“*A smile is worth a thousand words”*

.

INTRODUCTION:

A bright, radiant smile is often viewed as a symbol of health and beauty, enhancing both self-confidence and positive first impressions. In the past, achieving a white smile and addressing teeth discoloration was a challenge. However, with modern dentistry, we now have a variety of solutions, from professional whitening treatments to veneers, and more conveniently, whitening toothpastes. These options address discoloration caused by both external and internal factors.

Teeth whitening can be approached in three main ways: through professional treatment in a dental clinic, with take-home products prescribed by a dental professional, or by using over-the-counter whitening products. Among the most accessible commercially purchased whitening products are toothpastes, mouthwashes, strips, and gels, which have grown in popularity as they don't require a prescription. Manufacturers now offer a wide range of whitening toothpastes, each boasting different active ingredients designed to deliver visible results in just 2 to 4 weeks of use. Common ingredients include dicalcium phosphate dihydrate, calcium pyrophosphate, alumina, perlite, sodium bicarbonate, and activated charcoal, which help remove surface stains and biofilm, brightening the teeth's appearance.

Given this background, the study aims to assess the efficacy of various commercial whitening toothpastes on extracted human teeth. By comparing them side by side in a controlled lab environment, we aim to understand how effective each one is at achieving visible whitening results.

CHAPTER 1: DENTAL ENAMEL

The teeth are located in the oral cavity and are anchored to the jaw bones by the periodontal ligament. Their shape allows the distribution of forces from the crown to the root and, through the periodontal ligament, to the underlying bones.

A tooth can be divided into three parts [1]:

- *Crown:* the visible portion of the tooth that protrudes above the gum in the oral cavity. It is covered by a hard, acellular, avascular, and non-sensitive dental tissue called enamel.
- *Root:* the portion of the tooth, which is covered with the highly mineralized, avascular tissue called cementum. Different teeth groups have varying numbers of roots and shapes and are anchored to the alveolar bone via the periodontal ligament. Inside the dental roots, there are root canals containing blood vessels, lymphatic vessels, and nerves.
- *Dental collar:* the bridge element of the teeth that is usually located at the level of the gum and separates the crown from the root.

Enamel, dentin, and cement make up the hard dental tissues. In this *in vitro study* for the thesis, will the primary focus on enamel, as it is the tissue most affected by the action of whitening products.

Enamel is the outermost layer that covers the crown of the tooth and is the hardest tissue in our body. Its physical characteristics such as size, color, shape, and even malformation of the structure of enamel are inherited. Despite its thinness, enamel is the most resistant tissue in the body that protects the inner tissues of the teeth such as dentin and vital dental pulp. Since it is avascular, it cannot regenerate itself [2,3].

1.1: Development of Dental Enamel

The mechanism of the formation of enamel is called Amelogenesis. Enamel is produced by a specific type of cylindrical cell of ectodermal origin, called ameloblasts. These cells secrete an organic matrix that, through a maturation process, becomes mineralized tissue primarily composed of calcium hydroxyapatite, which gives enamel its characteristic hardness.

Amelogenesis has five main stages [3]:

-*Presecretory stage:* One of the earliest events [4] , the differentiation of pre-ameloblasts into mature ameloblasts occurs along with the formation of structures such as the basal membrane and the cells of the enamel organ, preparing for the production of the enamel's organic matrix

-*Secretory stage:* In this phase, the enamel reaches its full thickness; however, it is composed of a soft, cheese-like substance that can be easily separated from the dentin by mechanical techniques [5]. Ameloblasts actively synthesize the organic matrix of enamel and develop the Tomes' process. An initial layer of non-prismatic enamel formed. The secretory stage ends when the enamel matrix is fully formed and reaches its full thickness.

-*Transition stage:* This is a short phase during which the immature enamel transforms into mature, fully mineralized enamel. At this stage, enamel secretion stops, and a large part of the matrix is eliminated. The number of ameloblasts decreases by approximately 25%, and the remaining ameloblasts reduce their secretion of proteins (amelogenin, enamelins, ameloblastin, tuftelin) and their organelles, such as the endoplasmic reticulum and Golgi apparatus [3,6]. This stage is a preparatory phase for maturation.

-Maturation stage: This phase is characterized by significant growth of crystals that make the enamel durable and well-mineralized, thanks to the transport of inorganic ions and matrix proteins. At the end of this stage, the ameloblasts undergo programmed cell death, and the protein content decreases, resulting in increased mineralization until the proteins are fully replaced by minerals [7].

-Postmaturation stage: The reduced enamel epithelium forms Nasmyth's membrane, which protects the enamel surface during eruption. After the eruption, the outer layer undergoes a mineralization process through interaction with saliva [3].

1.2 Composition and structure of dental enamel

The basic structure of dental enamel consists of hydroxyapatite crystals with the chemical structure $Ca_{10}(PO_4)_6(OH)_2$ [8] *(Figure 1).* This is the main component responsible for enamel's hardness, strength, and resistance to wear. The hydroxyapatite crystals are tightly packed and organized into enamel prisms or rods, which form a three-dimensional pattern. Most of these crystals have a hexagonal shape when viewed in cross-section [3].

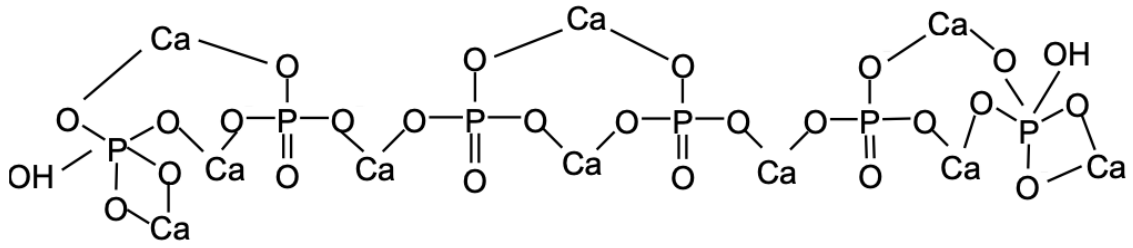

Figure 1: Chemical structure of hydroxyapatite, Ca10(PO4)6(OH)2 [8]*.*

Enamel consists of 96% inorganic substances, primarily calcium phosphate that crystallizes in the form of carbonated hydroxyapatite, and 4% organic components, including proteins and water. The hydroxyapatite crystals are aligned parallel to one another and organized into nanometer-sized rods, approximately 25 nm thick and 100 nm wide. These rods systematically combine to form elongated keyhole (fish-

like) patterns known as enamel prisms, which are 4–8 microns in diameter. The diameter of the prisms widens near the enamel surface [2,9]. (*Figure 2)*

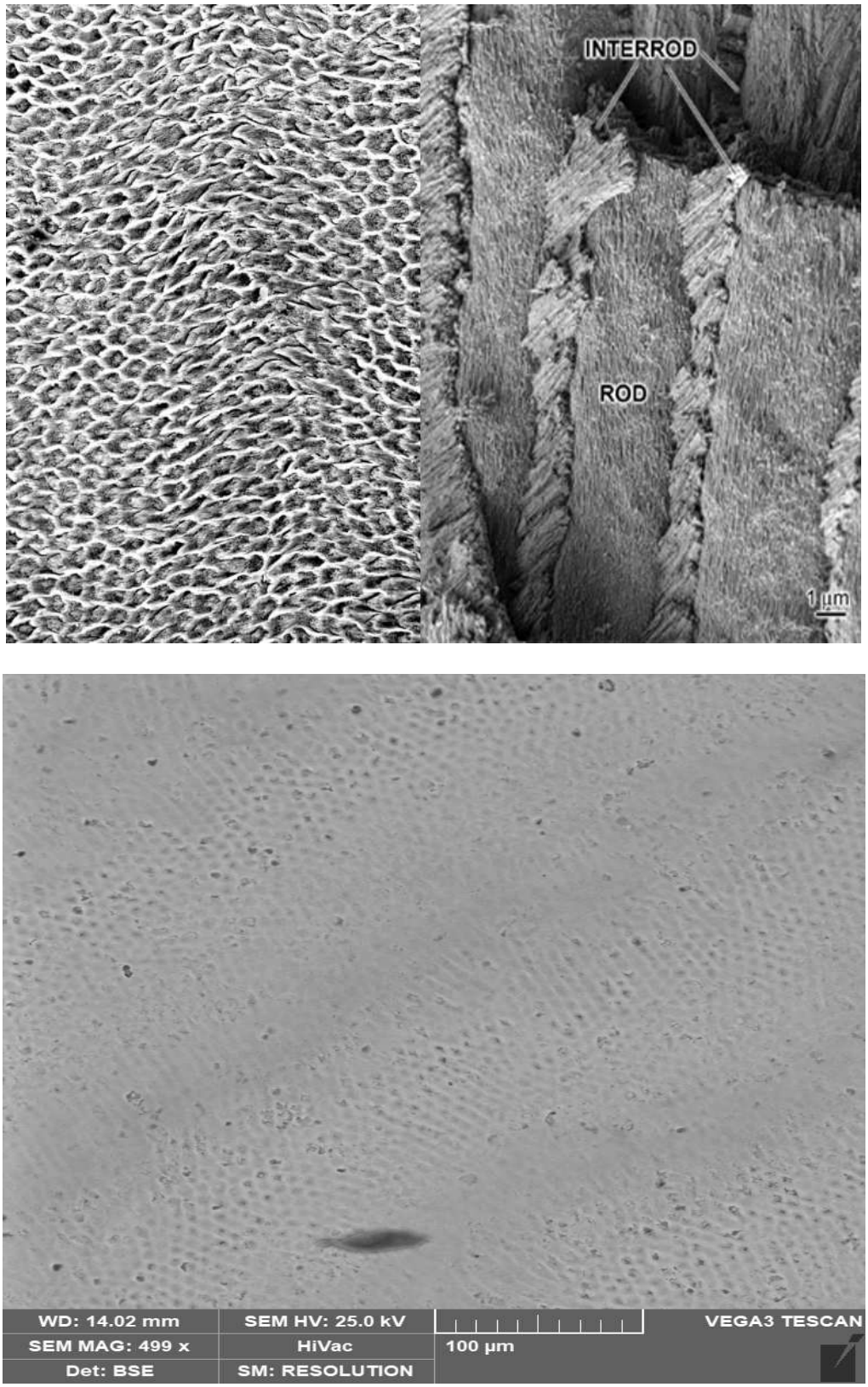

Figure 2: Structure of the sound enamel surface (SEM)

CHAPTER 2: ENAMEL WEAR AND DEGRADATION

Enamel wear is a complex process and is most commonly observed in older individuals due to aging. However, in recent times, cases of dental wear are also increasing among younger generations [10]. For a long time, tooth wear was believed to be a physiological process because it did not affect the function of the teeth. However, once the pulp is exposed due to the wear of hard dental tissues, the process is considered pathological. Enamel is the first tooth tissue to lose its structure, and this loss can be associated with metabolic factors, such as bacterial plaque, or a combination of wear-related factors. The occlusal and cervical surfaces of the teeth are primarily affected by tooth wear. Erosion, abrasion, attrition, and abfraction are the key mechanisms that can lead to tooth wear [10,11]. However, in the context of this study, we will primarily focus on abrasion and attrition, as these are the most relevant mechanical processes related to tooth wear.

