Universidad Europea

GRADUATION PROJECT

Degree in Dentistry

EVALUATION OF STAINING BEVERAGES AFTER IN OFFICE BLEACHING. A PILOT STUDY.

Madrid, academic year 2022/2023

Numero indentificativo: 005

Abstract and keywords

Introduction: Knowledge of different types of staining is vast, however, there remains uncertainty regarding the influence of staining beverages upon teeth that were bleached in office under hydrogen peroxide 40%.

Objectives: The objective of the study is to assess the feasibility of conducting a larger scale research study on the effect of staining beverages on teeth after in-office bleaching. Secondary objectives are to identify the most potent staining beverage, evaluating its negative impact on the results after hydrogen peroxide bleaching, and assess hydrogen peroxide's efficacy at increasing teeth value.

Methodology: 25 extracted incisors and premolars were separated into 5 groups containing 5 teeth. Initial images at T0 were taken before staining of all groups. A bleaching procedure using hydrogen peroxide 40% was used to bleach the teeth. Photos were taken at T1, 1 week post bleaching. 4 groups were stained for 15 minutes per day for 14 days using coffee, tea, red wine, and coke. The fifth group acted as a control, with the teeth being submerged in distilled water. Images were taken at T2, directly after the 14 days. The images were taken using a Canon eos 700 camera using a dentalize polarizing filter. The images from T0, T1, T2 were processed through photoshop, which recorded the L*a*b* values. Delta E values were calculated using the online Jose Pereira delta E calculator.

Results: The beverage with the strongest staining potency was tea, it presented a ΔE value of 24.55, coke with a ΔE of 20.206, red wine with a ΔE of 14.172 and coffee with a ΔE of 12.04.

Conclusions: Staining beverages negatively impact in-office bleaching results. Tea has the strongest staining potency followed by coke, red wine, and coffee. Hydrogen peroxide, 40%, used in-office bleaching was found to reduce L* values causing the tooth to lose brightness.

Keywords: Dentistry, Aesthetics; Staining; Bleaching; Colour

Resumen y palabras clave

Introducción: El conocimiento de los diferentes tipos de manchas es amplio, sin embargo, existe incertidumbre con respecto a la influencia de las bebidas que manchan los dientes que fueron blanqueados en la oficina con peróxido de hidrógeno al 40%.

Objetivos: Evaluar viabilidad de gran estudio sobre bebidas que manchan dientes tras blanqueamiento en oficina. Identificar bebida más manchadora, evaluar su impacto negativo en resultados de blanqueamiento con peróxido de hidrógeno y su eficacia en aumentar valor dental.

Metodología: 25 incisivos y premolares se dividieron en 5 grupos de 5 dientes. Se sacaron fotos iniciales en T0 antes de teñir los grupos. Se usó peróxido de hidrógeno al 40% para blanquear los dientes. Las fotos se tomaron en T1, 1 semana después. 4 grupos se tiñeron durante 15 minutos diarios durante 14 días con té, café, vino y coca-cola. Un grupo control se sumergió en agua destilada. Las fotos en T2 se tomaron directamente después de los 14 días y fueron capturadas con una cámara Canon eos 700 utilizando un filtro polarizador dentalizado. Photoshop procesó las imágenes de T0, T1, T2 y registró los valores L*a*b*. Se calculó Delta E en línea.

Resultados: El té es la bebida que mancha más, presentando un valor de ΔE de 24,55, la coca-cola con un ΔE de 20,206, el vino tinto con un ΔE de 14,172 y el café con un ΔE de 12,04.

Conclusiones: Las bebidas que manchan los dientes tienen un impacto negativo en los resultados del blanqueamiento en la oficina. El té tiene la mayor capacidad de manchar, seguido de la coca-cola, el vino tinto y el café. Se encontró que el peróxido de hidrógeno al 40%, utilizado en el blanqueamiento en la oficina, reduce los valores L* haciendo que el diente pierda brillo.

Palabras clave: Odontología, Estética; Manchas; Blanqueamiento; Color

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1. INTRODUCTION

In 1936, a clinician named Pilkington defined dental aesthetics as "the science of copying or harmonizing our work with that of nature, making our art inconspicuous". (1). Aesthetics is the field of dentistry that is focused on improving the physical appearance and functionality of a patient's teeth, it forms a marriage between scientific and artistic elements, with patients being provided with a vast array of constantly evolving restorative options to improve the appearance of their teeth. A notable surge in demand for aesthetic treatments has emerged in recent times, with social media thought to be a big influence (2).

The desire to enhance one's face and teeth has existed since ancient times, due to the innovative work of individuals like Pierre Fauchard, dentistry evolved into a distinct medical field in the 18th century (3), enabling specialised treatment of both functional and cosmetic oral defects. The 20th century saw the most important advancements in cosmetic dentistry, despite subsequent improvements in preventative measures, materials used to restore teeth, and production methods for fabricating partial and complete dentures. (4).

In modern times, "aesthetic" teeth are judged by a few elements, with the maxillary incisors and canines being the most visible teeth in the mouth; they derive the confidence of someone's smile significantly and a lot of aesthetic procedures are directed at restoring those teeth. With aesthetic analysis being composed of a myriad of angles, measurements, landmarks, references, and ratios; challenges and inaccuracies can arise when adopting the old, much more holistic approach to aesthetic analysis (5).

Nowadays, aesthetics is analysed on two separate levels, macroaesthetics and microaesthetics. Macroaesthetics concerns the features of the face, gingiva, and the teeth, whereas microaesthetics focuses on the teeth individually.

1.1 MACROAESTHETICS & MICROAESTHETICS

The analysis derived from macroaesthetic assessment allows the dentist to assess the general aesthetic needs of each patient and it provides a foundation upon which microaesthetics can further enhance the aesthetic nature of a patients smile. Facial analysis comprises of assessing the shape of the face and lips, measuring the facial midline, interpupillary line, incisal plane, and labial line (6). Gingival analysis focuses on the gingival margins, the contour of the gingiva and the interdental papillae. Tooth analysis involves recording the incisal curvature, dental alignment, dental midline, axis of the teeth, the proportions of the teeth, the labial corridor, and the incisal and cervical embrasures (7).

