritation of the eyes, nose and throat. Increased cancer risk has been noted in occupations other than welding. Also associated with dermatitis and lung problems. |
| Vanadium | Some steel alloys, iron, stainless steel, nickel alloys. | Acute effect is irritation of the eyes, skin and respiratory tract. Chronic effects include bronchitis, retinitis, fluid in the lungs and pneumonia. |
| Zinc | Galvanized and painted metal. | Metal Fume Fever. |

**Table II**
**Source and Health Effect of Welding Gases**

| ***GAS TYPE*** | ***SOURCE*** | ***HEALTH EFFECT*** |
|---|---|---|
| Carbon Monoxide | Foormed in the arc | Absorbed readily into the bloodstream, causing headaches, dizziness or muscular weakness. High concentrations may result in unconsciousness and death |
| Hydrogen Fluoride | Decomposition of rod coatings | Irritating to the eyes and respiratory tract. Overexposure can cause lung, kidney, bone and liver damage. Chronic exposure can result in chronic irritation of the nose, throat and bronchi. |
| Nitrogen Oxides | Formed in the arc | Eye, nose and throat irritation in low concentrations. Abnormal fluid in the lung and other serious effects at higher concentrations. Chronic effects include lung problems such as emphysema. |
| Oxygen Deficiency | Welding in confined spaces, air displacement by shielding gases | Dizziness, mental confusion, asphyxiation and death. |
| Ozone | Formed in the weldin arc, especially during plasma arc, MIG and TIG process. | Acute effects include fluid in the lungs and hemorrhaging. Very low concentrations (e.g., one part per million) cause headaches and dryness of the eyes. Chronic effects include significant changes in lung function. |

**Table III**
**Source and Health Effect of organic Vapours from Welding Process**

| *GAS TYPE* | *SOURCE* | *HEALTH EFFECT* |
|---|---|---|
| Aldehydes (such as Formaldehyde) | Metal coating with binders and pigments. Degreasing solvents | Irritant to eyes and respiratory tract. |
| Diisocyanates | Metal with polyurethane paint. | Eye, nose and throat irritation. High possibility of sensitization, producing asthmatic or other allergic symptoms, even at very low exposures. |
| Phosgene | Metal with residual degreasing solvents. (Phosgene is formed by reaction of the solvent and welding radiation.) | Severe irritant to eyes, nose and respiratory system. Symptoms may be delayed. |
| Phosphine | Metal coated with rust inhibitors. (Phosphine is formed by reaction of the rust inhibitor with welding radiation.) | Irritant to eyes and respiratory system, can damage kidneys and other organs. |

## Chapter VI – The Effect of Welding Fumes on Soft Structures in the Oral Cavity

Occupational health associations estimate that approximately 5 million welding workers are exposed to welding fumes, which contain components such as chromium or nickel.

This chronic exposure to these substances can have serious consequences for the health of these workers, as these components are considered essential for the corrosion resistance of metals.
Environmental contaminants can cause genetic changes in the human body, leading to mutations at the cellular level. Exposure to chromium and nickel can lead to enzyme inhibition and cellular oxidative stress, resulting in serious conditions such as cell death.

The preventive measures involved in monitoring the health of workers exposed to these contaminants are extremely important for preventing negative health effects on them. An important biomarker for this is the micronucleus test performed on the oral mucosa, which can highlight any genetic damage caused by exposure to welding fumes. A recent study conducted on welding workers in Brazil confirmed the frequency of mutagenic abnormalities and the toxicity of welding fumes on their health.

The study analyzed the effects of contact between metals from welding processes and the oral cavity, highlighting an increase in nuclear anomalies, causing tissue necrosis and apoptosis. It has been found that the body reacts to these metals, and hydroxyl radicals can play a role in the defense and repair of cellular molecules. The results of the study showed that exposure to welding fumes may be responsible for damage to the human genome and even for the onset of oral cancer.

Although a direct correlation between welding fumes and oral cancer could not be established, the study confirms an increased tendency for abnormal cells to develop in individuals exposed to these substances, compared to those who are not exposed.

Considering the complex composition of the oral cavity, including dental elements, it is important to conduct further studies to understand the consequences of the contact between these and welding fumes on the entire organism.

Thus, research must also focus on the impact on teeth, as possible components of the damage caused by exposure to welding fumes, in order to obtain a more complete picture of the consequences of this phenomenon on oral and overall health.

# PERSONAL CONTRIBUTION

## Chapter I. The Hardness of Dental Enamel

The hardness of dental enamel is the ability of this hard tissue to withstand mechanical forces without deforming. Dental enamel has a hardness comparable to that of medium steel and precious metals.

On the Mohs scale, it reaches level 5, being the strongest tissue in the human body. This is due to a complex organization of the component structures, which optimizes resistance to physical, chemical, and thermal actions.The enamel contains on average 96% mineral substances, with hydroxyapatite as the main mineral.

This develops even during intrauterine life and continues to grow long after birth and childhood. Nevertheless, the qualities of enamel can deteriorate in the absence of optimal development conditions, such as changes in the pH of the oral cavity or alterations in the natural biofilm of the oral cavity.

## Chapter II. The Motivation for Choosing the Topic

Through this study, I aim to highlight the morphophysiological disturbances of the hard dental structures (enamel) under the influence of various chemical aggressions (demineralizing factors).

I wish for the study conducted to be original, both in terms of the theme addressed and the techniques used, and to complete the picture of the harmful impact of chemical aggressions on the hard dental structures.

Furthermore, the work itself should serve as a wake-up call to limit, as much as possible, the exposure of welding workers to these harmful factors (welding fumes), with the aim of improving overall health as well as oral health.

## Chapter III. General Methodology

The general methodology used in the studies presented in this paper is outlined, which were conducted on recently extracted teeth, but with periodontal or orthodontic pathology. Patients were selected from the Perfect Dent Dental Medicine Center in Constanța, and the exclusion criterion was the presence of carious processes.

After the testing of the teeth, they were cut transversely and longitudinally with a diamond disc and then fixed in acrylic resin and polished to be prepared for testing.

The hardness of the enamel of the tested teeth was determined using a microhardness tester HV-1000 (fig. III.1), where the processed samples were secured to avoid measurement errors. The Vickers test, developed by Smith and Sandland, was designed to measure the hardness of materials, being easier to use than other hardness tests.

This test can be used on all types of materials, including biological samples, and the results are given in Vickers Pyramid Value. (HV). Hardness is not a property of the material, but an empirical value interpreted according to the experimental method used. This method can be applied to determine the hardness of various materials, including dental enamel.

**Figura III.1 – D**evice for microhardness measurements. Microdurimeter HV-1000.

# Chapter IV - STUDY I

## Analysis of enamel hardness in patients with gastroesophageal reflux

### IV.1 Introduction

The study conducted is an in-vitro one, aiming to assess the impact on the enamel surface of recently extracted teeth, following pH changes simulated in the study methodology. The obtained results will then be compared with previously reported findings in the specialized literature, thus creating a context for discussions regarding the oral health of individuals suffering from this gastroesophageal reflux pathology.

### IV.2 Material and Method

The basic principle of enamel hardness testing consists of pressing a diamond pyramid penetrator onto the surface of the sample for a specific period of time and at a certain pressure. After its release, the hardness is calculated by measuring the diagonal of the mark made by the penetrator.

The study utilized 6 recently extracted molars (M1, …, M6). Initially, for each sample, the hardness values of the dental enamel were determined (following the analysis of 10 indentations per sample), along with the average value and standard deviation. The average hardness values were compared using the One-Way ANOVA test, at a significance level of $\alpha = 0.05$. Since the probability associated with the test statistic value was $p > 0.05$, it was considered that we cannot speak of significant differences between the analyzed mean values, which is why the 60 values determined for the 6 molars (M1, …, M6) were included in the values of the Control group.

Each wisdom molar was sectioned longitudinally, thus forming 12 samples (M1A, M1B; …; M6A, M6B), which were grouped into two batches (Batch 1, Batch 2), each

containing 6 samples. Six solutions were prepared with the following pH values: 6.5, 5.5, 5.0, 4.5, 3.5, and 2.5, solutions designed to simulate the pH of gastric juice and saliva. In these solutions, two samples were introduced for 3 minutes, one from each batch, according to the scheme presented in Table II.