2.1 Mechanical Wear: Abrasion

Abrasion is the loss of hard tooth tissue due to friction from external abrasive agents. This damage can occur when toothpaste or toothbrushes are used incorrectly. A common cause is excessive brushing with hard-bristled toothbrushes, overly abrasive toothpaste, or improper use of dental floss. Toothpaste commonly contains abrasive particles such as silicates, aluminum, activated charcoal, calcium phosphates, and calcium carbonates, which can be spherical or angular in shape. Toothpaste with an RDA (Relative Dentin Abrasion) index above 90 can be harmful to enamel, especially when used after consuming acidic foods [12]. Although brushing usually causes minimal changes, much depends on the choice of toothbrush and the level of abrasiveness in the toothpaste.

It is important to consider the composition of toothpaste, as it directly affects the degree of wear on hard tooth tissue. Abrasive lesions are usually deeper vertically than horizontally and often appear on the cervical surfaces of the teeth as smooth, shiny concavities or irregularities [3,13]. *(Figure 3)*

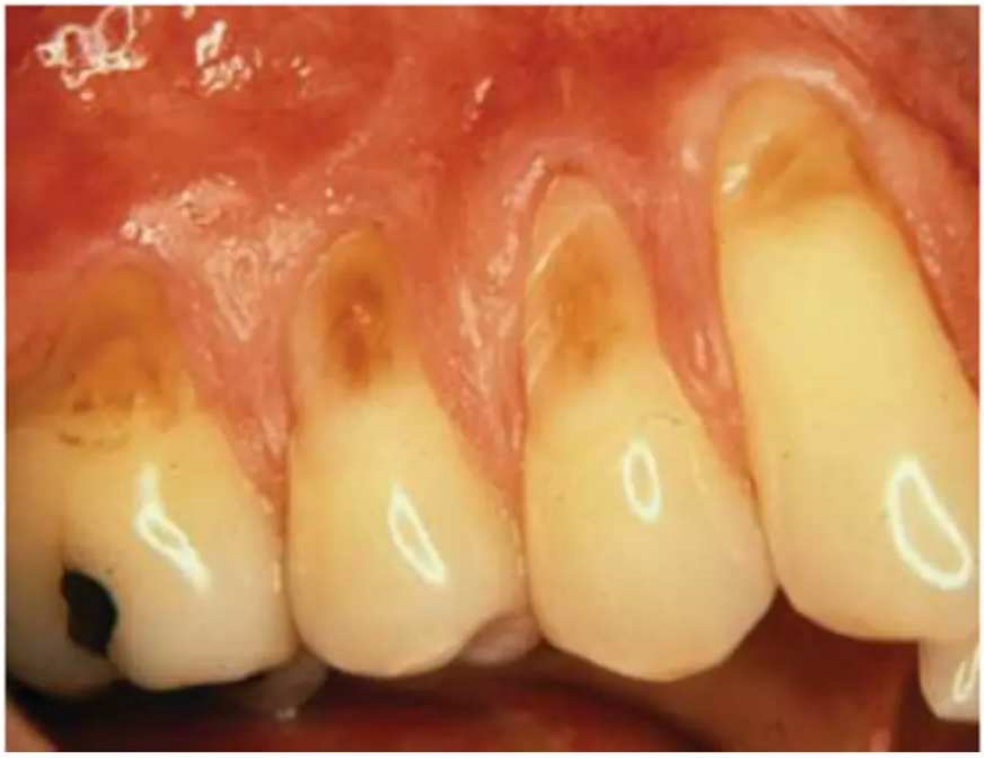

Figure 3: Dental abrasion

2.2 Gradual wearing down of tooth surfaces: Attrition

Attrition comes from the Latin word “attritum”, meaning “friction or rubbing [14]. Attrition is caused by constant tooth-to-tooth contact between antagonistic teeth, especially on the cutting edges and occlusal surfaces. This process can be physiological, as in normal tooth wear, or pathological, as in parafunctional habits such as bruxism. It leads to the formation of translucent surfaces and potential exposure of dentin, causing hypersensitivity and shortening of the teeth. *(Figure 4)*

Physiological attrition results in a gradual loss of tooth structure. Incisors are usually affected first, followed by the occlusal surfaces of molars, the palatal cusps of maxillary canines, and the buccal cusps of mandibular canines. As wear progresses, contact points between the teeth expand into broader contact zones. Pathological attrition can be caused by bruxism, bad habits such as chewing tobacco, and diseases like amelogenesis imperfecta or dentinogenesis imperfecta [3].

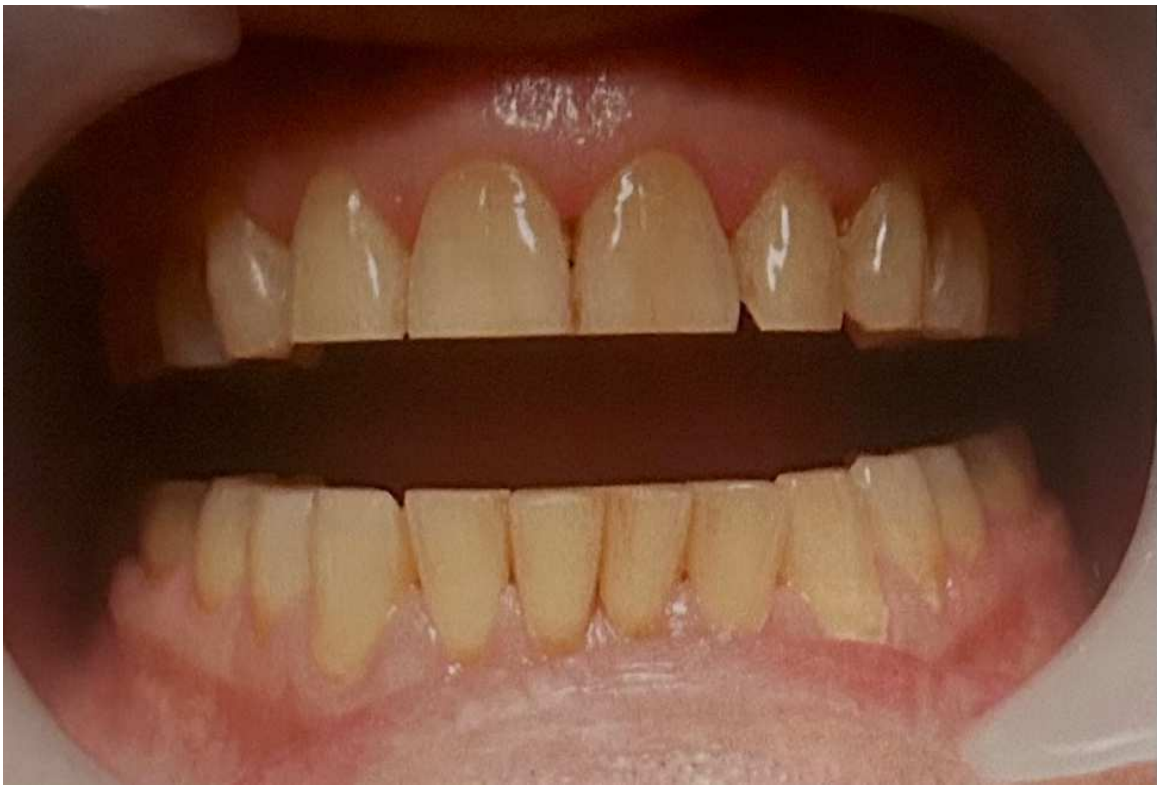

Figure 4: Attrition in a patient suffering from bruxism (tooth grinding) [3].

CHAPTER 3: TEETH WHITENING

Teeth whitening is one of the most in-demand dental treatments today and has been growing in popularity. Whitening is an effective way to remove tooth discoloration. Whitening products generally fall into three categories: professional treatments, at-home whitening kits, and over-the-counter products. These differ in the concentration of active ingredients, the method of application, and the duration of use.

Although various whitening agents are available, hydrogen peroxide is the most commonly used. It works by breaking down organic compounds that cause discoloration. However, the most common side effect of hydrogen peroxide treatments is tooth sensitivity, which can occur after the procedure. Maintaining good oral hygiene before starting whitening sessions is also crucial. Before undergoing a whitening procedure, it's essential to check if the teeth are suitable for the treatment. If there is existing tooth sensitivity, cavities, or other oral health issues, these need to be addressed first. Whitening treatments are only effective on natural teeth, as artificial dental materials like dentures, veneers, or fillings do not allow the whitening chemicals to penetrate and change color.

3.1 History of dental whitening.

Throughout history, teeth whitening has been an important aspect of many societies. In ancient Egypt, teeth were whitened using a mixture of potassium carbonate and sunlight. A similar method was used in the Roman Empire, where natural wax compounds were applied to make teeth appear brighter.

To highlight the significance of teeth whitening over the centuries, let’s review the methods used in various historical periods [15]:

-*Ancient Egyptian Civilization:* Use of potassium carbonate (K_2CO_3) and sunlight.

-*Ancient Roman Civilization:* Natural compounds based on wax.

-*12th Century:* Healthcare practitioners recommended using pastes containing sage powder and salt as an abrasive agent.

-*17th Century:* Barbers used nitric acid (HNO_3) and metal wires. This technique was highly effective but tremendously damaging to the enamel.

-*18th Century:* Use of potassium carbonate (K_2CO_3) and lactic acid ($C_3H_6O_3$).

19th Century: Hypochlorous acid ($HClO_3$) and hydrogen peroxide (H_2O_2) became popular.

-*20th Century:* Dentists discovered that hydrogen peroxide and heating lamps could accelerate the whitening process.

-*Second Half of the 20th Century:* Sodium perborate ($NaBO_3$) and water were used for treating devitalized teeth, alongside peroxide radicals produced by the alkaline decomposition of hydrogen peroxide. The introduction of carbamide peroxide ($CH_6N_2O_3$) also occurred during this period.