Microaesthetics studies a patients smile on a much more microscopic level. Individual tooth analysis allows the clinician to assess the specific needs of the teeth. The anatomy of the teeth is studied, with the golden ratio used as an aesthetic reference point to judge their shape. The texture, translucency and colour of the enamel are also essential aspects of microaesthetic evaluation (7)

1.1.1 SHAPE/ ANATOMY: GOLDEN RATIO

The use of the golden ratio extends back in history to the time of the ancient Greeks, who described the golden ratio as “divine proportions” as they believed beauty and harmony within nature were divinely inspired (8). The specific value of the golden ratio is the irrational number phi, 1.61803399... (9). This unique number has been utilised by architects to guide building design, and it has remained relevant ever since its conception. Fibonacci was the individual responsible for creating a sequence of numbers derived from phi, which perfectly quantifies beauty (9) The concept of proportionate teeth was initially proposed by a dentist, Lombardi, in 1973. However, he dismissed the use of golden ratio to rehabilitate aesthetics (10) It was only until the subsequent work of Levin and Ricketts which established golden ratio as being the standardised measurement of aesthetics. Levin created a tooth calliper to determine if a person's teeth are in perfect alignment and a diagnostic grid to ensure that the teeth are spaced correctly as seen in figure 1 (10). With an appropriate crown width to length ratio, we can establish symmetrical looking teeth, with the “gold standard” thought to lie between 1.618 and 1.0 as seen in figure 2.

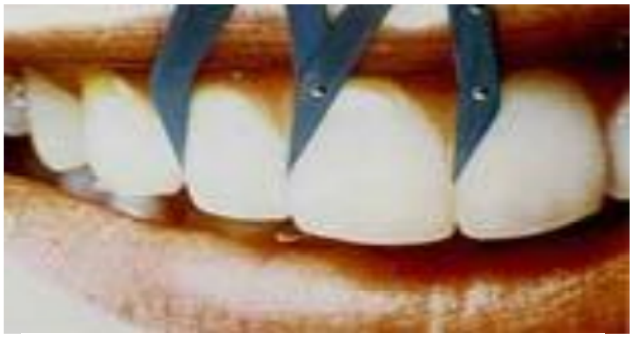


Figure 1. Levin's tooth calliper (10).

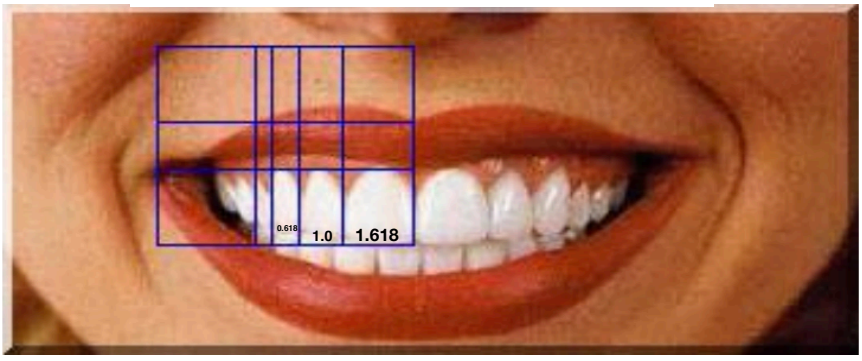


Figure 2. Golden ratio proportions (10).

1.1.2 TEXTURE & TRANSLUCENCY

The texture of the teeth is another important component of dental aesthetics since it dictates how bright the tooth appears. This surface texture is determined by the enamel, which is a tissue which must withstand regular physical and chemical stressor (11)When we are young, our teeth have much more pronounced surface topography, which reflects a lot of light and makes the teeth appear bright. But as we age this surface topography is attenuated and the enamel appears a lot darker since it reflects less light, as demonstrated in the figures 3 and 4.

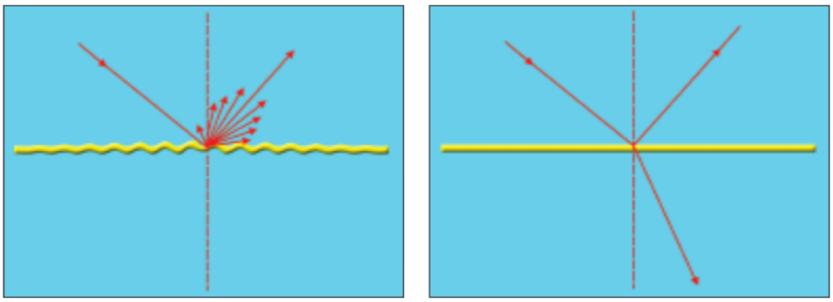


Figure 3. Roughened and smooth surface light interaction (57).

Figure 4. Teeth values with age (11).

1.1.3 COLOUR (CHROMA/VALUE)

The colour of a tooth can change due to various physical and chemical interactions that occur within the dental tissues. These changes can be caused by extrinsic factors, which come from outside the tooth, or intrinsic factors, which originate from within the tooth (12)

1.2 THE INTERPRETATION OF COLOUR

As humans we interpret colour through an intricate and dynamic process that is mediated by specialized cells called cones. Cone cells are located within the retina, in the posterior portion of the eyeball. These cells contain pigment which are sensitive to different parts of the visible spectrum of light. Visible light is a small portion of the electromagnetic spectrum, ranging from approximately 380 to 750 nanometres in wavelength (13)

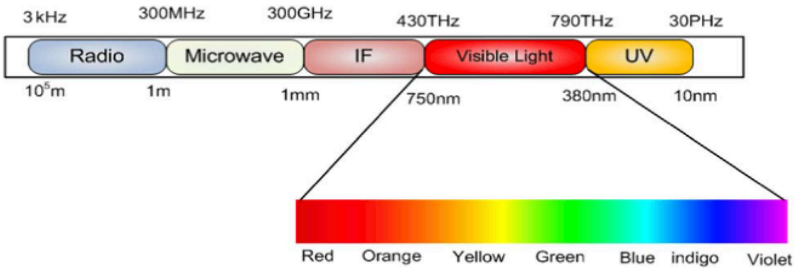


Figure 5. Visible light communication frequency (58).