**Table II –** pH values solutions for experiment

| **pH** | **6.5** | **5.5** | **5** | **4.5** | **3.5** | **2.5** |
|---|---|---|---|---|---|---|
| **Batch 1** | $M_{1A}$ | $M_{3A}$ | $M_{5A}$ | $M_{2B}$ | $M_{6B}$ | $M_{4B}$ |
| **Batch 2** | $M_{2A}$ | $M_{6A}$ | $M_{1B}$ | $M_{4A}$ | $M_{3B}$ | $M_{5B}$ |

In each test, 15 fingerprints were taken, and the corresponding hardness values were determined. The average hardness values obtained were compared within each batch to determine the influence that the pH of the solution has on the hardness of dental enamel. Additionally, the average hardness values at each pH were compared between the samples of Batch 1 and those of Batch 2.

## IV.3 Results obtained from enamel hardness testing

### VI.3.1 Analysis of the hardness of dental enamel for untreated chemical samples

The samples (M1, …, M6) were subjected to a load of 100gf for 10 seconds. In each test, 10 impressions were practiced (5 on 2 rows) placed at a distance of at least 2.5 times the diagonal of an indentation. The diagonals d1 and d2 were measured using the Test Engineer for HV program. The indentations made in the enamel were carefully analyzed in terms of their asymmetry. (figures IV.4 si IV.5). Thus, a measurement was considered valid only if the absolute difference between diagonals d1 and d2 was less than 10%.

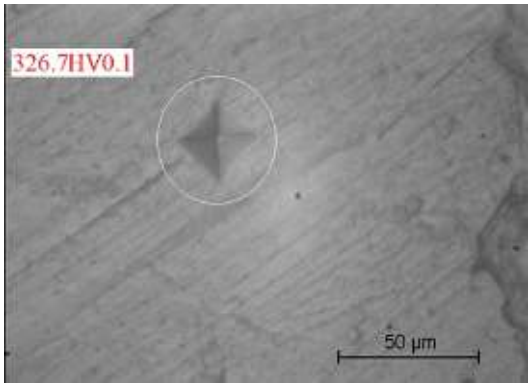


**Figure IV.4 –** Enamel indent, specimen $M_1$ (Timp = 10s, Încărcare = 100gf)

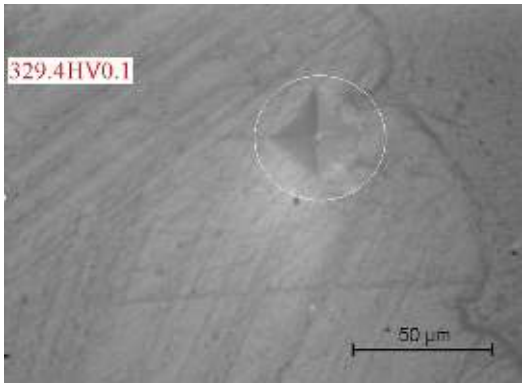


**Figura IV.5 -** Enamel indent, specimen $M_6$ (Timp = 10s, Încărcare = 100gf)

The hardness values of dental enamel for samples (M1, …, M6) ranged between 320-334 Kgf/mm², values that are consistent with data from the specialized literature. The results obtained are summarized in the Summary Statistics table, which presents the mean, standard deviation (SD), minimum and maximum values, as well as the result of the Shapiro-Wilk normality test.

In all cases, the probability associated with the statistical value of the test was $p > 0.05$, which is why it can be stated that, for all the samples under study, the values are normally distributed. (tabel III, figure IV.6)

**Table III.**
Statistic values for Control group samples

| | **Enamel Hardness** | | | | | |
|---|---|---|---|---|---|---|
| Sample | M1 | M2 | M3 | M4 | M5 | M6 |
| Mean | 327.346 | 326.190 | 328.573 | 329.646 | 327.311 | 325.919 |
| SD | 3.5127 | 3.3366 | 2.4614 | 2.4598 | 3.8573 | 4.1661 |
| Minimum | 320.540 | 322.000 | 325.000 | 325.530 | 322.000 | 321.000 |
| Maximum | 332.000 | 332.850 | 333.370 | 334.000 | 332.150 | 332.000 |
| S-W test | P=0.6950 | P=0.5964 | P=0.7109 | P=0.9386 | P=0.0963 | P=0.2324 |

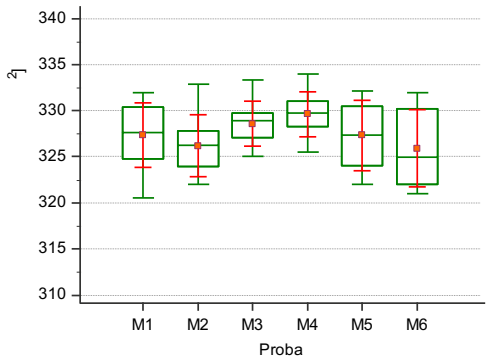


**Figure IV.6 –** Graphic representation Box-and-whisker and Error-Bar (Medie ± SD) for hardness values of enamel, samples ($M_1$, …, $M_6$), Time = 10s, Load = 100gf.

**Tabelul IV.** One-Way ANOVA test and PostHoc Student Newman-Keuls analysis for Control group samples

**Levene's test for equality of error variances**

| | |
|---|---|
| Levene statistic | 2.344 |
| df 1 | 5 |
| df 2 | 54 |
| Significance level | P = 0.054 |

**ANOVA**

| Source of variation | Sum of Squares | DF | Mean Square |
|---|---|---|---|
| Between groups (influence factor) | 100.3171 | 5 | 20.0634 |
| Within groups (other fluctuations) | 610.3445 | 54 | 11.3027 |
| Total | 710.6615 | 59 | |
| F-ratio | 1.775 | | |
| Significance level | P = 0.134 | | |

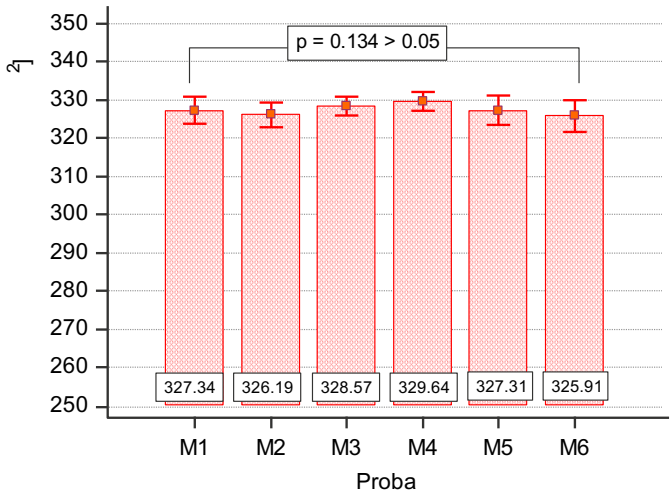


**Figura x.** Graphic representation of Bar and Error-Bar (Medie ± SD) for hardnes values of dental enamel, samples ($M_1$, …, $M_6$), Time = 10s, Load = 100gf.

Thus, the ANOVA test shows that there are NO statistically significant differences (F = 1.775, p = 0.134 > α = 0.05) between the average hardness values determined for the samples (M1, …, M6), which is why the 60 values determined for the 6 molars were included in the values of the Control group.

### IV.3.2. Analysis of the hardness of dental enamel for chemically treated samples

Each wisdom molar was sectioned longitudinally, thus forming 12 samples (M1A, M1B; …; M6A, M6B), which were grouped into two batches: Batch 1 (M1A, M3A, M5A, M2B, M6B, M4B) and Batch 2 (M2A, M6A, M1B, M4A, M3B, M5B), with 6 samples each.

Six solutions were prepared with the following pH values: 6.5, 5.5, 5.0, 4.5, 3.5, 2.5. In these solutions, two samples, one from each batch, were introduced for 3 minutes.

The samples corresponding to the two batches were subjected to a load of 100gf for 10 seconds. In each test, 15 impressions were made (5 each on 3 rows) placed at a distance of at least 2.5 times the diagonal of an indentation, and the corresponding hardness values were determined. The indentations made in the enamel were carefully analyzed in terms of their asymmetry, with a measurement being considered valid when the absolute difference between diagonals d1 and d2 was less than 10%.