3.2 Color

Color is a visual perception resulting from the interaction between a light source and an object. In this case, the hard tissues of the teeth are being studied for their color properties. The color of the teeth is determined by how light interacts with the surface of the teeth.

The human eye captures and processes color, but it can only detect the visible spectrum, which includes wavelengths between 380 nm and 760 nm. When light strikes a tooth, the unabsorbed and reflected light is picked up by photoreceptors (cones and rods) in the retina. These photoreceptors convert the light into electrical

signals, which are sent to the brain, where the signals are interpreted to perceive color.

The color of teeth is genetically determined. In areas where enamel is thinner, teeth tend to appear more yellow due to the visibility of the underlying dentin. As enamel thickens, particularly in areas like the incisal edge, teeth appear more bluish-white. To address tooth discoloration, bleaching products containing hydrogen peroxide or carbamide peroxide are commonly used. These agents lighten teeth through a chemical process in which free radicals are released. The radicals break down organic molecules, penetrating the enamel and reaching the dentin, resulting in a brighter appearance.

The whitening process begins approximately 5 minutes after application, as discoloration starts to fade [16]. Hydrogen peroxide-based products typically require shorter application times, ranging from 30 to 60 minutes, and maintain a pH of 5.0. If the pH drops below this level, it surpasses the critical threshold for enamel, which can lead to the onset of cavities. Carbamide peroxide-based products, on the other hand, are designed for longer application periods of up to 10 hours. Within the first two hours, around half of the peroxide is released, but due to the presence of urea in the formula, the pH remains stable, minimizing the risk of tooth decay.

The chroma (intensity) of enamel varies depending on its thickness and translucency. Enamel's mineralization and translucency are directly proportional—higher mineralization increases translucency. Even small changes in the degree of mineralization can cause noticeable shifts in color and increase the porosity of the enamel.

Tooth color is determined by a combination of optical properties. When electromagnetic radiation hits the surface of a tooth, several phenomena occur: light

is transmitted through the tooth, reflected both regularly and diffusely on its surface, and absorbed and scattered within the tooth tissues. The overall result of this interaction is an 'off-white' color, influenced by the absorption coefficient of the tooth tissues and the length of the light absorption path [17]. Aesthetic dentistry relies on a three-dimensional color model defined by the Munsell system, developed in the late 19th century. These three dimensions are [17]:

- ***Hue****:* The characteristic that defines the color family (e.g., red, blue, yellow).
- ***Chroma****:* The saturation or intensity of the base color, indicating how vibrant or dull the color appears.
- ***Value (brightness)****:* A measure of how light or dark the color is, with black representing the lowest brightness and white representing the highest.

To determine a tooth's color, dentists compare the tooth to a standard color shade guide, typically made of ceramic or synthetic tooth samples representing various shades. By placing the shade guide close to the patient's tooth, the dentist identifies the closest matching shade. This guide functions as a standard reference, showing a range of possible tooth colors. Black indicates the lowest brightness, while pure white represents the highest, providing a way to measure the tooth’s brightness level [18] .

Thus, when you notice someone’s smile, the brightness and radiance stand out, especially if they have undergone teeth whitening.

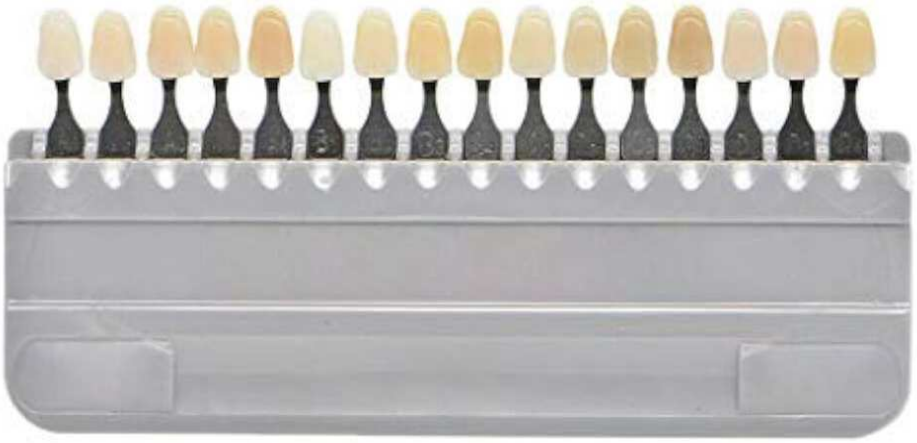

Figure 5. VITA Shade Guide

3.3 Dental Discoloration

Tooth discoloration is a common esthetic concern affecting people of all ages [19]. It is defined as a coloration of the teeth that differs from the normal white to yellowish-white shade. It can be classified as either extrinsic or intrinsic and may occur in different parts of the tooth [20] [21].

The cause of extrinsic discoloration can be: pigmented plaque caused by chromogenic bacteria, frequent consumption of coloring drinks such as tea, coffee, red wine [22], smoking, improper oral hygiene, and certain medicated mouthwashes containing chlorhexidine and fluorine stannous. The high pH of the oral cavity favors pigment accumulation on teeth [23]. *(Figure 6)*

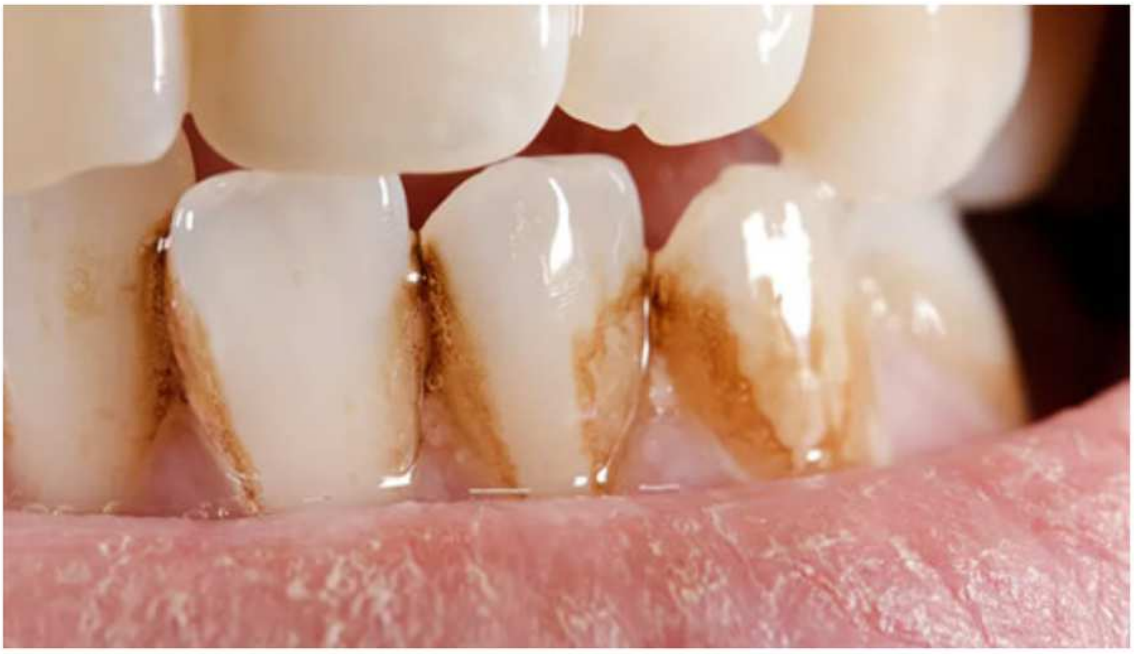

Figure 6. The extrinsic discoloration caused by the use of chlorhexidine mouthwash.

Intrinsic tooth discoloration emerges during the development of the tooth due to various metabolic disorders, which can alter the light-transmitting properties of the tooth. The main causes of internal discoloration include [3,23]:

Age-related changes: Teeth take on a grey or yellowish color due to the deposition of organic compounds in the dentin tubules.

Dental pulp hemorrhage: The rupture of red blood cells leads to iron and other byproducts infiltrating the dentin, causing the tooth to darken or turn greyish, yellow, or brown. Teeth may turn grey or reddish-grey.

Fluorosis: The color of the enamel can vary from white or grey to dark brown and is associated with constant and prolonged ingestion of excessive amounts of fluoride.

Hematological diseases: Conditions such as fetal erythroblastosis, thalassemia, sickle cell anemia, and various forms of porphyria can cause dyschromia.

Amelogenesis imperfecta: The enamel becomes yellow to brown due to a disturbance in enamel development. *(Figure 7)*

Dentinogenesis imperfecta: Blue-grey or yellow-brown discoloration that is c caused by tooth developmental disturbance.

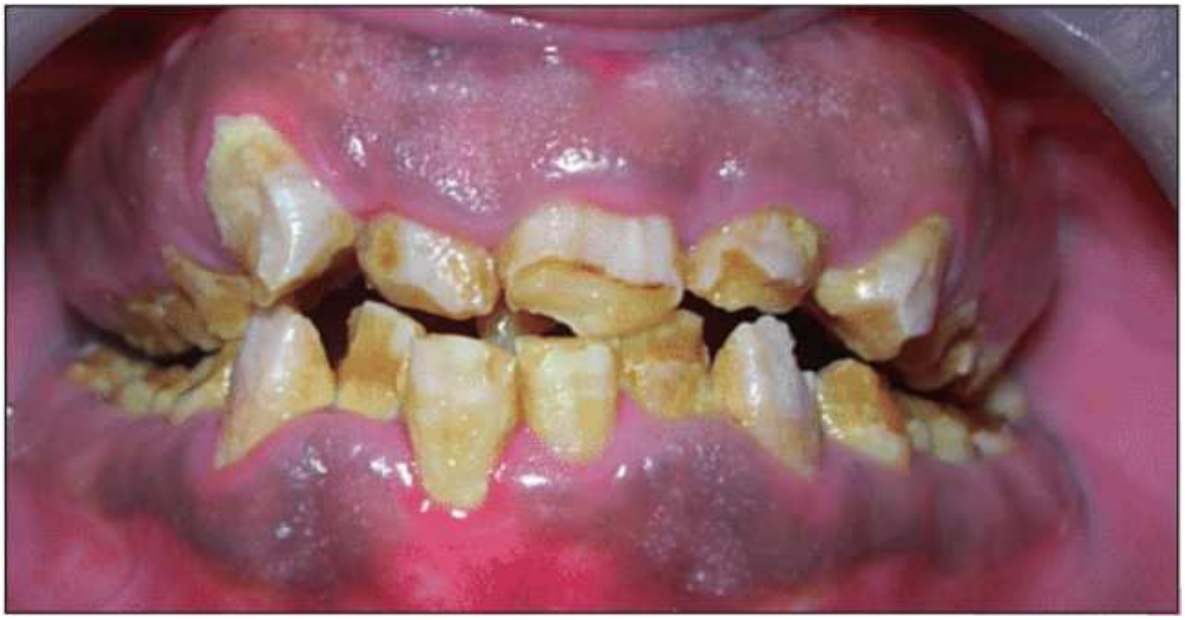

Figura 7: Intrinsic tooth discoloration *caused by amelogenesis imperfecta. Labial view of maxillary and mandibular teeth* [24]*.*

3.4 Methods of teeth Whitening

There are currently, four main techniques of teeth whitening:

- professional whitening: is performed in dental clinics.