When light enters the eye, the cones are responsible for absorbing the light and generating an electric signal which travels to the brain via the optic nerve. The brain then processes this information and interprets it as colour. Three essential and interrelated factors—the illuminant, the object, and the observer—must all be present for a colour to be perceived. The illuminant source emits radiation that the item will either reflect or transmit to the observer's eye. This physical energy is captured by the eye and converted into chemical impulses that the optical centre of the brain can understand (14)

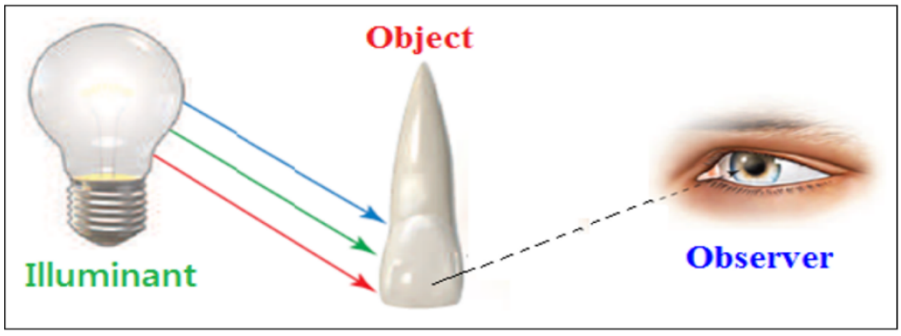


Figure 6. Human observation (14).

Interpreting colour is a subjective process, with colour perception varying greatly from person to person. Age, gender, cultural background, and personal experiences are just a few examples of the variables that might affect how someone perceives colour (13) Due to this, what one person sees as "red" may be seen by another as more of an "orange" colour. The lighting conditions also heavily influences colour interpretation of an object. Colours may appear brighter or more muted depending on the type and quality of background light such as natural sunlight, fluorescent light, or incandescent light.

Despite the difficulty of perceiving colour, there are established systems like the Munsell colour system that offer a common language for defining and measuring colour (15). When describing a colour, three parameters are considered: hue, chroma and value. These parameters were established at the beginning of the 20th century by Albert Munsell who developed a three-dimensional model that organizes colours based on those three key characteristics, as seen in figure 7 (16) Within modern day clinical practice to assess the colour of a tooth, shade guides such as the VITA classic shade guide follow Munsell's colour system.

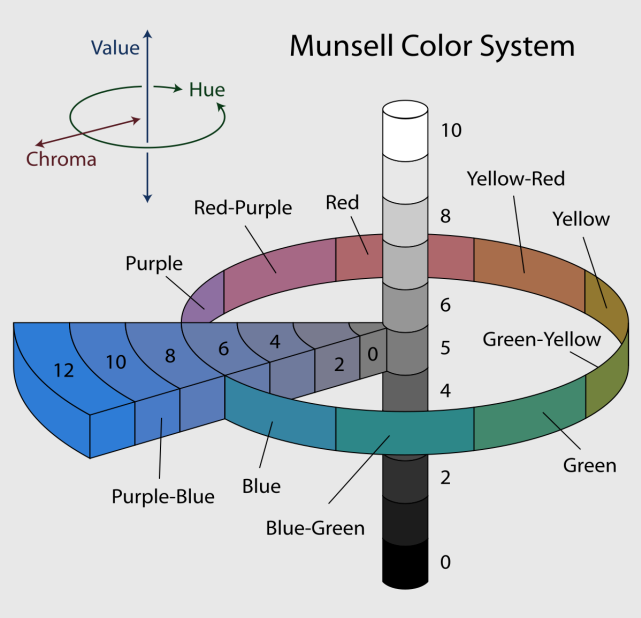


Figure 7. The Munsell colour system (59).

1.2.1 INTERNATIONAL COMMISSION ON ILLUMINATION COLOUR SPACE

The International Commission on Illumination (CIE) have defined a colour space which allows for accurate and consistent colour measurements. This three-dimensional colour space is derived from sets of L*a*b* values which allows quantification of a colour.

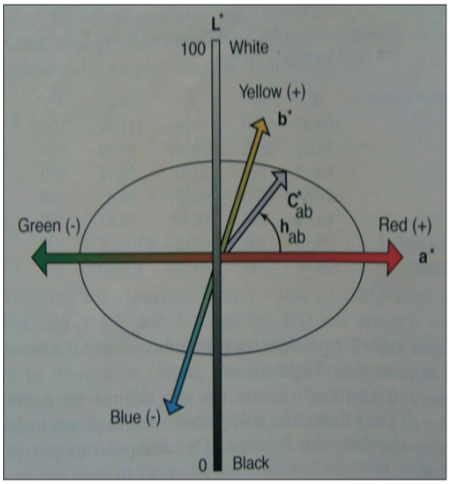


Figure 8. CIE lab system (60).

It was developed upon a theory that colours have opponents, which can never exist at the same time. The two sets of opponents are red and green and yellow and blue (17)The L* component represents lightness, and it is measured from 0 (black) to 100 (white). The a* component represents the colours position upon the red-green axis. The b* component represents the colours position upon the yellow-blue axis (18) The deltas for L* (ΔL*), a* (Δa*), and b* (Δb*) can be either positive (+) or negative (-). However, the total difference, Delta E (ΔE*), is always positive. If the delta for a* is positive, then it indicates the colour is red and if it negative, it is green. If the delta for b* is positive, then it indicates the colour is yellow and if it is negative, it is blue (18)

To determine the color change, the L*a*b* values will be compared. The equation below allows colour change to be derived into a number:

$$\Delta E^*_{ab} = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2}$$

Regarding how we clinically perceive the Delta E value, some authors argue that under laboratory lighting, a human observer can distinguish changes in colour with a Delta E value of 1. But another study argues that a human eye cannot clinically interpret (17)

1.3 HUE

Hue is a property of colour that refers to the dominant wavelength of light that is reflected by an object. In other words, hue describes the actual colour of an object, such as red, blue, green, or yellow. Within dentistry, shade guides are used to assess the colour of the teeth. The VITA shade guide is divided into four groups corresponding with the basic hue categories: A (reddish-brown), B (yellow), C (grey), and D (reddish-grey) (19). As seen in below figure 9.