The average hardness values obtained were compared within each batch to determine the influence that the pH of the solution has on the hardness of dental enamel. Additionally, the average hardness values at each pH were compared between the samples from Lot 1 and Lot 2. (figures IV.8 and IV.9)

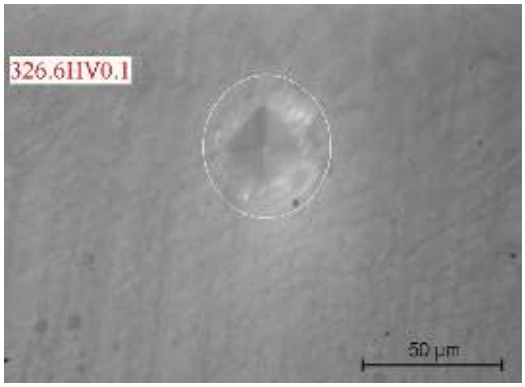


**Figure IV.8 –** Enamel indent, Group$_1$ - sample $M_{1A}$ la pH 6.5 (Time = 10s, Load= 100gf).

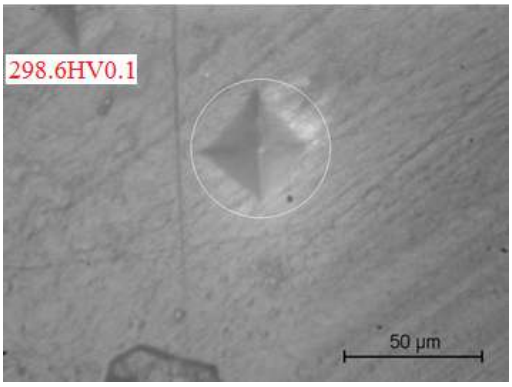


**Figure IV.9 –** Enamel indent, Group$_1$ - sample $M_{4B}$ la pH 2.5 (Time = 10s, Load = 100gf)

**A. Statistic Analysis for samples in Group 1**

**Table V.** Statistic values and Shapiro-Wilk test for samples in Group 1

| | **Enamel hardness [Kgf/mm$^2$] – Group 1** | | | | | | |
|---|---|---|---|---|---|---|---|
| Sample | 2.5pH | 3.5pH | 4.5pH | 5.0pH | 5.5pH | 6.5pH | Martor |
| N | 15 | 15 | 15 | 15 | 15 | 15 | 60 |
| Mean | 301.465 | 311.861 | 319.994 | 323.646 | 326.417 | 327.578 | 327.498 |
| SD | 2.7255 | 2.8891 | 3.2837 | 4.1989 | 2.6686 | 3.8956 | 3.4706 |
| Miniumm | 297.000 | 307.000 | 315.000 | 319.000 | 322.000 | 322.700 | 320.540 |
| Maximum | 306.000 | 316.000 | 325.000 | 330.500 | 330.000 | 333.000 | 334.000 |
| Test S-W | W=0.9679 | W=0.9494 | W=0.9218 | W=0.9521 | W=0.9292 | W=0.9539 | D=0.1022 * |
| Test K-S* | P=0.8257 | P=0.5156 | P=0.2049 | P=0.0610 | P=0.2657 | P=0.0697 | P>0.10 |

The average hardness values of the samples from Lot 1 and the Control were compared using the One-Way ANOVA test, a test that allows for the comparison of means from three or more samples. The test was supplemented by the PostHoc analysis (Student-Newman-Keuls test), an analysis that allows, in the case of obtaining a significant difference between the analyzed mean values, to specify which of the means differ from each other.

**Table VI.** One-Way ANOVA test and PostHoc Student Newman-Keuls analysis for Group 1 samples

**Levene's test for equality of error variances**

| | |
|---|---|
| Levene statistic | 1.828 |
| df 1 | 6 |
| df 2 | 143 |
| Significance level | P = 0.098 |

**ANOVA**

| Source of variation | Sum of Squares | DF | Mean Square |
|---|---|---|---|
| Between groups (influence factor) | 10539.7840 | 6 | 1756.6307 |
| Within groups (other fluctuations) | 1641.4643 | 143 | 11.4788 |
| Total | 12181.2483 | 149 | |
| F-ratio | 153.033 | | |
| Significance level | P < 0.001 | | |

**Student-Newman-Keuls test for all pairwise comparisons**

| Factor | Different (P<0.05) from factor nr |
|---|---|
| (1) 2.5PH | (2)(3)(4)(5)(6)(7) |
| (2) 3.5PH | (1)(3)(4)(5)(6)(7) |
| (3) 4.5PH | (1)(2)(4)(5)(6)(7) |
| (4) 5.0PH | (1)(2)(3)(5)(6)(7) |

| | |
|---|---|
| (5) 5.5PH | (1)(2)(3)(4) |
| (6) 6.5PH | (1)(2)(3)(4) |
| (7) MARTOR | (1)(2)(3)(4) |

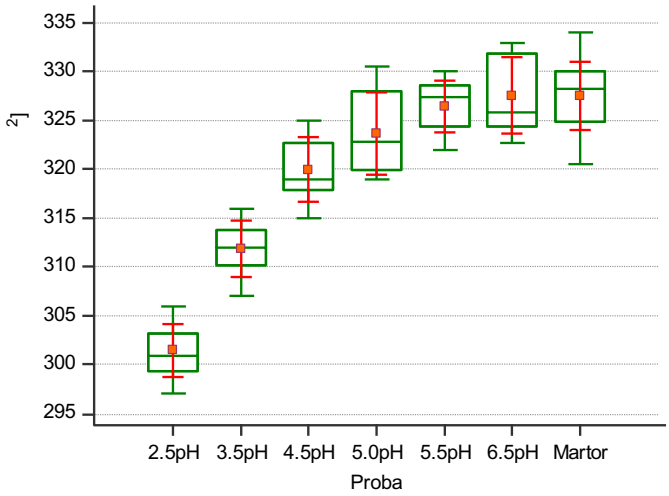


**Figure IV.10.** Graphic representation Box-and-whisker and Error-Bar (Medie ± SD) for hardness values of enamel, samples ($M_1$, …, $M_6$), Time = 10s, Load = 100gf.

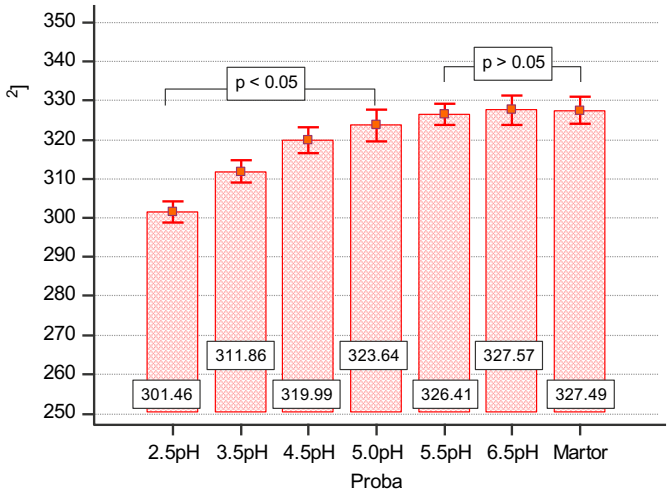


**Figure IV.11.** Graphic representation of Bar and Error-Bar (Medie ± SD) for hardnes values of dental enamel, samples ($M_1$, …, $M_6$), Time = 10s, Load = 100gf.

Thus, the ANOVA test shows that there are statistically significant differences between at least two of the average hardness values of the compared samples ($F = 153.03$, $p < 0.001 < \alpha = 0.05$). According to the PostHoc analysis (Student-Newman-Keuls test), it can be stated that there are no statistically significant differences ($p > 0.05$) between the mean hardness values of the samples immersed in solutions with pH = 6.5, 5.5, and the control sample, while there are statistically significant differences ($p < 0.05$) among all other samples immersed in solutions with pH = 5.0, 4.5, 3.5, 2.0, and the samples immersed in solutions with pH = 6.5,

5.5 along with the control sample. As the pH value decreases, the hardness of dental enamel depreciates. (figures IV.10 si IV.11).