- home whitening usually is done with the prescribed whitening kits.

- a combination of professional and at-home whitening.

-whitening with over-the-counter products that are available without a prescription.

3.4.1 Professional whitening

Professional whitening, also known as in-office bleaching, is performed in a dental clinic. The patient relies on the skills of the dental professional, who, prior to beginning the procedure, takes photographs to assess the before-and-after results. The professional ensures that the soft tissues are isolated with a rubber dam or a liquid barrier. Afterward, the whitening agent, such as hydrogen peroxide, is applied for a duration ranging from 20 to 60 minutes, depending on the concentration of the product and the dosage recommended by the manufacturer.

Certain companies, depending on the specific whitening products, may also recommend catalytic breakdown using heat or light produced by plasma arc lamps, halogen lamps, or light-emitting diode (LED) lasers to trigger the photoactivation of the whitening agent and accelerate its effect [25,26] *(Figure 8).*

This method is regarded as safer because the professional oversees the entire process, requires minimal patient involvement, and the results are instantly noticeable. However, the drawbacks include high costs, lengthy sessions, and the potential onset of side effects such as dentin hypersensitivity [27]. However, the drawbacks include high costs, lengthy sessions, and the potential onset of side effects like dentin hypersensitivity [28].

Figure 8. Professional Teeth Whitening with LED Light

3.4.2 Home Whitening

Home whitening is a procedure that uses products containing lower concentrations of hydrogen peroxide and carbamide peroxide compared to professional, in-office methods. In this approach, the patient performs the whitening treatment at home, but always under the supervision of a dental specialist, who evaluates the progress and effectiveness of the treatment during follow-up visits.

This whitening method requires the use of custom-fitted trays filled with whitening agents (10-20% carbamide peroxide, which is equivalent to 3.5-6.5% hydrogen peroxide) [29]. The patient decides when and for how long to wear the trays, depending on their schedule. Nighttime whitening allows the agent to work for an extended period, reducing the number of applications, while daytime whitening requires shorter, more frequent applications.

The process involves taking impressions of the patient's dental arches to create custom trays, as well as taking photographs of the teeth before and after treatment

to track progress. The dental professional provides the patient with the trays and syringes containing the whitening gel, offers detailed instructions for use, and schedules follow-up visits to monitor the results. *(Figure 9)*

The advantage of this method is that the patient can apply the treatment independently. However, it requires more active participation and responsibility from the patient. In many cases, patients do not follow the instructions consistently, leading to a higher failure rate with this technique.

The relatively lower cost is a key reason why this method is popular, as it is less financially demanding. However, side effects, such as moderate tooth sensitivity, can occur. In terms of effectiveness and safety, home whitening does not achieve the same level of whiteness in a single session as clinical treatments [30].

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Figure 9. Custom-fitted trays

3.4.3 Combined whitening

This procedure is recommended for patients with significant dental discoloration, such as that caused by tetracyclines, where 'in-office bleaching' or 'at-home bleaching' alone does not yield satisfactory results. The procedure occurs in two stages: The first is the 'in-office bleaching' phase, where the dental professional applies the whitening product at a variable concentration, depending on the shade of the tooth to be lightened. According to the literature, the recommended hydrogen peroxide concentration is 35%. In the second phase, 'at-home bleaching,' custom-fitted trays are used with carbamide peroxide syringes at concentrations of 10%, 15%, or 20%, as suggested by the literature [31].

Research shows that combining both in-office and at-home whitening treatments generally leads to better results than using the in-office method alone. However, it is also noted that this combined approach can cause increased tooth sensitivity, especially compared to using only professional whitening in the clinic [28].

3.4.4 Whitening with counter-top products

Over-the-counter teeth whitening products are available without the need for a dentist or dental hygienist. These products can be found in pharmacies and grocery stores and come in various forms, with different ingredients and application times. Their effectiveness is generally achieved with long-term use, though the duration and results vary depending on the product [29].

There are several types of whitening products, including toothpaste, strips, gels, pens, mouth rinses, and floss, all designed to remove surface stains and brighten teeth. While some may show quicker results, most require regular use for noticeable improvement. Whitening gums and toothbrushes are also available. *(Figure 10)*

Whitening strips, for example, often contain hydrogen peroxide in varying concentrations. These strips, made of flexible polyethylene, adhere to the teeth when pressed onto the surface. They can be used without professional supervision, but improper use might pose some risks [32].

It's important to be aware of potential downsides. Whitening strips can sometimes irritate the gums, affect speech, or cause uneven results if misapplied. While they can help brighten teeth, achieving perfect results may not always be possible, so careful consideration before use is advised [33].

Whitening gels or pens, which typically contain hydrogen peroxide or carbamide peroxide, are also effective options. These products are applied by brushing the gel onto the tooth surface with an applicator. Once applied, the gel forms a thin layer that adheres to the enamel, slowly releasing peroxide to whiten the teeth.

These products are popular for several reasons:

- The gel is easy to apply, similar to brushing on a thin layer of nail polish.
- It can be applied to multiple teeth at once.
- Many come with disposable applicators, offering added convenience and hygiene.

Overall, these over-the-counter whitening products provide a practical and accessible way to whiten teeth at home [34].

-*Mouthwashes* are liquid, water-based products used for teeth whitening. They typically contain low concentrations of hydrogen peroxide (1.5%) and sodium hexametaphosphate. These ingredients help prevent new stains from forming while controlling and removing existing surface stains. Additionally, they inhibit plaque buildup, contribute to a whiter appearance, and promote overall oral hygiene [35].

- *Whitening toothpastes* are specially formulated to brighten teeth and prevent stains from forming. The goal is to restore the teeth's natural whiteness and prevent yellowing. These toothpastes often contain bleaching agents like hydrogen peroxide or carbamide peroxide, which target organic molecules in biofilm to reduce plaque. They also contain abrasive components, such as silica and dicalcium phosphate, which help remove surface stains and impurities.

The effectiveness of a whitening toothpaste largely depends on its abrasive properties. However, the abrasiveness must be carefully balanced to avoid damaging the tooth enamel. Excessive abrasiveness can harm both the enamel and the dentin layer underneath. Regular use of these toothpastes can lead to whiter teeth and a healthier mouth, but it’s important to follow the label instructions and avoid overuse [36].

The abrasiveness of toothpaste is measured by its RDA (Relative Dentin Abrasivity) value, which indicates how much the toothpaste wears down the tooth surface. While toothpastes with higher RDA values may be more effective at removing plaque and polishing teeth, they may not always meet whitening expectations due to the short contact time between the gel and the tooth surface. Additionally, using reusable applicator brushes can increase the risk of microbial contamination.

Some manufacturers include information about the toothpaste's abrasiveness on their packaging, helping consumers understand the product's abrasiveness. While a higher RDA value suggests better plaque removal, excessive abrasiveness can damage tooth enamel, so careful use is recommended.

Whitening floss is another innovative product. Made of fine plastic filaments coated with silica abrasive particles, it is designed to remove stains between the teeth and under the gumline. Similarly, whitening gums release agents like sodium

hexametaphosphate when chewed, helping to prevent and remove stains while providing a whitening effect. These products have been developed to meet the growing demand for whiter teeth [37].

- *Whitening pens* are another option, containing a whitening gel in a pen-like device. The gel is housed in a reservoir within the pen, designed for easy application. While convenient, some users have reported tooth sensitivity and discomfort in the oral mucosa during use. Scientific studies have also shown that light erosion areas can form on healthy teeth with prolonged use [38].

Figura 10. Whitening with counter-top products

CHAPTER 4: WHITENING AGENTS

Teeth whitening can be performed in two main ways:

-*Mechanical Methods:* Fine abrasive powders found in toothpaste act through friction to remove stains on the surface of teeth.

-*Chemical Methods:* Chemical whitening using active ingredients such as hydrogen peroxide and carbamide peroxide occurs when these components interact with dental tissues and release oxygen molecules. This process enables the breakdown (oxidation) of the molecules responsible for pigments.

Toothpastes are specially formulated for teeth whitening function to prevent and remove the formation of additional stains. However, it has been shown that toothpastes with low abrasive properties do not adequately remove the stained film that accumulates on the tooth surface. On the other hand, toothpastes with higher abrasive properties have been widely documented to effectively eliminate or prevent the formation of external stains [39].

It is understood that the degree of abrasiveness plays an important role in the effectiveness of toothpaste. Proper selection of abrasive content is critical to maintaining dental health while enhancing the whitening effect.

Studies confirm that the abrasive properties of the main components play a significant role in stain removal. Based on these properties, whitening agents can generally be divided into three main categories:

-*Low Abrasive:* These products are used to clean light stains without damaging tooth enamel.

-*Medium Abrasive:* These products, containing moderate abrasive components, are effective in removing more prominent stains.

-High Abrasive: Such products are designed to remove dense stains but should be used with caution, as they carry a risk of damaging tooth enamel.

Abrasive properties depend on the structure of the ingredients, typically found in toothpastes and other whitening products. The effectiveness of the whitening process is associated with the correct formulation, as well as the abrasive properties of the products used.

Common abrasive components found in toothpaste include perlite, hydrated silica, alumina, calcium carbonate, dicalcium phosphate dihydrate, calcium pyrophosphate, and sodium bicarbonate. Chemicals used in bleaching processes include hydrogen peroxide, carbamide peroxide, calcium, sodium pyrophosphate, sodium tripolyphosphate, sodium citrate, and sodium hexametaphosphate. Additionally, some research suggests that enzymes, such as papain, are also effective in removing extrinsic stains [40].

4.1 Hydrogen Peroxide

Hydrogen peroxide is the most commonly used active ingredient in whitening products. Its chemical formula is H_2O_2, where the two oxygen atoms are joined by a single covalent bond, and each hydrogen atom is individually bonded to an oxygen atom.