Figure 9. Hue of teeth (61).

Figure 10. Chroma of teeth (61).

1.4 CHROMA

The chroma or saturation of a colour refers to the purity and intensity of a hue, which is determined by the amount of pigment present in it, as seen in figure 10. A high chroma colour has a pure, vivid, and intense hue, while a low chroma colour is more subdued and muted. In the context of the VITA shade guide, which is a commonly used dental shade-matching system, the chroma of a tooth colour is divided into four levels, ranging from 1 to 4. Each level represents a different level of colour intensity, with 1 being the least saturated and 4 being the most saturated (20). As seen below in figure 11.

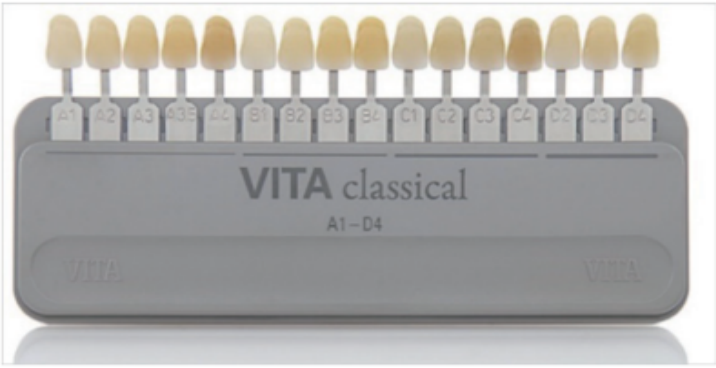


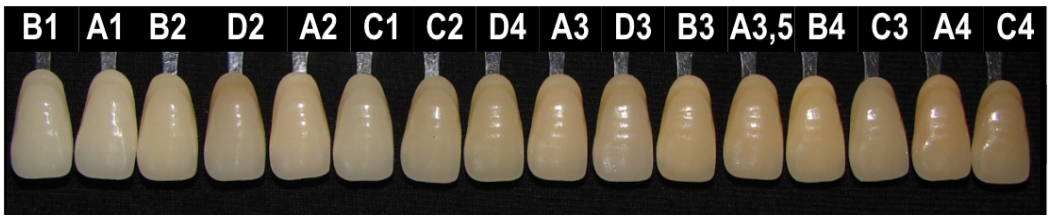
Figure 11. VITA classical dental shade guide (62).

1.5 VALUE

Value refers to the lightness or darkness of a colour and is determined by the amount of light reflected by a surface. A high-value colour is lighter and brighter, while a low-value colour is darker and less bright, as seen in figure 12. Value is an important parameter of how colour is perceived because it can have a significant impact on the overall appearance and visual effect of a colour (20)

Within dental tissue, value is represented in terms of brightness. Brightness can be characterized in a few ways. For example, a tooth with a higher value or “brightness” is opaquer and appears whiter. A tooth with a lower value is more translucent and appears greyer. Within anterior teeth, we can observe different values within the same tooth. Dividing the tooth into thirds, the incisal third has low value and a translucent appearance, the middle third is the brightest with a high value and the cervical third has a medium value. The VITA shade guide is ordered following increasing saturation, however as seen in figure 13, it has been rearranged by order of descending brightness.

Figure 12. Value of teeth (61).



F**igure 13**. VITA shade guide rearranged in order of descending value (12).

1.6 OPTICAL PROPERTIES OF TEETH

The optical properties of a tooth refer to its ability to reflect, transmit or absorb light. This phenomenon is known as translucency, opalescence, and fluorescence.

1.6.1 TRANSLUCENCY

Translucency, as seen in figure 14 and 15, is related with the previously mentioned parameter, value. It is an optical property which describes how much light can pass through an object. Translucency can be affected by factors such as enamel dehydration, surface texture and the wavelength of light that hits the tooth. The aging process heavily influences the translucency of teeth over time. With more enamel wear resulting in thinner enamel and smoother surfaces, underlying dentin is more visible, creating a much darker looking tooth (21).

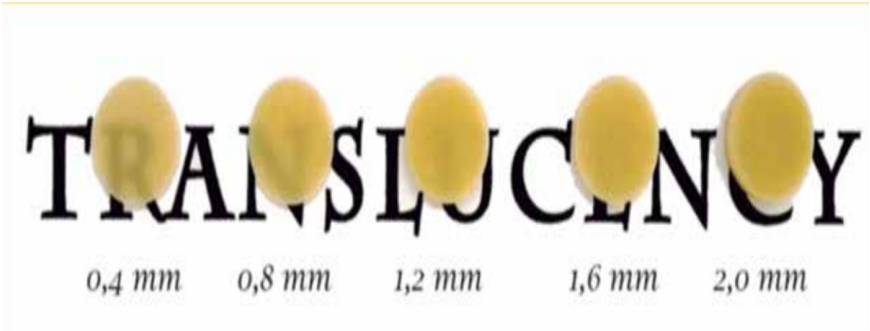


Figure 14. Translucency visualised (61).

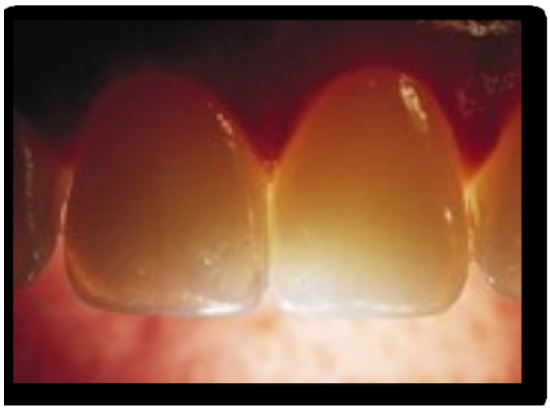


Figure 15. Translucency of the natural dentition (61).

1.6.2 OPALESCENCE

Opalescence is the optical property that relates to when light strikes the enamel and the light beam scatters and refracts upon the microcrystals and organic substances on the enamel surface. It is named opalescence since this optical phenomenon was first observed in opal stones (11) Opal stones can be observed under direct reflected light and transmitted light, as seen in figure 16. Reflected light is characterised by a blue shade and transmitted light is characterised by an orange shade.