**B. Statistic Analysis for samples in Group 2**

**Tabelul VII.** Statistic values analysis for samples in Group 2.

| | **Enamel Hardness [Kgf/mm$^2$] – Group 2** | | | | | | |
|---|---|---|---|---|---|---|---|
| Sample | 2.5pH | 3.5pH | 4.5pH | 5.0pH | 5.5pH | 6.5pH | Martor |
| N | 15 | 15 | 15 | 15 | 15 | 15 | 60 |
| Mean | 299.995 | 312.585 | 320.463 | 322.883 | 325.802 | 326.971 | 327.498 |
| SD | 3.4476 | 1.9796 | 2.4078 | 3.7705 | 2.7511 | 3.3283 | 3.4706 |
| Minimum | 293.000 | 309.840 | 317.920 | 317.000 | 321.000 | 320.500 | 320.540 |
| Maximum | 304.820 | 317.500 | 326.000 | 329.000 | 330.000 | 331.010 | 334.000 |
| Test S-W | W=0.9462 | W=0.9346 | W=0.9527 | W=0.9440 | W=0.9599 | W=0.9519 | D=0.1022 |
| Test K-S | P=0.4673 | P=0.3190 | P=0.0650 | P=0.4356 | P=0.6899 | P=0.0600 | P>0.10 |

The average hardness values of the samples from Lot 2 and the Control were compared using the One-Way ANOVA test, a test that allows for the comparison of means from three or more samples. The test was supplemented by the PostHoc analysis (Student-Newman-Keuls test), an analysis that allows, in the case of obtaining a significant difference between the analyzed mean values, to specify which of the means differ from each other. (table VIII.)

**Table VIII.** Results for One-way ANOVA test and PostHoc Analysis Student-Newman-Keuls test for all pairwise comparisons in Group 2.

**Levene's test for equality of error variances**

| | |
|---|---|
| Levene statistic | 2.385 |
| df 1 | 6 |
| df 2 | 143 |
| Significance level | P = 0.032 |

**ANOVA**

| Source of variation | Sum of Squares | DF | Mean Square |
|---|---|---|---|
| Between groups (influence factor) | 11038.1749 | 6 | 1839.6958 |
| Within groups (other fluctuations) | 1473.1744 | 143 | 10.3019 |
| Total | 12511.3493 | 149 | |
| F-ratio | 178.578 | | |
| Significance level | P < 0.001 | | |

**Student-Newman-Keuls test for all pairwise comparisons**

| Factor | Different (P<0.05) from factor nr |
|---|---|
| (1) 2.5PH | (2)(3)(4)(5)(6)(7) |
| (2) 3.5PH | (1)(3)(4)(5)(6)(7) |
| (3) 4.5PH | (1)(2)(4)(5)(6)(7) |
| (4) 5.0PH | (1)(2)(3)(5)(6)(7) |
| (5) 5.5PH | (1)(2)(3)(4) |
| (6) 6.5PH | (1)(2)(3)(4) |
| (7) MARTOR | (1)(2)(3)(4) |

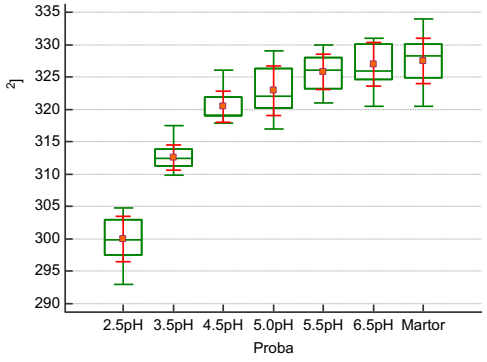


**Figure IV.12 -** Graphic representation Box-and-whisker and Error-Bar (Medie ± SD) for hardness values of enamel, samples Group 2, Time = 10s, Load = 100gf.

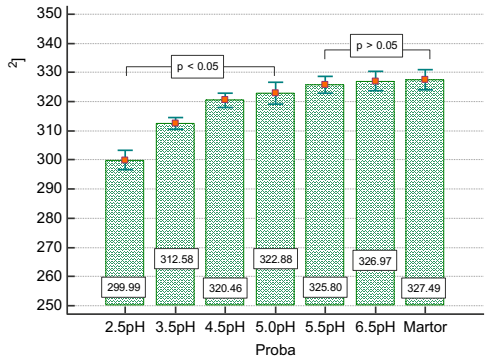


**Figure IV.13 -** Graphic representation of Bar and Error-Bar (Medie ± SD) for hardnes values of dental enamel, samples Group 2, Time = 10s, Load = 100gf.

Thus, the ANOVA test shows that there are statistically significant differences between at least two of the average hardness values of the compared samples ($F = 178.57$, $p < 0.001 < \alpha = 0.05$). According to the PostHoc analysis (Student-Newman-Keuls test), it can be stated that there are no statistically significant differences ($p > 0.05$) between the mean hardness values of the samples immersed in solutions with pH = 6.5, 5.5, and the control sample, while there are statistically significant differences ($p < 0.05$) among all other samples immersed in solutions with pH = 5.0, 4.5, 3.5, 2.0, and the samples immersed in solutions with pH = 6.5, 5.5 along with the control sample. As the pH value decreases, the hardness of dental enamel depreciates. (figures IV.12 si IV.13).

## IV.4. Discussions.

As studies in the specialized literature demonstrate, a decrease in the pH level in the oral cavity below the critical threshold of 5.5 affects the structure of dental enamel and, consequently, impacts its ability to withstand mechanical forces.

In the conducted study, I obtained similar results, starting from normal conditions, by testing an initial control group that was not exposed to acid attack, with the average normal hardness value of enamel in this case being 326 – 327, a value that decreases significantly once the oral pH falls below 5.5. Thus, at a pH value of 5.0, the hardness of the enamel decreases to 323, then at pH 4.5, the hardness drops to 319. At pH 3.5, the difference is already quite significant, with the hardness value reaching 311, and at the lowest testing pH of 2.5, the hardness of the enamel has significantly depreciated, reaching an average value of 301.

## IV.5. Conclusions

This study has once again highlighted that, with the decrease in pH value, the hardness of enamel depreciates in direct proportion, leading to the idea that patients suffering from gastroesophageal reflux disease, whose salivary pH frequently falls below the critical value of 5, are at a much higher risk of developing cavities due to the increased acidity of fluids in the oral cavity.

That being said, clinicians must conduct a thorough examination of patients predisposed to this condition, especially since not all patients present with the classic signs of gastroesophageal involvement. Most often, it is even the dentists who might notice signs of dental erosion, correlate all known data, and guide the patient in question towards a specialized examination. Therefore, a close collaboration between the two medical specialties, gastroenterology and dentistry, is necessary and even advisable when it comes to such situations, in order to address both the underlying condition and the secondary (extraesophageal) issues that arise.

# Chapter V - STUDY II

## Analysis of the Effects of Welding Fumes on Dental Enamel Hardness

### 1. Introduction

Welding fumes (WFs) pose significant health risks to workers, ranging from acute respiratory/neurological symptoms to chronic pathologies like bronchitis and lung cancer. Exposure to these pollutants, both long-term and short-term, can lead to various adverse effects on general health. Recent scientific discoveries focus on the correlation between metals used in welding processes, health conditions, and emerging diseases, aiming to advance preventive measures that benefit welders' health.

The impact of fumes from welding processes on the respiratory system is particularly significant. Contaminants of the environment can distort and modify the human organism's genome, causing mutations. Occupational exposure to these contaminants occurs through direct contact with the skin, ingestion, or inhalation. The mechanisms of work toxicity of chromium and nickel can be direct and indirect, generating free radicals that damage DNA structure and cause cell death.

The cytotoxicity of WFs can lead to damage to the human genome, leading to carcinogenesis, teratogenesis, and premature cell aging. The study confirms previous studies showing an increased tendency to develop abnormal cells in subjects exposed to WFs.

In the dental field, intensifying scientific studies on the phenomena resulting from contact between dental units and WFs is necessary to describe a complex and complete picture of diseases caused by these contaminants on the whole organism.

### 2. Materials and Methods

The study was carried out on a number of 15 teeth recently extracted (from patients with advanced chronic marginal parodontopathy or from patients with orthodontic condition who are required to extract certain dental units in order to obtain the necessary space for the start of the ortodontic treatment), imposing the exclusion criterion for the experiment, whereby the dental units affected by caries were not taken into account for testing, in order not to distort the results obtained.