The mechanism of action of hydrogen peroxide is based on an oxidative process that allows it to penetrate the enamel and dentin. This process can sometimes cause dental sensitivity, which is directly proportional to the concentration of the peroxide itself [41].

Hydrogen peroxide is effective in three stages during the teeth whitening process:

-*Stage One:* Peroxide penetrates the interprismatic spaces in the tooth enamel due to its high permeability. From the moment of application, it continues to circulate inside the tooth for about two weeks.

-*Stage Two:* During circulation, hydrogen peroxide decomposes, producing oxygen-free radicals and interacting with organic chromophores. In this process, reactive ions such as hydroxyl, hydroperoxyl radicals, superoxide radical cation, and superoxide radical anion are formed through the breakdown of chemical bonds. The reactivity of these radicals is influenced by factors such as the presence of metal cations, pH, light, and temperature.

-*Stage Three:* After the action of peroxide, the tooth surface reflects light differently, causing a change in the perceived color. The whitening process can also lead to a slight roughness on the tooth surface, which increases light reflection, making the teeth appear brighter [42].

While this process provides a whitening effect, it can also lead to side effects such as increased tooth sensitivity. Gels containing remineralizing compounds, such as nano-hydroxyapatite and calcium fluoride, can be used to prevent post-treatment sensitivity [41].

4.2. Carbamide Peroxide

Carbamide peroxide ($CH_6N_2O_3$) is a stable compound commonly used in teeth whitening, similar to hydrogen peroxide. When it reacts with water, this compound breaks down chromogenic molecules by releasing free radicals, providing a whitening effect on enamel and dentin.

Carbamide peroxide is structurally more stable and, therefore, lasts longer than hydrogen peroxide, which tends to degrade quickly due to its instability.

Research indicates that carbamide peroxide can decompose into ammonia and carbon dioxide. During this process, it promotes teeth whitening by increasing pH. Carbamide peroxide stands out as an effective alternative in teeth whitening treatments [43].

4.3 Activated charcoal

Activated charcoal is a fine black powder produced through controlled heating or chemical treatment of various carbon-containing materials such as bamboo, walnut shells, peat, and coconut shells. This thermal process, referred to as 'activation,' enhances the adsorption capacity of activated carbon.

Adsorption is a mechanism that allows molecules to stick to the surface of a solid substance. This property of activated charcoal is particularly beneficial in various fields, such as environmental (water purification) and health (detoxification). However, scientific evidence on the efficacy of activated charcoal in oral hygiene products for reducing tooth stains is limited. Therefore, additional research is required to better understand the role and influence of activated charcoal on dental health.

Carbon interacts with surface stains on teeth, aiding in their removal and seemingly forming chemical bonds with chromogenic molecules. Nevertheless, there is some uncertainty regarding the application of activated charcoal in toothpastes. While some studies suggest that activated charcoal in toothpastes can bind to all deposits on the tooth surface, there is insufficient scientific data to fully validate this claim. Although activated charcoal may help remove plaque, bacteria, and stained substances by trapping them in its pores, the extent of its effectiveness in eliminating deposits from tooth surfaces remains uncertain [44,45]. *(Figure 11)*

Figure 11: Activated charcoal toothpaste

4.4 Perlite

Perlite is a volcanic-origin material that is chemically inert and an amorphous glass silicate with a neutral pH. When exposed to high temperatures, it expands into a foamed structure after being ground into fine particles, increasing in volume during the process. In dental prophylaxis, perlite is frequently used as an abrasive ingredient in toothpastes, effectively removing pigmented chromophores and biofilms while enhancing the gloss of the tooth surface. Research indicates that perlite particles align parallel to the tooth surface, thereby offering protection against scratching [46].

4.5 Blue covarine

Covarine blue, when incorporated into toothpaste, is a chemical that creates an immediate teeth-whitening effect by shifting the yellow-blue color axis. It significantly increases the perception of whiteness. This color shift occurs when the pigment is deposited on tooth surfaces, forming a film that evens out, enhancing the

appearance of whiteness. Recent studies, as verified in the literature, have shown that incorporating substances like Covarine blue into whitening agents is an important innovation. The optical effect produced by this pigment allows for a visible and detectable whitening result every time the toothpaste is used [47]. *(Figure 12)*

(Figure 12): Blue covarine toothpaste

CHAPTER 5: EXPERIMENTAL PART

5.1 The Aim of Study

This study focuses on evaluating the difference in whitening effectiveness of three commercial charcoal-based toothpastes on stained, extracted human teeth over 28 days. A total of 20 human incisors (lateral and central), extracted for orthodontic or periodontal reasons, were collected, stained in a coffee solution, and randomly divided into four groups (n = 5 per group). Group CTR (control group) was brushed without toothpaste, while Groups CPX, CPT, and CLG were brushed with the whitening toothpastes *Curaprox Black & White, Curasept Black Lux, and Colgate Max White Charcoal.* The whitening effects of these toothpastes on stained teeth were measured using a spectrophotometer.

The aim of this in vitro study is to determine which toothpaste is more effective in terms of whitening performance and to observe whether the whitening toothpastes significantly improved enamel color after daily brushing.

Null Hypothesis: There is no significant difference in the whitening effect between the toothpastes studied across the groups used in the study.

5.2 Materials and Methods

This in vitro study received ethical approval by the Department of Clinical Sciences and Stomatology (DISCO) at Università Politecnica delle Marche, Ancona, Italy.

5.2.1 Specimens' Preparation

For research purposes, 20 extracted sound human incisors (central and lateral) were collected for orthodontic or periodontal therapeutic reasons [48]. According to the Local Ethical Committee guidelines and the 1964 Helsinki Declaration, informed consent

was obtained from the subject aware that their hard-dental tissues, as discarded of the surgical procedures, would have been used for research purposes [49].

Teeth with lesions, caries, restorations, discoloration, or hypoplastic defects were excluded from the study. Each tooth was examined under the light of a dental unit and with a dental probe. The teeth were carefully washed under running water and immersed in an ultrasonic bath with distilled water for 4 minutes to remove blood and biological residues. Specimens were then carefully cleaned of any debris and extrinsic stains using a piezo ultrasonic scaler (Mectron Piezosurgery Touch with a standard tip), followed by a low-speed handpiece with a brush, without prophylaxis paste. After the cleaning procedure, the teeth were stored in a 0.5% w/w chloramine solution (NH_2Cl) at room temperature until the start of the study [50]. The teeth samples were randomly divided into four groups (n = 5 per group) using a simple randomization technique.

Group Names:

1. **Group CTR** (Control Group)
2. **Group CPX** (Curaprox Black & White, Curaden-Switzerland
3. **Group CPT** (Curasept Black Lux, Curasept S.p.A. Italy
4. **Group CLG** (Colgate Max White Charcoal, Colgate-Palmolive- US)

The detailed ingredient list for all the tested toothpastes can be found in *Table 1*.

Photo protocol of the control group's teeth was taken before and after the staining protocol to observe and compare the changes in enamel color. *(Figure 13)*

Figure 13:Photo protocol of teeth before staining (1) and after staining in coffee solution (2)

Table 1: The ingredient list of the toothpastes evaluated in the study, as provided by the manufacturers

Group Name	Brand Name	Type	Ingredients	Active Whitening Ingredient(s)
CPX	Curaprox Black Is White	Whitening Toothpaste	Aqua, sorbitol, glycerin, hydrated silica, charcoal powder, aroma, argilla, decyl glucoside, cocamidopropyl betaine, sodium monofluorophosphate, tocopherol, mica, xanthan gum, hydroxyapatite (nano), titanium dioxide, microcrystalline cellulose, maltodextrin, potassium acesulfame, sodium benzoate, potassium chloride, potassium sorbate, menthyl lactate, methyl diisopropyl propionamide, ethyl menthane carboxamide, zea mays starch, stearic acid, cetearyl alcohol, citrus limon peel oil, citric acid, lactoperoxidase, glucose oxidase, amyloglucosidase, tin oxide, sodium bisulfite, hydrogenated lecithin, limonen, CI75810, CI77289.	Hydrated silica, Charcoal powder, Hydroxyapatite (nano), Titanium dioxide, Sodium monofluorophosphate
CPT	Curasept Black Lux	Whitening Toothpaste	Aqua, Sorbitol, Hydrated Silica, Glycerin, Cellulose Gum, Sodium Methyl Cocoyl Taurate, Aroma, Xylitol, C.I. 77499, Sodium Lauroyl Sarcosinate, Mg-Sr Carbonate Hydroxyapatite Conjugated with Chitosan, Ozonized Olive Oil, Hydrogen Peroxide, Pvp, Urea Peroxide, Glycyrrhetinic Acid, Solum Diatomeae, Sodium Fluoride, VP/VA, Copolymer, Charcoal Powder, O-cymen, 5-OL, Decyl Glucoside, Tocopheryl, Acetate, Potassium Acesulfame, Sodium Benzoate, Citric Acid.	Hydrogen Peroxide, Urea Peroxide, Charcoal Powder, Hydrated Silica, Mg-Sr Carbonate Hydroxyapatite Conjugated with Chitosan
GLG	Colgate Max White	Whitening Toothpaste	Aqua, Sorbitol, Hydrated Silica, PEG-12, Tetrapotassium Pyrophosphate, Sodium Lauryl Sulfate, Aroma, Potassium Hydroxide, Cellulose Gum, Phosphoric Acid, Cocamidopropyl Betaine, Sodium Fluoride, Sodium Saccharin, Xanthan Gum, Mica, Charcoal Powder, Limonene, CI 77891, Contains: Sodium Fluoride (1450 ppm F^-).	Hydrated Silica, Tetrapotassium Pyrophosphate, Charcoal Powder

Staining Protocol

Tea and coffee, two of the most popular drinks around the globe, are well-known for their tendency to stain teeth [19]. For this reason, freshly brewed coffee was chosen as the staining solution. The coffee solution was prepared using Illy's well-known Italian Arabica coffee. After the scaling and polishing procedures were completed, the specimens were immersed in the coffee solution. During the two-week staining protocol, the staining solution was shaken once a day, and the coffee solution was replaced with freshly brewed coffee every three days *(Figure 14).*

Preparation of coffee: Fifteen grams of coffee were brewed with 500 ml of hot, filtered tap water [51]. The solution was allowed to cool to room temperature before the samples were soaked for two weeks. During the two-week staining protocol, the staining solution was shaken once a day, and the coffee solution was replaced with freshly brewed coffee every three days.

Firgure 14: Staining Protocol. The specimens are immersed in a coffee solution for two weeks.

Brushing Protocol

Each specimen in its assigned group was brushed using brand new Curaprox Soft manual toothbrushes, with a different color designated for each group to avoid mixing the toothpastes.