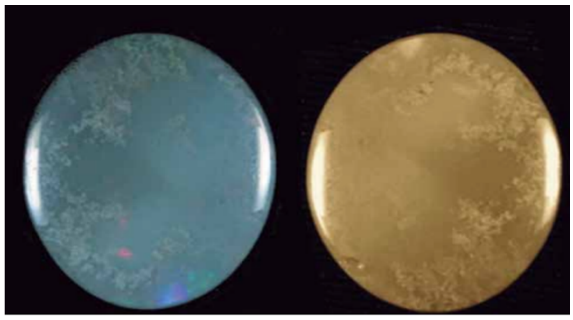


Figure 16. Opal stones- opalescence in nature (61).

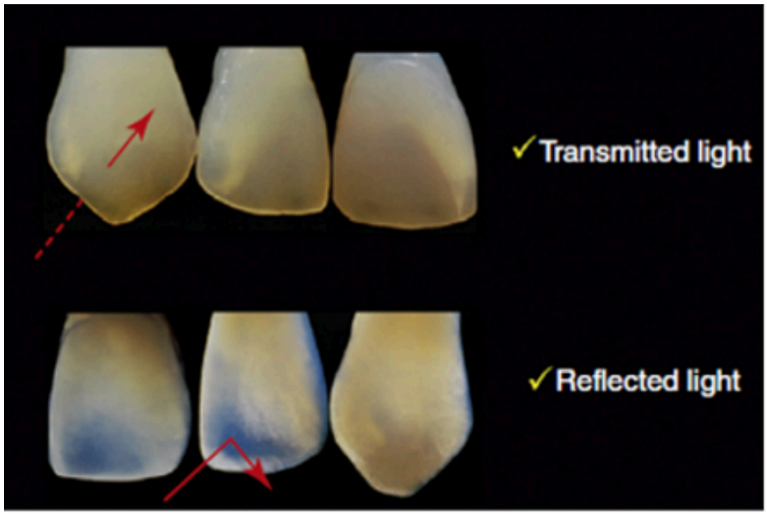


Figure 17. Transmitted light vs reflected light (61).

When natural light enters the enamel, the longer wavelengths in the red-orange range are transmitted and refracted through the enamel crystallites, while the shorter wavelengths in the blue, green, and purple range are reflected and dispersed, as seen in figure 17. This results in a distinctive opalescent shimmer in the enamel, which can vary in colour depending on the thickness of the enamel and the angle of the light (21)

Opalescence is particularly noticeable in the incisal third of the tooth, as seen in figure 18, where the enamel is thinner, and the opalescent effect is more pronounced. In this area, the enamel may appear blue greyish in colour due to the way the light is reflected and transmitted through the crystallites. In the cervical area of the tooth, where the enamel is thicker, with the opalescent effect appearing more orange in colour (21)

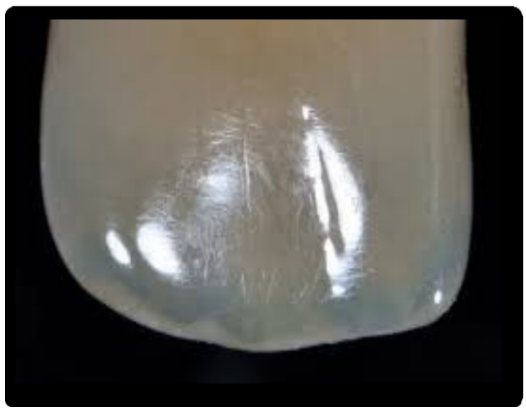


Figure 18. Opalescence observed in the incisal third (61).

1.6.3 FLUORESCENCE

Fluorescence is another optical property of teeth, which refers to the ability of a structure to absorb shorter wavelengths of light outside the visible spectrum (such as UV light) and emit light energy within the visible spectrum.

Both enamel and dentin are fluorescent, but the fluorescence is more pronounced in dentin, which contains more organic pigment that is photosensitive to UV rays. As a result, dentin is about three times more fluorescent than enamel (22)

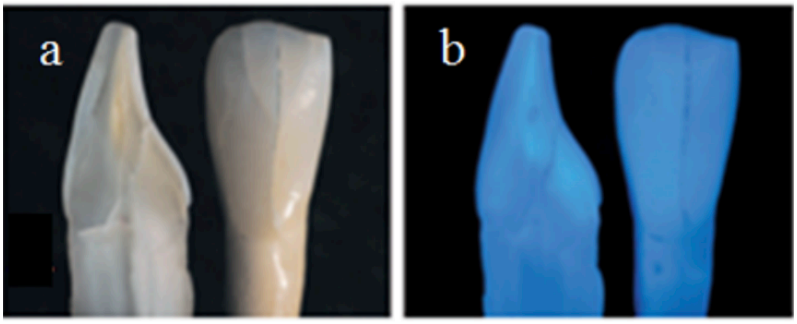


Figure 19. Teeth under normal light (a) and teeth glowing under UV light due to their natural fluorescence (b) (61).

When natural teeth are exposed to UV light, such as sunlight, they absorb and return the UV light as an intense white and light blue fluorescence, which makes them appear more brilliant and whiter during the day. This fluorescence is a complementary characteristic that adds to the aesthetic appeal of healthy, natural-looking teeth (23)

Current knowledge and literature regarding tooth staining indicates that there can be a variety of factors which can influence how and why a tooth may become stained. The aetiologies of staining can be categorised into intrinsic and extrinsic staining (24)

1.7 INTRINSIC STAINING

Changes in the structure or thickness of the tooth's hard tissues can lead to intrinsic tooth discolouration. The blue, green, and pink shades of the enamel, which are accentuated by the yellow to brown shades of the dentin below it, determine the natural colour of teeth (23)

Many conditions, including metabolic abnormalities, systemic variables, and local circumstances like injury, might result in this kind of discolouration. A small number of teeth may be stained intrinsically, or it may impact widespread areas of both temporary and permanent teeth (25)