A testing device was then designed to simulate as accurately as possible the conditions of contact of the teeth with the fumes resulting from the welding processes. (figure V.1)

**Figure V.1**. Test device for the contact between teeth and WFs

The testing device is composed of the following components:

1. Testing chamber – a container with both input and output in order to fill it up with WFs and adjust the air pressure inside

2. A relative pressure manometer (pressure gauge) – to keep track of the air pressure inside the testing chamber
3. Vacuum pump – used to reach negative pressure inside the testing chamber enabling the capture of WFs inside the testing container
4. Vacuum manometer – to monitor the negative pressure inside the testung chamber
5. Two faucets to adjust the pressure
6. Electronic valve – to maintain a constant pressure inside the testing chamber
7. Electronic fan to recirculate the contaminated air flux inside the testing chamber
8. Heating resistance to rise up the temperature inside the testing chamber to simulate the homeostatic temperature from inside of the oral cavity (aproximately $37^0$ C)
9. Temperature probe for long-time measurements of the temperature inside the testing unit
10. A thermostat connected to the temperature probe and to the heating resistance to set up the testing temperature (aproximately $37^0$ C)
11. Carbon dioxide tank with a pressure reducer – used to create the mixture of WFs
12. Device for capturing the WFs and direct them inside the testing container
13. Fixing support for the teeth probes to be tested
14. Support plate to put up together the entire assembly.

The operating principle of this testing device consists of the following steps and it is based on reproducing the work enviornmental conditions of a welding worker:

Step 1. Fix the tooth to be tested in the dedicated support.
Step 2. Connect of the vacuum pump to create nagative pressure inside the testing chamber to – 0.6.
Step 3. Initiate the welding arc using a rutilic electrode and a galvanized steel bar, followed by the production of the WFs. The inlet valve to the testing chamber is opened throughout this procedure.
Step 4. Collect the WFs using the dedicated device and direct them inside the testing container, up to 0 value of pressure inside the chamber.
Step 5. Connect the carbon dioxide tank and release gas inside the testing chamber, using the pressure reducer valve up to the value of 0.5 of the relative pressure manometer.
Step 6. Waiting time for each testing.

During this procedure with this testing method, introducing “fresh” WFs each 24h was required. The study conducted considered an average of 4 hours exposure to WFs / working day of 8 hours.

**Preparing tooth enamel samples for microhardness testing**

The 15 teeth (both exposed and unexposed to WFs) used for this study were sectioned lenghtwise and crosswise using a diamond disc motor in the manner of obtaining clear cuts of the cusps or the incisal edge. The enamel cuts were then fixed into acrylic resin and polished using ultrafine granulations discs, in order to obtain clean cut edges and very smooth surfaces of the specimens, after each polishing course the specimen probes being washed thoroughly with distiled water.

**Figure V.9** – lengthwise and crosswise sections of teeth samples to expose the enamel for further hardness testing after exposure tooo welding fumes

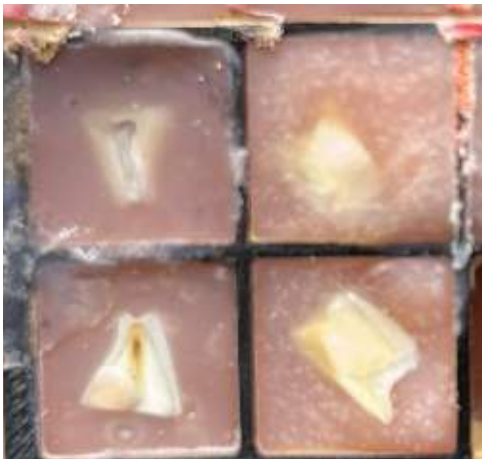

**Figure V.12** – Fixed enamel samples in acrylic resin

From all the specimens obtained and prepared for testing, 25 probes were randomly selected, being devided into 5 groups, as following:
Group A = {G1-A, G2-A, G3-A, G4-A, M-A}
Group B = {G1-B, G2-B, G3-B, G4-B, M-B}
Group C = {G1-C, G2-C, G3-C, G4-C, M-C}
Group D = {G1-D, G2-D, G3-D, G4-D, M-D}
Group E = {G1-E, G2-E, G3-E, G4-E, M-E},

Where:
G1 - A,B,C,D,E – specimens exposed for 48h to WFs, simulating 10 working days for a welder;
G2 - A,B,C,D,E – specimens exposed for 96h to WFs, simulating 20 working days for a welder;
G3 - A,B,C,D,E – specimens exposed for 168h to WFs, simulating 40 working days for a welder;
G4 - A,B,C,D,E – specimens exposed for 336h to WFs, simulating 80 working days for a welder;
M - A,B,C,D,E – specimens unexposed to WFs.

A reunited lot was considered for analysis – Lot $_{reunited}$ = {G1, G2, G3, G4, M}.

### 3. Results for Enamel Hardness Analysis after Welding Fumes Exposure

**Table 1.** Summary Statistics - Dental Enamel Hardness [Kgf/mm$^2$] Group A to E

| | Dental Enamel Hardness [Kgf/mm$^2$] – Group A | | | | |
|---|---|---|---|---|---|
| | G1-A | G2-A | G3-A | G4-A | M-A |
| N | 15 | 15 | 15 | 15 | 15 |
| Mean | 321.183 | 316.693 | 311.199 | 307.410 | 326.899 |
| SD | 2.958 | 3.192 | 2.948 | 2.332 | 2.420 |
| Minimum | 315.230 | 311.600 | 307.110 | 303.781 | 322.894 |
| Maximum | 325.000 | 321.200 | 316.110 | 312.230 | 331.489 |
| Shapiro-Wilk test | W=0.9077 | W=0.9359 | W=0.9181 | W=0.9748 | W=0.9769 |
| | p=0.1250 | p=0.3338 | p=0.1800 | p=0.9211 | p=0.9442 |
| | Dental Enamel Hardness [Kgf/mm$^2$] – Group B | | | | |
| | G1-B | G2-B | G3-B | G4-B | M-B |
| N | 15 | 15 | 15 | 15 | 15 |
| Mean | 320.577 | 314.123 | 312.510 | 305.952 | 327.075 |
| SD | 3.674 | 3.214 | 2.876 | 3.386 | 3.454 |
| Minimum | 314.250 | 309.600 | 308.240 | 301.220 | 320.340 |
| Maximum | 326.370 | 318.920 | 317.560 | 311.434 | 332.692 |
| Shapiro-Wilk test | W=0.9728 | W=0.9183 | W=0.9596 | W= 0.9413 | W=0.9684 |
| | p=0.8967 | p=0.1816 | p=0.6861 | p=0.3996 | p=0.8336 |
| | Dental Enamel Hardness [Kgf/mm$^2$] – Group C | | | | |
| | G1-C | G2-C | G3-C | G4-C | M-C |
| N | 15 | 15 | 15 | 15 | 15 |
| Mean | 321.059 | 315.869 | 310.722 | 306.252 | 327.346 |
| SD | 2.447 | 2.918 | 2.572 | 2.531 | 3.361 |
| Minimum | 317.250 | 310.250 | 306.580 | 302.554 | 321.436 |
| Maximum | 325.370 | 319.920 | 315.664 | 310.110 | 332.876 |
| Shapiro-Wilk test | W=0.9627 | W=0.9620 | W=0.9633 | W=0.9322 | W=0.9587 |
| | p=0.7391 | p=0.7264 | p=0.7490 | p=0.2945 | p=0.6704 |
| | Dental Enamel Hardness [Kgf/mm$^2$] – Group D | | | | |
| | G1-D | G2-D | G3-D | G4-D | M-D |
| N | 15 | 15 | 15 | 15 | 15 |
| Mean | 319.335 | 314.263 | 311.425 | 305.354 | 326.463 |
| SD | 2.869 | 2.567 | 3.143 | 3.323 | 3.678 |
| Minimum | 314.250 | 310.600 | 307.240 | 302.110 | 319.340 |
| Maximum | 323.370 | 318.780 | 317.110 | 311.110 | 331.898 |
| Shapiro-Wilk test | W=0.9477 | W=0.9429 | W=0.9410 | W=0.8289 | W=0.9632 |
| | p=0.4891 | p=0.4207 | p=0.3958 | p=0.0089 | p=0.7470 |
| | Dental Enamel Hardness [Kgf/mm$^2$] – Group E | | | | |
| | G1-E | G2-E | G3-E | G4-E | M-E |
| N | 15 | 15 | 15 | 15 | 15 |
| Mean | 318.620 | 313.689 | 309.918 | 304.596 | 327.247 |

| | | | | | |
|---|---|---|---|---|---|
| SD | 3.524 | 3.930 | 3.381 | 3.037 | 3.048 |
| Minimum | 314.100 | 308.450 | 305.120 | 299.120 | 321.665 |
| Maximum | 326.622 | 320.920 | 316.980 | 309.908 | 331.658 |
| Shapiro-Wilk test | W=0.8954 | W=0.9443 | W=0.9624 | W=0.9695 | W=0.9506 |
| | p=0.0810 | p=0.4395 | p=0.7332 | p=0.8506 | p=0.5344 |