Group CTR (Control Group): Five incisal teeth were brushed only with a Curaprox Soft manual toothbrush (blue) moistened in artificial saliva. *(Figure 15)*

Group CPX (Curaprox Black & White): Five incisal teeth were treated using Curaprox Black & White whitening toothpaste with a Curaprox Soft manual toothbrush (brown). *(Figure 16)*

Group CPT (Curasept Black Lux): Five incisal teeth were treated using Curasept Black Lux whitening toothpaste with a Curaprox Soft manual toothbrush (yellow) *(Figure 17)*

Group CLG (Colgate Max White): Five incisal teeth were treated using *Colgate Max White* whitening toothpaste with a Curaprox Soft manual toothbrush (green). *(Figure 18)*

The modified Bass technique was chosen for brushing, as it is considered the most effective brushing technique [52].

Each sample underwent brushing twice a day (morning and evening), simulating normal brushing sessions over the course of one month. Each brushing session lasted 75 seconds per group, which is 15 seconds per tooth, for a total of 3 minutes per day. The toothpaste was diluted with artificial saliva in a ratio of 1:2 (1 g of toothpaste to 2 ml of artificial saliva).

The diluted toothpaste was poured over the samples to simulate brushing in an aqueous environment. *(Figure 19)* Tooth brushing was performed in a humid environment, with an operator loading the toothbrush heads with a fresh mixture

each time. After brushing, the specimens were washed, dried, and submitted to the study measurement [53].

After every brushing cycle, teeth were rinsed thoroughly and placed in artificial saliva Biotene Oralbalance Gel, GSK, England from the first day of the brushing cycle protocol.

The composition of the artificial saliva: Sorbitol. Water. Glycerin, Xylitol, Butylene Glycol, Sodium Polyacrylate, Polyacrylic Acid, Hydroxyethylcellulose, Sorbic Acid, Glucose, Benzoic Acid, Lactoperoxidase, Lysozyme, Lactoferrin, Disodium Phosphate, Glucose Oxidase, Potassium Thiocyanate.

Figure 15: Group CTR (Control Group)

Figure 16: Group CPX (Curaprox Black & White)

Figure 17: Group CPT (Curasept Black Lux)

Figure 18: Group CLG (Colgate Max White Charcoal)

Figure 19: Toothpaste diluted with artificial saliva in a 1:2 ratio on the left, pure toothpaste on the right.

5.2.2 Color measurement

After-staining color measurement: The color of the specimens was evaluated at five different time points: the baseline (T0), after 7 days (T1), 14 days (T2), 21 days (T3), and 28 days (T4) from the start of the brushing cycle with whitening toothpaste for groups CPX, CPT, CGT and without toothpaste for CTR group. The color measurements were performed using a spectrophotometer SpectroShade-Micro (MHT S.p.a., Verona, Italy). *(Figure 20):*

The spectrophotometer was allowed to warm up for 10 min after turning on and before each measurement [54]. Before the color measurement, each sample was rinsed with distilled water and during each measurement session, the teeth were kept slightly moist to prevent optical changes from dehydration. The spectrophotometer was calibrated according to the manufacturer's recommendations using the provided white and green calibration standards. The color measurements were taken in CIELAB color space, which measures the lightness L* (lightness, where 100 represents white and 0 represents black), chroma c* (red-green chromatic coordinate), and hue angle h* (blue-yellow chromatic coordinate). The measurements were taken by placing samples on non-reflective black background (L = 9.20; c = 1.47; h = −3.83). The measurements were repeated three times for each specimen on the center of the vestibular surfaces of teeth, and the average values of L*, c*, and h* were calculated and recorded in an Excel file. (*Table 2,3)*

ΔE^*ab was calculated between the colors at five time points

(T0, T1, T2, T3 and T4) using the following equations [49] :

1. Color change between baseline (T0) and 7 days (T1):

$$\Delta E^*_{ab}(T0 \rightarrow T1) = \sqrt{(L^*_{T1} - L^*_{T0})^2 + (c^*_{T1} - c^*_{T0})^2 + (h^*_{T1} - h^*_{T0})^2}$$

2. Color change between baseline (T0) and 14 days (T2):

$$\Delta E^*_{ab}(T0 \rightarrow T2) = \sqrt{(L^*_{T2} - L^*_{T0})^2 + (c^*_{T2} - c^*_{T0})^2 + (h^*_{T2} - h^*_{T0})^2}$$

3. Color change between baseline (T0) and 21 days (T3):

$$\Delta E^*_{ab}(T0 \rightarrow T3) = \sqrt{(L^*_{T3} - L^*_{T0})^2 + (c^*_{T3} - c^*_{T0})^2 + (h^*_{T3} - h^*_{T0})^2}$$

4. Color change between baseline (T0) and 28 days (T4):

$$\Delta E^*_{ab}(T0 \rightarrow T4) = \sqrt{(L^*_{T4} - L^*_{T0})^2 + (c^*_{T4} - c^*_{T0})^2 + (h^*_{T4} - h^*_{T0})^2}$$

The variation in L*, h*, and c* values was calculated using the following equation [49]:

$$\Delta L^* = L^*_{Tn} - L^*_{T0}$$

$$\Delta c^* = c^*_{Tn} - c^*_{T0}$$

$$\Delta h^* = h^*_{Tn} - h^*_{T0}$$

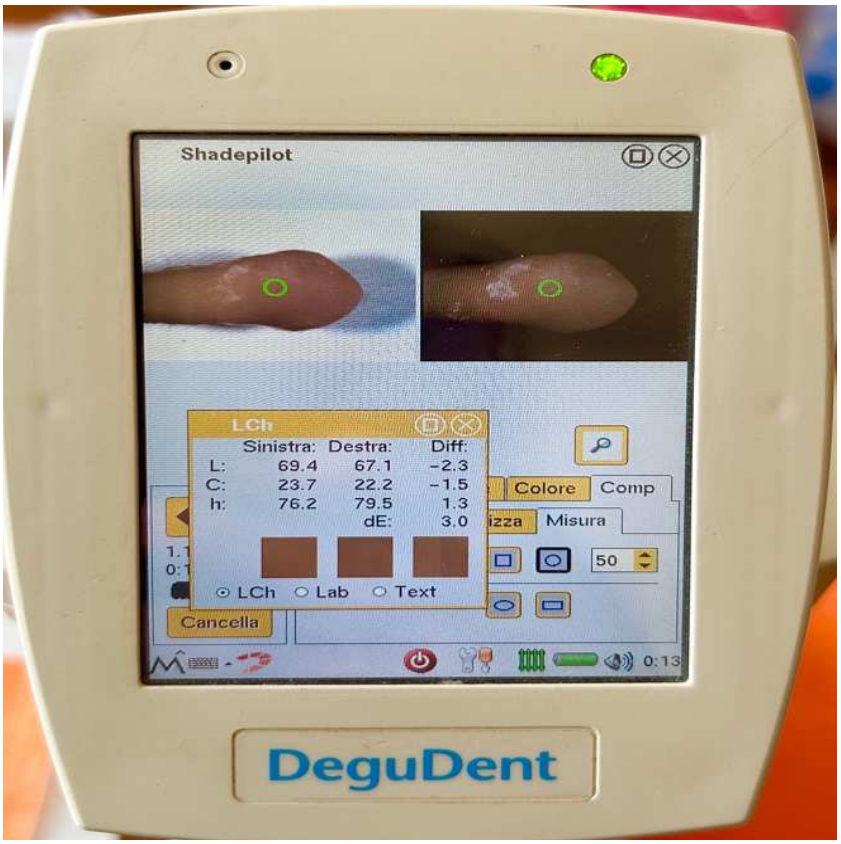


Figure 20: Dental spectrophotometer

Table 2. Color Measurement and data collection before starting the staining protocol of control group CTR.

Name	N	Surface	L	c	h
CTR	Sample 1	Incisal	68,4	17,9	90,2
		Central	68,7	25,5	87,1
		Cervical	70,8	21,3	89,7
	Sample 2	Incisal	63,8	13,7	81,3
		Central	68,2	23,4	84,1
		Cervical	69,0	24,0	84,2
	Sample 3	Incisal	66,3	24,6	78,8
		Central	68,9	23,5	80,4
		Cervical	71,3	19,7	82,9
	Sample 4	Incisal	58,0	13,9	71,6
		Central	66,7	20,7	84,1
		Cervical	71,2	22,9	86,5
	Sample 5	Incisal	68,5	15,3	84,0
		Central	70,3	19,7	84,8

Table 3. Data collection after the staining protocol of CTR, CLG, CPT,CPX groups.