While widespread discoloration shows a divergence from normal tooth development, localised discoloration may be caused by events before or after tooth eruption. Knowledge of the stages of tooth development, such as the calcification and eruption sequences, can assist to explain why some teeth are internally stained (25)

1.7.1 DENTAL FLUOROSIS

Dental fluorosis is a condition characterised by tooth pitting and discoloration as seen in figure 20. Fluorosis is caused when consuming water with a high fluoride level during the formation stage of the teeth, it causes the creation of enamel to become disrupted, and as it matures, it becomes hypomineralized (26). The amount, timing, and duration of fluoride exposure have a significant impact on the development of fluorosis. The risk of enamel fluorosis is highest when exposure occurs during both the secretory and maturation periods, with the risk of exposure occurring just during the secretory stage being the lowest(27)

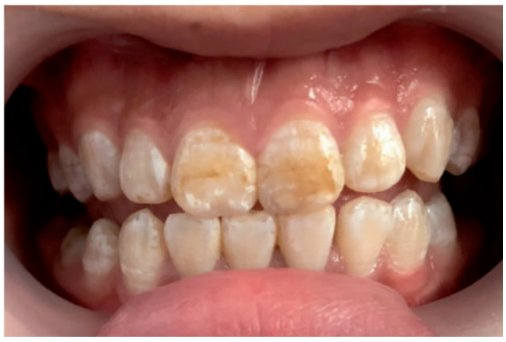


Figure 20. Fluorosis in the adult dentition (14).

1.7.2 MINOCYCLINE STAINING

Minocycline is a tetracycline derivative that can cause a green-grey or blue-grey intrinsic staining and it can be staged from mild to severe, a severe case can be seen in figure 21. It can affect both sets of dentitions, although the primary teeth generally present more staining if it is taken over an extended period of time. There are four hypotheses which debate the mechanism behind minocycline staining (28)

1st hypothesis: Minocycline is thought to attach to the acquired pellicle's glycoprotein. A subsequent oxidation reaction occurs due to either exposition to air or as a biproduct of bacterial activity. This results in the degradation of the aromatic ring and the formation of an insoluble black chemical. The integration of the pigment into the dentin may be caused by a demineralization-remineralization process (29,30)

2nd hypothesis: plasma proteins associated to minocycline are deposited in collagen-rich tissues, such as the teeth. This progressively oxidises when exposed to light over time (29,30)

3rd hypothesis: Minocycline chelates iron to create an insoluble complex which causes the staining (29,30)

4th hypothesis: During secondary dentinogenesis, minocycline is deposited in dentin, which is a process accelerated in bruxists (29,30)

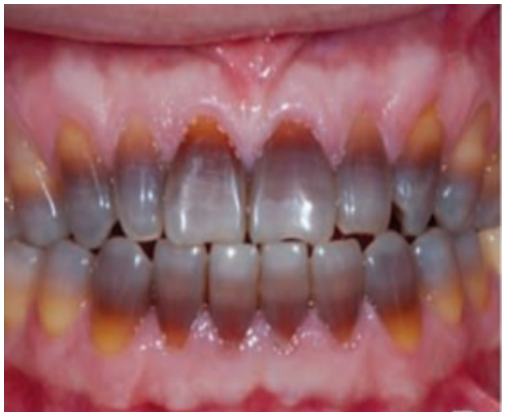


Figure 21. Severely tetracycline-stained teeth (28).

1.7.3 PULPAL NECROSIS

Acute dental trauma can result in pulpal necrosis, which causes the tooth's crown to become dark grey or black in colour as seen in figure 22. To what extent and how seriously the tooth's neurovascular supply has been damaged, however, is dependent on the severity of the accident (31)

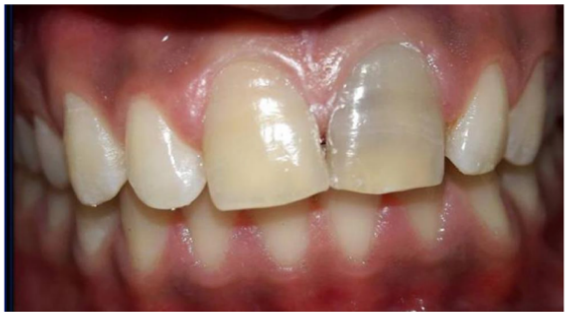


Figure 22. Coronal discoloration due to trauma and subsequent pulpal necrosis (31).

1.8 EXTRINSIC STAINING

Extrinsic discoloration can present in a variety of colours, being mediated by several factors. Chromogenic bacteria from soft bacterial plaque generate yellow, green, and orange discolorations that are typically seen in young individuals and those with poor dental hygiene (32) Most often, a brown or black discoloration is found around the gingival border. They are frequently found in children and adults with good dental

hygiene and minimal caries incidence, and they are strongly bonded to the tooth surface (33)

Dark patches comprised of insoluble iron salt and large concentrations of calcium and phosphate ions are characteristic of black extrinsic stains, which are often biofilms developed on the vestibular and lingual surfaces of teeth (25)

The creation of pellicle is the first stage of tooth discolouration. Bacterial colonisation results in plaque development a few hours after it forms. Bacterial plaque, which is firmly adhered to the tooth surface, is a soft coating of microorganisms in a matrix rich in polysaccharides and glycoproteins. Both internal (genetic tooth development, composition, and saliva production) and environmental variables influence plaque composition (33)

1.8.1 NATHOO CLASSIFICATION OF EXTRINSIC STAINING

The development of the Nathoo classification system, allows extrinsic staining to be categorised based upon the substance's chemical mechanism causing the discoloration (25)

N1 type: Refers to chromogenic substances such as food and drinks, which directly precipitate chromogens upon the surface of the teeth, leading to discoloration (34)

N2 type: Refers to chromogenic substances that, after adhering to the tooth surface, changes colour. As a result, the proximal and gingival tooth surfaces develop yellowish pigmentation, and as people age, these pigmentations become brown in colour.

N3 type: Refers to a colourless substance, named a prechromogen, which adheres to the surfaces of the teeth and causes discoloration mediated through a chemical reaction. These types of stain are known to be caused by carbohydrate-rich meals, stannous fluoride, and consumption of chlorhexidine.