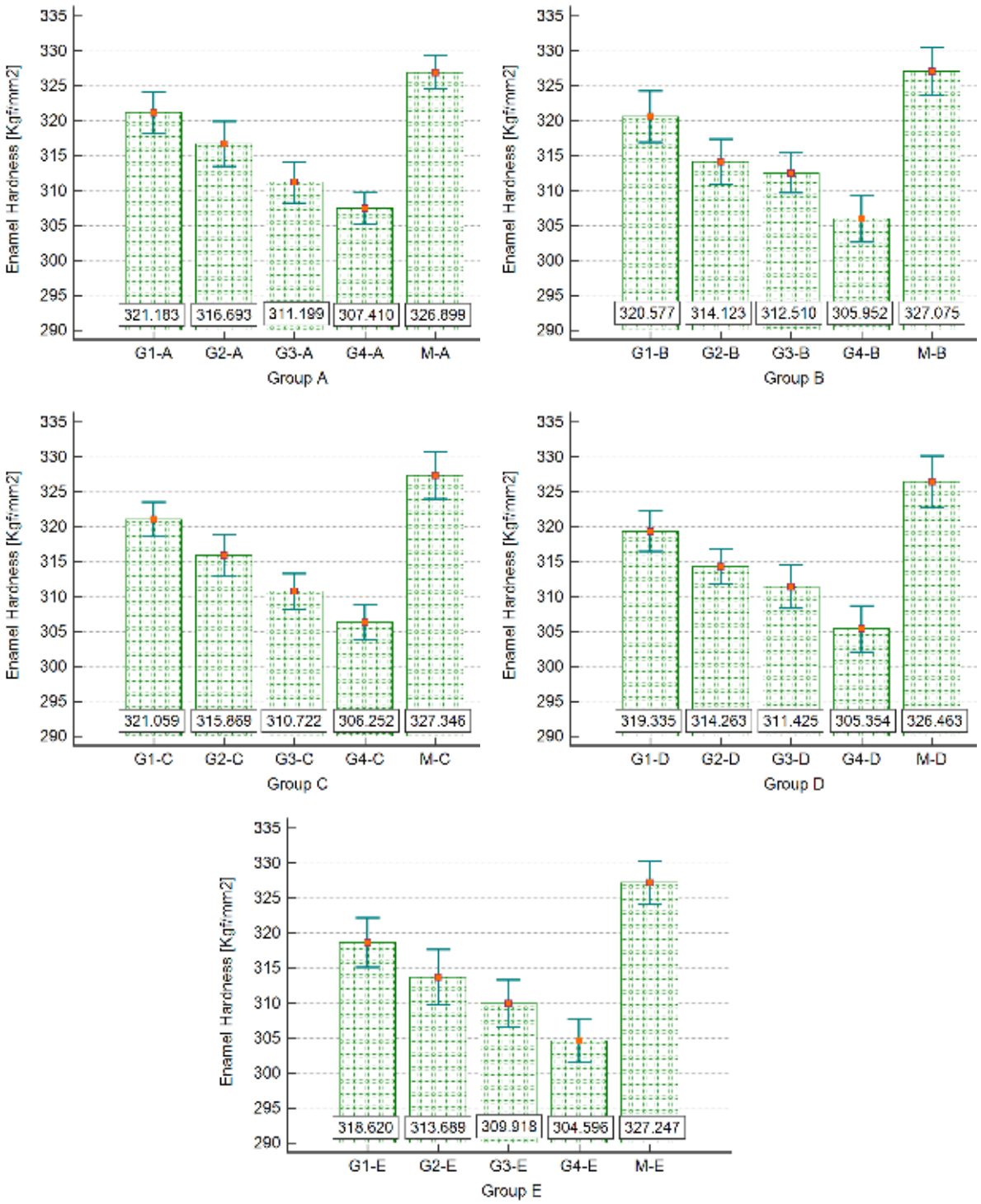


**Figure 1.** Bar and Error-Bar Chart (Mean ± SD) for dental enamel hardness values in Group A to E
Time = 10s, Load = 100gf.

For each group the result of the one-way ANOVA test shows that there are statistically significant differences between at least two of the mean hardness values of the compared samples (Table 2).

**Table 2**. The output of the ANOVA analysis for each Group A to E

| | Test statistics | Probability |
|---|---|---|
| Group A = {G1-A, G2-A, G3-A, G4-A, M-A} | F = 115.947 | p < 0.001 |
| Group B = {G1-B, G2-B, G3-B, G4-B, M-B} | F = 87.942 | p < 0.001 |
| Group C = {G1-C, G2-C, G3-C, G4-C, M-C} | F = 133.754 | p < 0.001 |
| Group D = {G1-D, G2-D, G3-D, G4-D, M-D} | F = 97.383 | p < 0.001 |
| Group E = {G1-E, G2-E, G3-E, G4-E, M-E} | F = 96.896 | p < 0.001 |

According to the PostHoc analysis (Student-Newman-Keuls test) applied to each group it can be stated that there are statistically significant differences in mean hardness values between all samples of each group ($p < 0.05$). The longer the duration of exposure, the more the value of the tooth enamel hardness is depreciated compared to the test hardness value.

All samples from control group M and all samples from groups G1, G2, G3, G4 were compared with each other. For each group the result of the one-way ANOVA test shows that there are no statistically significant differences ($p > \alpha = 0.05$) between the mean hardness values of the compared samples. (Table 3)

**Table 3**. The output of the ANOVA analysis for group M and groups G1, G2, G3, G4

| | Test statistics | Probability |
|---|---|---|
| Group M = {M-A, M-B, M-C, M-D, M-E} | F = 0.175 | p = 0.950 |
| Group G1 = {G1-A, G1-B, G1-C, G1-D, G1-E} | F = 1.948 | p = 0.112 |
| Group G2 = {G2-A, G2-B, G2-C, G2-D, G2-E} | F = 2.433 | p = 0.059 |
| Group G3 = {G3-A, G3-B, G3-C, G3-D, G3-E} | F = 1.516 | p = 0.207 |
| Group G4 = {G4-A, G4-B, G4-C, G4-D, G4-E} | F = 1.896 | p = 0.121 |

Considering the results presented in table 3, the data were grouped into five groups M, G1, G2, G3, G4, each group having 75 values. The result of the One-way ANOVA test shows that there are statistically significant differences between at least two of the mean hardness values of the compared samples ($F = 500.571$, $p < 0.001 < \alpha = 0.05$). According to the PostHoc analysis (Student-Newman-Keuls test) it can be stated that there are statistically significant differences regarding the mean hardness values between all samples ($p < 0.05$). The longer the exposure time increases, the more the tooth enamel hardness value decreases compared to the hardness value of the control sample M.

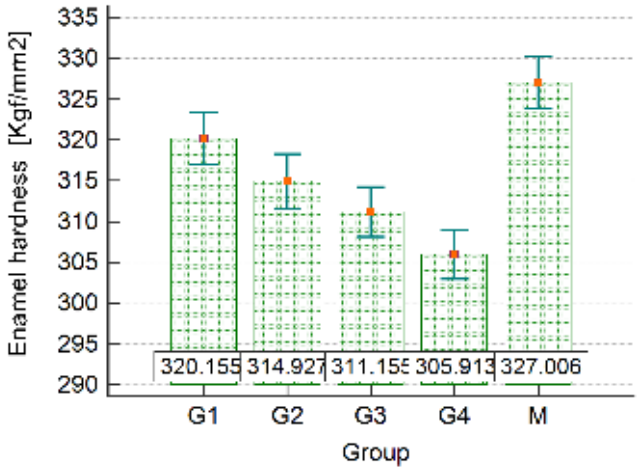


**Figure 1.** Bar and Error-Bar Chart (Mean ± SD) for dental enamel hardness values in Groups M, G1, G2, G3, G4, Time = 10s, Load = 100gf.