			T0			T1			T2			T3			T4		
Name	N	Surface	L	c	h	L1	c1	h1	L2	c2	h2	L3	c3	h3	L4	c4	h4
CTR	Sample 1	Incisal	60.8	16.8	82	60-2	15.2	86.9	60.7	14.2	86.3	59.1	15.0	85.0	59.7	14.9	83.8
		Central	62.1	18.2	80.1	63.6	21.7	82.8	62.4	22.4	82.4	63.0	21.7	82.3	62.7	19.6	82.1
		Cervical	63.5	23	80.8	65.5	20.5	83.7	63.6	20.3	84.2	65.0	20.2	84.3	62.6	20.4	82.4
	Sample 2	Incisal	60.7	13.3	81.5	59.2	13.2	82.6	58.3	8.6	85.4	56.0	12.3	81.9	58.3	10.2	81.3
		Central	63.3	21.7	80.9	63.1	23.9	83.1	63.4	22.0	83.0	60.8	22.4	83.5	61.4	20.2	83.5
		Cervical	60.9	24.2	77.2	57.6	24.8	80.3	60.7	24.6	77.1	57.4	27.2	80.2	57.3	26.2	78.9
	Sample 3	Incisal	63.5	21.6	75.5	60.0	19.0	81.5	58.8	17.2	77.0	56.0	12.7	82.0	57.0	22.0	74.8
		Central	66.4	18.9	75.5	60.0	28.9	80.9	60.8	25.4	77.8	61.3	22.6	83.7	60.2	23.5	77.5
		Cervical	64.7	23.9	78	62.0	27.3	80.2	61.0	23.9	77.5	58.0	26.9	79.3	60.5	27.0	77.0
	Sample 4	Incisal	60.5	10	68.5	60.0	9.8	74.7	58.9	12.6	76.8	58.9	17.9	77.0	57.3	12.1	74.2
		Central	66.4	15.6	76.8	67.5	23.0	74.3	64.1	17.9	79.3	61.5	25.8	78.4	64.0	19.3	78.3
		Cervical	69.7	20.3	80.4	68.4	23.3	78.7	65.7	22.3	81.0	59.5	24.2	78.3	63.0	22.2	79.6
	Sample 5	Incisal	61.4	22.4	77.6	59.5	22.5	88.5	59.6	17.6	81.7	57.1	12.5	76.7	60.7	19.6	78.9
		Central	62.4	23.5	78.2	60.0	15.9	82.7	62.6	22.8	83.3	63.3	22.5	82.4	63.6	21.9	81.6
		Cervical	62.4	22.5	79.8	62.5	22.6	82.7	62.3	23.0	83.7	61.9	22.7	82.7	61.2	22.2	82.4
CLG	Sample 1	Incisal	63	15.1	86.8	62.0	22.3	80.0	62.5	12.2	86.6	64.4	13.6	88.5	63.9	13.2	88.0
		Central	67.2	15.6	87	64.0	12.0	88.3	64.3	17.5	82.9	64.2	17.1	86.9	63.8	16.4	84.9
		Cervical	63.5	19.9	87.1	64.2	17.3	83.9	61.6	23.7	80.0	61.1	18.9	85.9	61.4	17.2	85.7
	Sample 2	Incisal	57.3	12.4	82.5	60.1	13.5	86.5	58.5	13.8	86.2	60.1	19.4	85.9	59.0	13.5	84.9
		Central	64.3	19.4	81.5	65.3	20.1	82.8	64.4	19.4	83.4	65.5	19.3	82.6	65.5	19l3	82.1
		Cervical	62.9	26.2	78.3	62.0	23.7	79.6	58.5	28.3	79.4	61.5	25.7	79.4	60.1	24.9	78.9
	Sample 3	Incisal	57.7	14.7	84.1	62.3	9.8	87.2	58.0	16.9	79.2	61.8	13.3	84.7	61.8	14.3	83.2
		Central	63.4	21.3	81.3	63.8	19.6	81.2	61.3	20.6	81.5	64.0	20.6	80.3	64.3	21.5	79.5
		Cervical	62.5	24.9	80.6	62.0	24.0	80.8	62.0	21.6	82.6	62.3	23.2	79.5	62.2	23.7	78.8
	Sample 4	Incisal	51.7	11.3	63.5	56.3	11.2	70.3	58.5	12.1	75.0	54.0	11.3	69.2	55.2	11.0	69.5
		Central	61.4	19.1	74.8	62.3	23.7	78.1	63.3	19.1	78.7	62.1	19.1	76.4	62.9	18.5	80.5
		Cervical	61.4	26.6	75.6	62.6	27.5	78.8	62.6	26.6	78.4	62.5	26.7	77.8	62.6	26.3	77.6
	Sample 5	Incisal	56	17.5	75.5	56.4	20.6	81.0	54.6	26.8	78.9	55.6	16.8	78.3	54.9	11.8	67.1
		Central	60.5	21.8	81	61.4	22.7	83.3	62.0	21.7	84.1	61.6	21.8	82.9	60.1	18.5	80.5
		Cervical	57.9	21.8	82.1	58.0	23.9	82.4	58.6	22.3	82.9	59.2	22.1	83.1	59.0	21.5	83.2

CPT	Sample 1	Incisal	60.7	9.9	85.6	63.3	9.6	95.3	63.4	10.4	93.9	56.0	6.2	71.5	56.4	7.0	70.6
		Central	67.8	18	85	70.5	18.0	86.8	71.3	18.5	88.0	58.6	11.7	80.6	59.1	12.5	80.4
		Cervical	67.4	20.4	81.5	65.6	19.6	81.8	66.0	19.2	80.6	55.4	13.5	81.1	54.5	14.3	81.3
	Sample 2	Incisal	58.4	8.2	72.7	55.9	6.8	74.2	56.0	8.0	76.8	57.2	12.9	87.4	57.7	14.3	87.5
		Central	60	12.6	80.1	58.1	13.7	81.7	57.9	13.0	80.7	68.1	22.8	84.4	68.1	22.4	84.0
		Cervical	57.7	15.1	80.8	55.3	16.2	82.4	53.7	17.1	82.5	59.8	19.4	76.2	61.7	19.0	76.8
	Sample 3	Incisal	58.4	14.2	90.3	51.4	16.5	78.0	51.2	16.5	77.6	53.7	18.5	77.4	52.2	16.9	77.1
		Central	67.6	22.9	84.2	60.0	24.7	80.0	58.8	26.6	80.2	59.8	25.0	80.0	60.3	24.8	80.1
		Cervical	63.6	21.6	77	63.1	57.7	22.2	77.8	22.3	78.4	58.7	22.1	78.6	58.5	21.9	78.1
	Sample 4	Incisal	51.8	14	76.3	53.9	17.9	77.0	53.7	18.0	77.0	62.0	10.3	95.4	60.6	10.3	96.4
		Central	60	26	79	60.2	24.3	79.6	69.1	18.7	86.5	70.7	18.6	87.4	68.6	20.3	85.8
		Cervical	60.8	22.4	78.6	58.0	22.7	79.0	60.0	22.4	79.0	64.0	18.8	82.2	66.0	20.4	83.4
	Sample 5	Incisal	60.8	16	77.9	60.0	15.8	79.0	60.4	15.6	78.9	60.0	13.5	81.0	60.3	15.4	79.7
		Central	64.9	21.3	80.4	65.0	23.0	82.2	65.0	22.9	82.4	63.9	22.3	82.4	65.2	23.0	83.2
		Cervical	62.3	23.4	78	62.7	22.9	80.6	58.9	23.9	79.7	59.9	23.3	80.0	62.8	24.0	81.3

CPX	Sample 1	Incisal	58.4	20.2	83.1	57.6	17.3	82.1	60.0	18.2	82.6	56.6	19.2	81.8	58.8	19.6	82.6
		Central	61	23.4	80.2	60.5	23.5	79.1	61.4	22.1	80.5	60.3	24.6	79.0	62.1	23.4	79.8
		Cervical	64.2	23.6	79.6	63.2	33.5	89.5	63.7	21.0	79.2	61.1	22.2	80.8	62.0	22.8	79.5
	Sample 2	Incisal	55.3	21.7	80.2	54.1	6.5	59.3	53.0	16.2	78.6	59.8	12.1	86.5	56.7	13.8	83.5
		Central	62	24	85	58.4	12.7	75.5	62.0	20.0	83.0	63.5	18.8	85.5	64.3	15.5	86.6
		Cervical	59.7	18.9	86.2	55.4	14.0	77.2	56.9	14.6	87.8	58.3	15.8	85.5	60.5	15.3	88.0
	Sample 3	Incisal	58.2	11.7	86.8	58.5	9.8	81.9	61.0	14.4	86.3	61.6	14.4	87.0	63.4	14.7	85.7
		Central	66.3	19.7	80.3	63.8	16.0	76.0	66.1	19.3	82.3	65.9	19.9	81.6	66.1	20.5	80.7
		Cervical	63.2	22.9	76	62.0	20.2	73.6	62.0	21.0	77.0	60.0	22.7	77.7	59.8	23.0	76.9
	Sample 4	Incisal	55.8	16.2	86	55.0	14.3	85.8	55.5	15.6	89.0	56.4	12.9	87.9	55.5	15.4	88.2
		Central	61.2	21.5	82.4	60.8	20.0	81.6	62.7	20.3	82.5	61.6	20.4	83.2	61.1	19.4	84.4
		Cervical	59.6	21.4	75.6	60.0	20.0	74.3	60.8	19.7	75.6	59.5	20.6	75.0	60.1	21.8	76.7
	Sample 5	Incisal	60.6	13.4	85.3	61.3	14.5	82.0	62.8	13.2	86.4	60.4	16.1	86.0	60.0	17.3	85.6
		Central	62	22.3	84.4	62.9	21.3	84.1	64.3	18.8	86.2	62.7	22.2	86.8	62.5	21.2	86.8
		Cervical	61.2	21.4	84.2	61.2	21.0	85.3	63.5	18.4	86.8	60.5	21.7	86.5	61.8	20.6	87.4

5.2.3 Statistical Analysis

The homogeneity and normality of the data were assessed. Statistically significant differences between the experimental groups (CTR, CLG, CPT, CPX) were identified through a one-way ANOVA, followed by Tukey's post-hoc test, using the Test Analysis tool in Excel (Office 365, Microsoft Corporation, Bellevue, WA, USA). The level of statistical significance will be set at $p < 0.05$.

The sample size was calculated in Excel based on the average and standard deviation of the ΔL^* values from a preliminary study.

CHAPTER: 6 RESULTS

The study analyzed the whitening efficacy of different toothpastes on four sample groups (CTR, CLG, CPT, CPX) by measuring ΔE values at five time points (T0, T1, T2, T3, T4). The results were subjected to statistical analysis using one-way ANOVA, followed by Tukey's post-hoc test for pairwise comparisons between the groups.

1. ANOVA Results:
    - A statistically significant difference was observed between the groups ($p = 0.0012$) for the factor "Group," indicating that the mean ΔE values varied significantly among the test groups.
    - The factor "Time" also showed a statistically significant effect ($p = 0.0222$), suggesting that color changes occurred over time. However, no significant interaction between "Group" and "Time" was found ($p = 0.9543$), meaning that the rate of color change over time was similar across all groups.
2. Tukey HSD Post-hoc Test:
    - Pairwise comparisons revealed a statistically significant difference between the CLG and CPT groups, with CLG showing higher ΔE values ($p = 0.0010$). Additionally, the CLG group also differed significantly from the CPX group ($p = 0.0369$).

      No significant differences were detected between the control group CTR and any of the test groups, indicating that the whitening effect of the tested toothpastes did not significantly differ from the untreated control.