1.8.2 FACTORS CONTRIBUTING TO EXTRINSIC STAINING

Many factors can contribute to extrinsic staining, with many patients being exposed to several of these risk factors, identifying the specific causes of extrinsic staining can be complicated. However, there are certain extrinsic stains who have unique characteristics either visually or where they accumulate. Various beverages have different capacities to

erode teeth. The higher the acidic capacity of the beverage, the more erosion it can produce upon tissue such as enamel. Along with the demineralizing and remineralizing process during the consumption of beverages, minerals and stains may be structurally incorporated into the enamel structure (35). Poor oral hygiene habits result in plaque and calculus accumulation which can contribute to brown or black staining. Tobacco consumption can generate brown and black staining upon the teeth, extending from the cervical third to the middle third (32) If used excessively beyond the recommended amount, cationic mouthwashes like chlorhexidine can cause teeth discoloration (32)

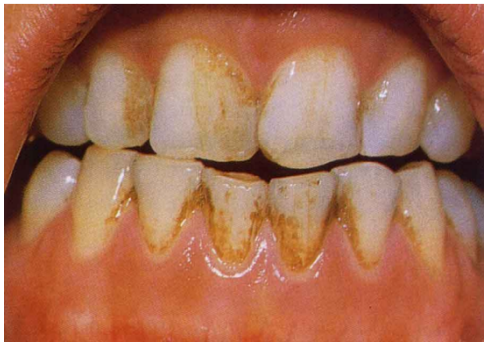


Figure 23. Extrinsic staining due to chlorhexidine (32)

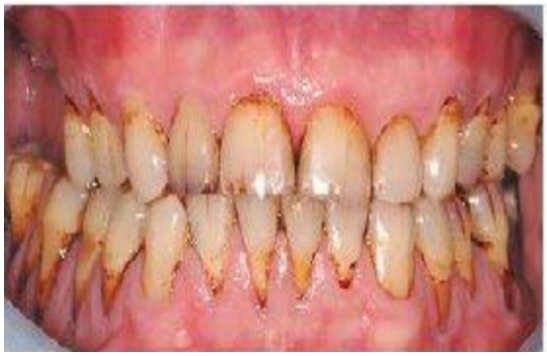


Figure 24. Extrinsic staining due to tobacco use (32).

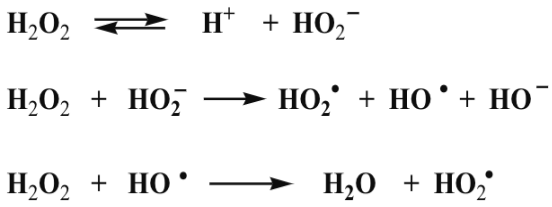
1.9 TREATMENT OF STAINING

Living in a society where we have easy access to a wide range of food and drink can be a blessing and a curse, as many tempting options are known to be very harmful to the health and aesthetics of teeth (36). As a result, in office tooth bleaching has emerged as one of the most highly requested aesthetic treatments due to its minimally invasive nature and its rapid aesthetic improvement of discoloured teeth (37) Dentists performing in office bleaching treatments use either hydrogen peroxide or carbamide peroxide, in high concentrations. The application of hydrogen peroxide in high concentrations needs to be under strict supervision since wrong application can easily lead to complications. Bleaching can also be performed at home by the patient, still

under the guidance of the dentist, but much lower concentrations of hydrogen peroxide or carbamide peroxide are used (38)

1.9.1 MECHANISM BEHIND BLEACHING PROCEDURES

The mechanism behind tooth bleaching follows a certain pathway. Carbamide and hydrogen peroxide, the principal bleaching agents, possess the ability to release both oxygen and perhydroxyl free radicals, as demonstrated by the chemical equation below. These molecules have the capability to diffuse through dental tissues.



Chromophores are large, pigmented molecules found in dental tissue and are responsible for tooth discoloration. The oxygen and perhydroxyl radicals produced by the bleaching solutions chemically degrade the double covalent bonds present in the chromophores structure (24). The resulting smaller, pigment containing molecules, diffuse to the surface of the enamel where they are eliminated. This modifies of the reflection of the wavelength of light, ultimately altering the original tooth colour and increasing the visual brightness of the enamel (39).

It has been observed that susceptibility to staining from dyes is higher after tooth whitening procedures. However, there is a general lack of information regarding the aesthetic damage caused by dyes during or shortly after the bleaching treatment in relation to the visual perception of colour change (2) When teeth are exposed to bleaching agents, whether in low or high concentrations, the changes in the enamel's microstructure may cause various micromorphological alterations. These changes include enamel erosion or porosity (40,41), increased surface roughness (35,42), and permeability (43), as well as diminished enamel microhardness and mineral content loss

(44,45). As observed in the figure below, the enamel experiences a number of morphological changes after being exposed to bleaching agents. These changes to the structure are characterised by pores, erosions, loss of aprismatic layer, craters, depressions, and increased depth of enamel irregularities (46) These morphological changes are summarised in figure 25 below:

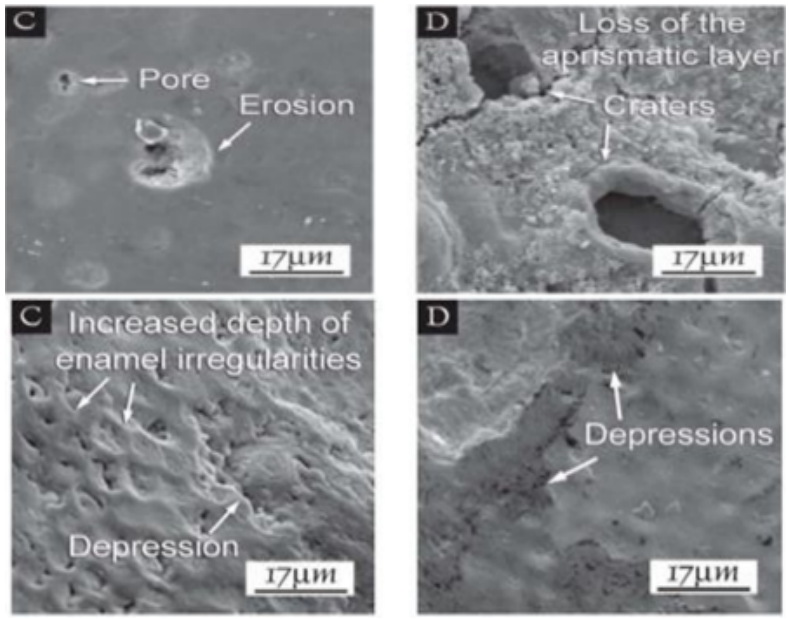


Figure 25. Enamel surface changes under scanning electron microscopy after hydrogen peroxide bleaching (46).