## 4. Discussion

Industrial fumes exposure has been a topic of debate in the scientific world due to global industrialization and technology development. A recent study conducted in the UK found a clear connection between exposure to weld smoke and the risk of developing squamous cell carcinomas. However, studies on the effects on teeth have been limited, leading to the need to explore these effects on tough dental structures.

A 2017 study found high concentrations of heavy metals in the structure of teeth, with some found at the level of molars and others at the level of incisives. The tough structure of teeth can be considered a true biological indicator of industrial pollution.

In 2020, a study by Ibrahim H.F. and Hassan G.S. demonstrated that these toxins have negative effects on the ultrastructure of the enamel, affecting the natural process of remineralization and the mechanical qualities of the hard substance (microhardness). The study was designed to simulate the inhalation of fumes produced during welding processes, with pressure and temperature conditions in the oral cavity and external environment.

Limitations of the study include the thickness of the enamel layer of teeth and the fact that the study was conducted in vitro, which may affect teeth outside the oral cavity's reactivity to stimuli.

## 5. Conclusions

This study found that exposure to welding fumes, particularly tobacco exhaled fumes, can significantly affect dental enamel hardness. After 48 hours, there was no significant difference in enamel hardness compared to the control group. However, after 336 hours, enamel hardness decreased significantly. This raises concerns about the toxicity of welding fumes (WFs), which have been shown to have harmful effects on respiratory health. Regulation of WF exposure falls under labor protection and occupational health department jurisdiction, aiming to improve industrial workers' health and quality of life.

# Chapter VI - STUDY III
# Analysis of the Effect of 65% Industrial Nitric Acid on Dental Enamel Hardness

## VI.1. Introduction

Dental erosion is characterized as a condition with multifactorial origins, among which one of the most recognized is exposure to the acidic atmospheric environment. Considering the harmful toxic environment in which those in the metallurgy and welding industry operate, exposure to nitric acid vapors can be a contributing factor to the development of dental erosion.

Exposure to acidic environments in workplaces, such as in the welding industry, can have a negative impact on workers' health. The present study focuses on the analysis of enamel hardness following exposure to industrial nitric acid.

The summary data from existing literature indicate the need for a more detailed investigation into this aspect, considering the possible harmful effects on the health of industrial workers.

## VI.2. Material and Method

The study was conducted on a number of 10 recently extracted teeth (from patients with advanced chronic marginal periodontitis or from patients with orthodontic conditions requiring the extraction of certain dental units to create the necessary space for the initiation of orthodontic treatment), with the exclusion criterion for the experiment being that dental units affected by carious processes were not considered for testing, in order to avoid distorting the obtained results.

The division of dental units for the experiment was done as follows: Group of samples with two dental units not subjected to the experiment, Group 1 with two dental units exposed to 65% nitric acid for 12 hours, Group 2 with two dental units exposed to 65% nitric acid for 24 hours, Group 3 with two dental units exposed to 65% nitric acid for 36 hours, Group 4 with two dental units exposed to 65% nitric acid for 48 hours (figures VI.1 and VI.6).


**Figure VI.1 –** Illustration of study execution

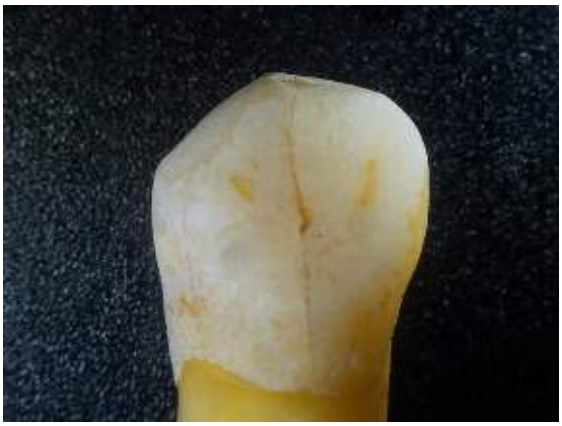

**Figure VI.6 –** Aspect of dental enamel after a 48 hour exposure to industrial nitric acid, $HNO_3$ 65%

The samples were washed with softened water for 10 minutes. From each tooth, four samples were processed. Out of all the samples obtained, 25 were randomly selected and divided into 5 groups as follows:
- Group A = {G1-A, G2-A, G3-A, G4-A, M-A}
- Group B = {G1-B, G2-B, G3-B, G4-B, M-B}
- Group C = {G1-C, G2-C, G3-C, G4-C, M-C}
- Group D = {G1-D, G2-D, G3-D, G4-D, M-D}
- Group E = {G1-E, G2-E, G3-E, G4-E, M-E}

where:
- G1-A,B,C,D,E are the samples exposed for 12 hours
- G2-A,B,C,D,E are the samples exposed for 24 hours
- G3-A,B,C,D,E are the samples exposed for 36 hours
- G4-A,B,C,D,E are the samples exposed for 48 hours (fig. VI.12 and VI.13)
- M-A,B,C,D,E are the control samples

The control samples and those exposed for the durations mentioned above have also been categorized for comparison into 5 batches:
- Group M = {M-A, M-B, M-C, M-D, M-E}
- Group G1 = {G1-A, G1-B, G1-C, G1-D, G1-E}
- Group G2 = {G2-A, G2-B, G2-C, G2-D, G2-E}
- Group G3 = {G3-A, G3-B, G3-C, G3-D, G3-E}
- Group G4 = {G4-A, G4-B, G4-C, G4-D, G4-E}

Additionally, the combined batch has been considered for analysis = {G1, G2, G3, G4, M}.

**Figure VI.12 –** Illustrations of dental enamel samples fixed in acrylic resin after the 12 hour and 24 hour exposures to 65% $HNO_3$ , prepared for hardness testing

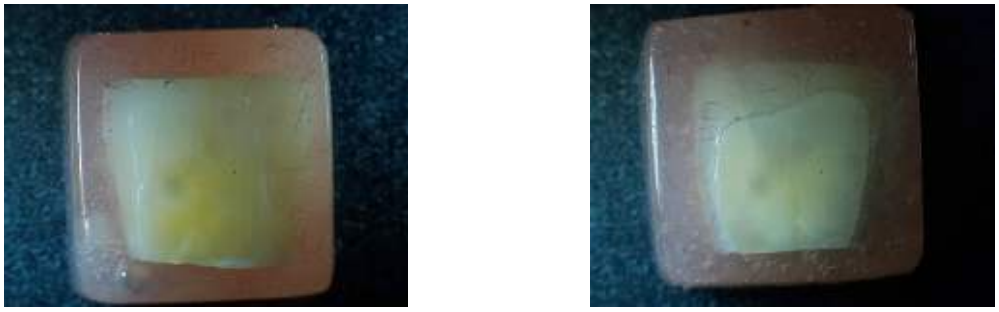

**Figure VI.13 -** Illustrations of dental enamel samples fixed in acrylic resin after the 36 hour and 48 hour exposures to 65 % $HNO_3$ , prepared for hardness testing

## VI. 3. Results obtained from the analysis of enamel hardness testing after exposure to 65% industrial nitric acid

To summarize the results obtained, I will present here only the statistical analysis results for the combined batch, which includes all samples subjected to the current experimental study.