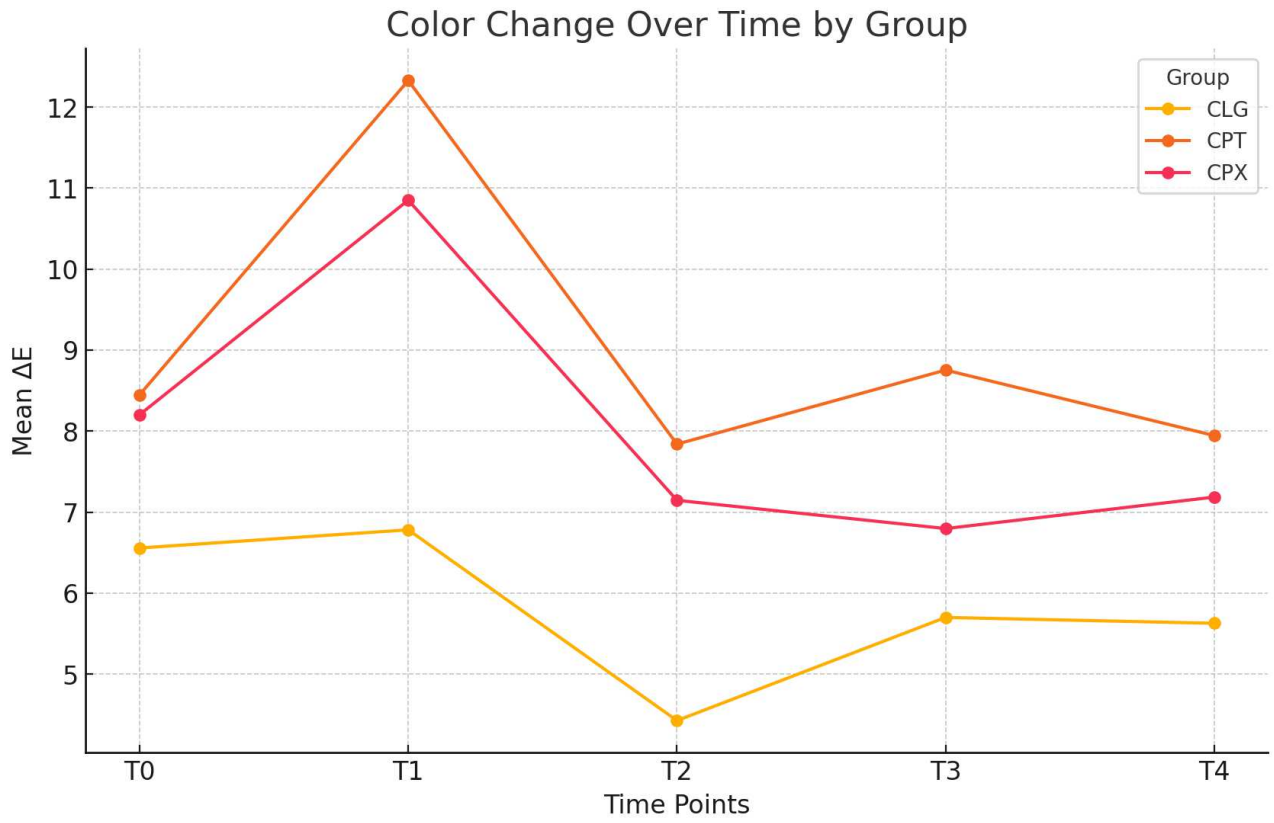
3. Mean ΔE Values:

   The mean ΔE values for each group were calculated over the time intervals, revealing that CLG consistently showed higher ΔE values than both CPT and CPX. Despite these differences, the mean ΔE values for the test groups did not significantly exceed those of the control group across the time periods.

4. Color Change Over Time:

   ΔE values varied across the time points, but the color changes did not show significant divergence between the test groups and the control group over time. This suggests that while some differences exist between the test groups, none of the toothpastes tested produced a consistently superior whitening effect compared to the control.

These results indicate that, although some differences were observed between the test groups, the toothpastes tested did not demonstrate a statistically significant improvement in whitening compared to the control group throughout the study.



Graph 1. The graph illustrates the mean ΔE values for each toothpaste group (CLG, CPT, CPX) measured at five different time points (T0, T1, T2, T3, T4), representing the progression of color change over the duration of the study.

CHAPTER 7. DISCUSSION

This study was conducted to evaluate the whitening efficacy of three commercial whitening toothpastes over time focusing on the following groups: *Curasept Black Lux* (CPT), *Colgate Max White Charcoal* (CLG), and *Curaprox Black Is White* (CPX). The results revealed that the *CPT* group exhibited a significantly higher whitening effect than the *CPX* group, while no significant differences were observed between the *CLG* group and the others. These findings are in line with previous studies, which consistently highlight the superior whitening action of peroxides like hydrogen peroxide and carbamide peroxide compared to non-peroxide alternatives [55,56]

Stability of Whitening Effect in the CPT Group.

One notable observation in the study was the stable whitening effect seen in the *CPT* group across the four-time points (T0 to T4). Peroxide-based agents are well-documented for their ability to produce visible whitening within the initial 2 to 4 weeks of use, with these results typically remaining stable for up to 8 weeks [55,57]

The sustained whitening seen in the *CPT* group corroborates findings from other research that suggests peroxide-based formulations, such as those found in *Curasept Black Lux*, provide long-lasting results with minimal decline in efficacy over time [58]. This stability is a major advantage for patients seeking reliable, long-term whitening effects.

Whitening Efficacy of Non-Peroxide Toothpastes.

In contrast, the *CLG* and *CPX* groups exhibited only moderate whitening effects, which did not match the efficacy observed in the *CPT* group. This is consistent with studies by Vural et al. (2021), who found that while charcoal-based toothpastes can

help reduce extrinsic stains, their overall whitening capacity is far less significant compared to peroxide-based formulations [54]. Charcoal-based products work primarily through the mechanical removal of surface stains, a process that does not penetrate deep enough to achieve the level of intrinsic stain removal offered by peroxides [59].

Franco et al. (2020) similarly highlighted the limited clinical effectiveness of charcoal-containing toothpastes, concluding that while they may enhance surface brightness, they are not sufficient for patients seeking deeper, more permanent whitening [59]. Dursun et al. (2023), reported that non-peroxide abrasives, including charcoal, offer only surface-level whitening and do not compare to the penetrating action of peroxides [55].

Clinical Relevance and Whitening Mechanism

The results of this study highlight that peroxide-based toothpaste that was used for *CPT* group performs better in clinical applications. Hydrogen peroxide functions by oxidizing chromophores deep within the enamel, which leads to more significant and lasting whitening results compared to charcoal-based products, which primarily rely on physical stain removal [54,56]. This explains why peroxide-based agents consistently outperform alternatives in clinical applications. For patients looking for substantial, long-term whitening results, peroxide-based products remain the preferred option.

Time Points and Whitening Progression

The sampling intervals used in the study align well with those commonly found in the literature. Clinical evaluations of whitening agents, particularly those based on peroxides, often assess efficacy at 2, 4, and 8-week intervals. Our results reflect

similar timelines, with *CPT* showing sustained whitening from T2 through T4, consistent with other research showing that peroxide-based whitening products maintain their effects over extended periods [57,58].

Colgate Max White Charcoal

The results obtained for *Colgate Max White Charcoal* in this study align closely with the findings reported by Vaz et al. (2019), where different whitening technologies, including activated charcoal, blue covarine, and hydrogen peroxide-based products, were compared [56]. In their investigation, the authors noted that while some products demonstrated initial whitening effects, charcoal-based formulations like *Colgate Max White Charcoal* did not show significant whitening results after prolonged use. Similarly, in our study, *Colgate Max White Charcoal* in the group (CLG) did not show better results in terms of whitening compared to the control group (CTR) or other groups (CPT, CPX). Although slight improvements in ΔE values were observed, these changes did not reach statistical significance, suggesting that the perceived whitening effect of this product may not be substantial.

Franco et al. (2020) also tested *Colgate Max White Charcoal*, confirming that while activated charcoal may be effective at removing surface stains, it does not provide the deep bleaching action offered by carbamide peroxide or hydrogen peroxide-based formulations [59]. The minor color changes recorded in both studies can likely be attributed to the abrasiveness of the charcoal rather than any intrinsic whitening capacity of the product itself.

Activated Charcoal and Whitening Potential

The use of activated charcoal in whitening products remains a topic of debate. Previous studies, such as those by Vertuan et al. (2023) and Franco et al. (2020), have demonstrated that while activated charcoal has a strong adsorption capacity for surface stains, its overall bleaching effect is minimal when compared to peroxide-based formulations [59,60]. Our study also found that while ΔE values for charcoal-based toothpastes improved slightly, the changes were not statistically significant. Like other studies, our results suggest that the perceived whitening benefits of activated charcoal are mostly limited to surface stain removal and do not provide the deeper bleaching action associated with peroxide-based treatments.

Study Limitation

Despite these promising findings, there are several limitations to our study. First, the relatively small sample size may limit the generalizability of our results. A larger sample could provide more robust conclusions and potentially reveal further differences between the groups. Additionally, our study focused on a relatively short-term assessment, spanning only the early weeks of treatment. Although our results showed that the whitening effect of the CPT group was stable over time, further research is needed to determine whether this effect persists in the longer term, beyond 8 weeks. Previous studies have suggested that whitening effects can diminish after three months without ongoing treatment [54].

Another key limitation is that this study did not evaluate the potential impact of these toothpaste formulations on enamel surface characteristics. Whitening agents, particularly those containing abrasives or peroxides, may affect the surface roughness and microhardness of enamel over time. Future studies should incorporate

tools like scanning electron microscopy (SEM) to examine any microscopic changes to enamel. SEM analysis could provide detailed images of the enamel surface before and after treatment, allowing researchers to assess whether these formulations cause erosion, increased porosity, or other surface alterations [54,57]

CHAPTER 8. CONCLUSION

This study evaluated the whitening efficacy of various toothpastes on four groups of samples (CTR, CLG, CPT, and CPX), with color variations measured using ΔE values across five-time points (T0-T4).

Differences Between Test Groups: The *Curasept Black Lux* (CPT) group demonstrated a statistically significant difference in ΔE values compared to the *Curaprox Black Is White* (CPX) group ($p = 0.0010$), suggesting that *Curasept* Black Lux provided a greater whitening effect. However, no significant differences were found between the *Curasept* Black Lux (CPT) and *Colgate Max White Charcoal* (CLG) groups, indicating that both products performed similarly.

No Significant Difference Compared to Control (CTR): No statistically significant differences were observed between any of the test groups (CLG, CPT, CPX) and the control group (CTR). This indicates that none of the tested toothpastes produced a whitening effect that significantly differed from the untreated control group, suggesting minimal overall impact.

Effect of Time on Color Change: Time had a statistically significant effect on ΔE values ($p = 0.0222$), indicating that tooth color changed over time. However, the interaction between time and the toothpaste groups was not significant, implying that the rate of whitening over time was similar across all groups.

Clinical Implications: While some differences were found between certain test groups, the absence of significant differences compared to the control group (CTR) raises questions about the practical effectiveness of these toothpastes in a clinical context. The data suggests that these products may not deliver meaningful whitening results when compared to no treatment at all.

Future Recommendations: Further studies with larger sample sizes and longer observation periods are needed to fully explore the whitening potential of these products. It would also be beneficial to examine these products for a longer period to better understand their overall whitening efficacy.

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