VI.3.9. Statistic Analysis for all samples in all Groups

**Table XLVII.** Statistic indicators & Shapiro-Wilk Test resultsfor all samples in all Groups.

| | Enamel Hardness [Kgf/mm$^2$] – Group | | | | |
|---|---|---|---|---|---|
| | G1 | G2 | G3 | G4 | M |
| N | 75 | 75 | 75 | 75 | 75 |
| Mean | 300.975 | 285.139 | 272.312 | 251.244 | 327.716 |
| SD | 3.145 | 3.2148 | 3.0218 | 3.6919 | 2.6338 |
| Median | 301.11 | 284.83 | 272.56 | 251.441 | 328.007 |
| Minimum | 293.67 | 278.407 | 266.1 | 243.107 | 321.49 |
| Maximum | 307.41 | 293.49 | 278.23 | 258.634 | 333.087 |
| IQR | 298.53 - 303.47 | 282.58 - 287.62 | 269.94 - 274.56 | 248.43 - 254.15 | 325.69 - 329.75 |
| S-W Test | W=0.9804 | W=0.9836 | W=0.9825 | W=0.9843 | W=0.9876 |
| | P=0.2967 | P=0.4444 | P=0.3865 | P=0.4786 | P=0.6796 |

**Table XLVIII.** Results for One-way ANOVA test and PostHoc Analysis Student-Newman-Keuls test for all pairwise comparisons in all Groups

**Levene's test for equality of error variances**

| | |
|---|---|
| Statistics Levene ($L_{calc}$) | 2.563 |
| df 1 | 4 |
| df 2 | 370 |
| Significance level | P = 0.038 |

**ANOVA**

| Source of variation | Variation (V) | (df) | Mean Square ($S^2$) |
|---|---|---|---|
| Between groups (influence factor) | 251221.8099 | 4 | 62805.4525 |
| Within groups (other fluctuations) | 3694.4171 | 370 | 9.9849 |
| Total | 254916.2270 | 374 | |
| F-ratio | | | 6290.036 |
| Significance level | | | P < 0.001 |

**PostHoc Analysis Student-Newman-Keuls test for all pairwise comparisons in all Groups**

| Group | N | Mean | SD | Different (P<0.05) from factor nr |
|---|---|---|---|---|
| (1) G1 | 75 | 300.9752 | 3.1450 | (2)(3)(4)(5) |
| (2) G2 | 75 | 285.1395 | 3.2148 | (1)(3)(4)(5) |
| (3) G3 | 75 | 272.3122 | 3.0218 | (1)(2)(4)(5) |
| (4) G4 | 75 | 251.2444 | 3.6919 | (1)(2)(3)(5) |
| (5) M | 75 | 327.7161 | 2.6338 | (1)(2)(3)(4) |

For the combined group = {G1, G2, G3, G4, M}, the result of the One-way ANOVA test shows that there are statistically significant differences between at least two of the mean hardness values of the compared samples ($F = 62805.452$, $p < 0.001 < \alpha = 0.05$).

According to the PostHoc analysis (Student-Newman-Keuls test), it can be stated that there are statistically significant differences in the mean hardness values among all samples ($p < 0.05$). As the exposure duration increases, the hardness value of dental enamel depreciates compared to the hardness value of the control sample M. (Tables XLVIII, Figures VI.28 and VI.29).

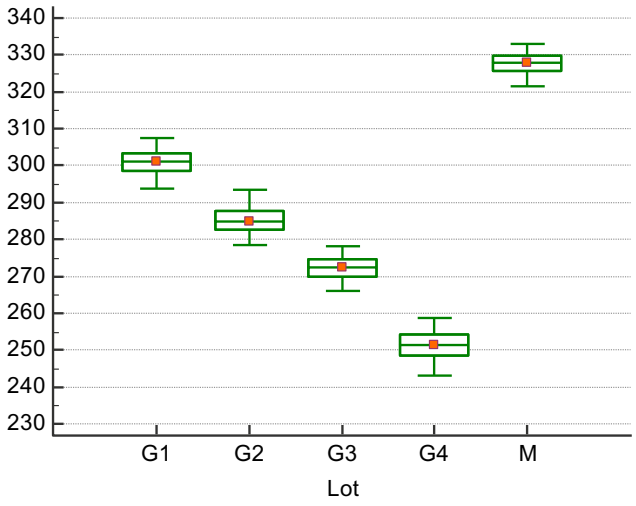


**Figure VI.28 –** Graphic representation Box-and-whisker (Mediana, IQR) for hardness values of dental enamel, Group = {G1, G2, G3, G4, M}, Time = 10s, Load = 100gf.

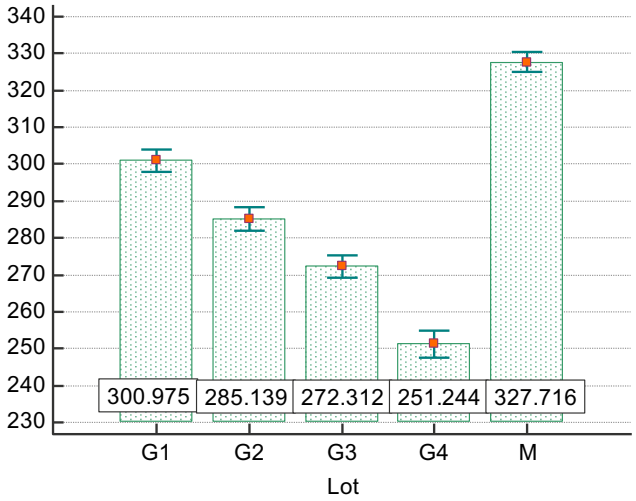


**Figure VI.29 –** Graphic representation Bar and Error-Bar (Medie ± SD) for hardness values of dental enamel, Lot = {G1, G2, G3, G4, M}, Time = 10s, Load = 100gf.

A 12-hour exposure of enamel to 65% nitric acid resulted in a reduction of its hardness to approximately 300 HV, while after 24, 36, and 48 hours of exposure, the hardness decreased to 285 HV, 272 HV, and 251 HV, respectively. Compared to the enamel samples from the control batch, which had a hardness of 327 HV, there is a significant deterioration in the quality of the enamel. These results should prompt increased attention regarding the impact on the oral health of workers who come into contact with this acid in the welding industry.

## VI.4. Discussions

Specialized studies have shown that those exposed to industrial acids in the workplace, such as sulfuric acid or hydrochloric acid, have an increased risk of dental enamel erosion. The lack of appropriate preventive measures and the disregard for protection rules contribute to this phenomenon.

Education on occupational risks, the promotion of general and oral health in such environments, as well as adherence to safety standards, such as wearing protective masks, is important. To prevent occupational dental erosion, it is recommended to implement free dental check-ups and preventive treatments for those exposed to industrial acids.

These measures can help reduce the negative impact of high-risk work environments on employees' oral health.

## VI. Conclusions

The study showed that tooth enamel can be affected by various industrial acids, such as sulfuric acid found in batteries. The recommendations include regular dental check-ups, promoting protective equipment in the workplace, a healthy diet, medicinal saliva stimulation, and adopting proper oral hygiene strategies.

## Chapter VII – General Conclussions

• Experimental studies conducted to analyze the hardness of dental enamel have reached several important conclusions.

• Endogenous and exogenous substances have a significant impact on enamel, causing erosion and demineralization.

Factors such as tobacco use, alcohol, carbonated beverages, and foods high in sugar are additional risk factors in the deterioration of dental enamel.

• The hardness of enamel is more significantly affected when exposure to chemical factors is prolonged and when the salivary pH drops below the normal limit.

• Welding fumes and acid vapors can affect dental structures, and patients exposed to these agents should be closely monitored, especially in collaboration between the specialties of pulmonology and dentistry.

Dental injuries caused by these factors should be detected and treated in the early stages to avoid costly and invasive interventions.

Additionally, pollutants in the workplace can exacerbate other pre-existing oro-dental conditions, such as periodontal disease or lesions of the oral mucosa.

It is important for both patients and doctors to be aware of the impact of internal and external chemical factors on dental health.

## Chapter VIII – Originality of the Thesis

The originality of this work lies both in the theme addressed in the presented experimental scientific studies and, more importantly, in the methodology and materials used in the three experiments to successfully determine the impact on dental enamel exposed to various harmful internal and external chemical factors.

The point of maximum interest regarding the originality of the thesis is represented by the study of the effects on dental enamel in the presence of welding fumes and 65% industrial nitric acid. Until now, in the specialized scientific literature, there have only been studies correlating the toxicity of welding fumes and the decapants used in metallurgy with the occurrence of various cellular anomalies in the soft tissues of the oral cavity.

However, as previously mentioned, the hard structures in the oral cavity should be viewed as individual elements that need to be studied and treated as distinct components of the dento-maxillary apparatus.

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