These negative effects are mediated by several factors such as the chemical structure of the bleaching agent, concentration of peroxide, the pH level, the time period it was applied, the medium in which it was stored (water or saliva), and the protocol used. Research has discovered that bleaching agents with higher hydrogen peroxide concentrations (43) and lower pH values (47) can modify the enamel's surface micromorphology, which increases the roughness, making the enamel surface more vulnerable to the attachment of biofilm and pigments. This, in turn, can reduce the effectiveness of bleaching.

Additionally, bleaching agents can lead to altered mineral content of the enamel. Studies have found that the use of high concentration bleaching agents can lead to a

reduction in enamel mineral content (45), which reduces the enamel's microhardness. These alterations to the strength of the enamel may increase the risk of damage, leading to higher tooth sensitivity and other adverse effects. There are still uncertainties regarding staining after bleaching procedures. This concern is mainly related to the time the patient should wait until consuming foods and beverages that contain staining agents (2).

1.10 PILOT STUDY

Pilot studies are investigations that determine whether a main study can be conducted. They allow researchers to evaluate the feasibility of a potential main study, enabling improvements in terms of quality and effectiveness (48). Preliminary data can be acquired from pilot studies to better understand the potential consequences of their intended investigation. Pilot studies are now frequently used in a variety of sectors to evaluate research procedures, enhance study designs, and gather preliminary data for more extensive studies. They are regarded as an essential part of the research process because they help reduce the possibility of failure and increase the likelihood that more extensive investigations will be successful. The Lancet published an article in 2009 that described the scale of research waste, which can account for up to 85% of research expenditures (49). This highlights the growing need to assess and investigate where improvements to the design of trials are needed, given the expense and time invested by researchers and major health research funders. Performing pilot studies allows researchers to become accustomed with the tools they will use for data collection and will equip them with the ability to handle any difficulties that may be encountered in the subsequent study. These difficulties could be researchers not adhering to protocol or following an insufficient methodology or using incorrect instruments (50) In order to prevent bias, pilot studies are generally carried out on individuals who closely resemble the target population but are not included in the final sample. (51)

2. OBJECTIVES & HYPOTHESES

With respect to the current literature and knowledge of bleaching procedures and the mechanisms of intrinsic and extrinsic staining, a study evaluating the various discolouring potencies of different staining beverages, will better inform dentists and patients about the potentially harmful effects of consuming these beverages after undergoing in-office bleaching. Keeping this in mind, the following Null hypothesis **(H_0)** for this study was formulated:

Consuming different staining beverages after undergoing in office bleaching, with hydrogen peroxide 40%, does not result in a significant discoloration of the enamel in comparison with the control group via assessment of CIE delta E values.

An alternative hypothesis **(H_1)** for this study:

Consuming different staining beverages after undergoing in office bleaching, with hydrogen peroxide 40%, results in a significant discoloration of the enamel in comparison with the control group via assessment of CIE delta E values.

Primary objective:

What is the feasibility of performing a larger scale research study which evaluates staining beverages after in office bleaching?

Secondary objectives:

1. To assess the negative impact of staining beverages (coke, coffee, red wine, and tea) on the results of in-office bleaching.
2. To identify the beverage with the strongest staining potency.
3. To evaluate the efficacy of in-office bleaching at in increasing the value of the teeth

3. MATERIALS & METHODS

In this study the evaluation of 4 staining beverages was performed on 25 extracted incisors and premolars. The teeth were separated into 5 groups containing 5 teeth. Each group was fixed into a block of silicon putty which facilitated repeatable imaging and staining. To avoid dehydrating the enamel, which can influence its colour stability, all groups were suspended in water in between each staining intervention. The 25 teeth were divided into 5 groups:

Group 0: Control group maintained in distilled water.

Group 1: Black coffee

Group 2: Black tea

Group 3: Red wine

Group 4: Coke

Inclusion criteria for the teeth used in the experiment:

- Central or Lateral maxillary or mandibular incisor
- First or second maxillary or mandibular premolar
- Crown fully intact, with no vertical or horizontal fractures
- No composite restorations
- No discolourations of the crown
- Caries free crown

3.1 STAINING PROCEDURE

To maintain consistent parameters, the same solutions were used to stain the teeth, seen in figure 26. The groups were always placed in 250ml of solution, enough to entirely submerge the crowns for the duration of the staining. The black coffee used in the experiment was Nescafe gold blend, 2 scoops of powder were mixed into 250ml of boiling water. The black tea used was PG tips, one tea bag was allowed to percolate in 250ml of boiling water for 2 minutes before being used to stain the teeth. The red wine used was a Chilean Merlot named Isla Negra, 250ml of red wine was decanted for the staining. The soda drink used was full sugar coca cola, again 250ml of this solution was used to stain the teeth. The staining procedure for the tea group can be seen in figure 27.

Figure 26. The commercial beverages used in the staining protocol

Figure 27. Image of the tea group during staining

3.2 BLEACHING PROCEDURE

The brand of bleaching product used in this experiment was opalescence boost™, hydrogen peroxide (H_2O_2) 40%, seen in figure 29. The protocol observed when performing the bleaching upon the extracted teeth was the same as the protocol used in office. Figure 28 shows the 5 groups during the bleaching procedure.

1) The teeth were rinsed with water and dried to clean the enamel surface of anything which could interfere with the bleaching procedure.

2) A 0.5-1mm thick layer of gel was placed on the labial surfaces of the teeth.

3) The gel was allowed to sit on the enamel for 30 minutes.

4) The gel was then suctioned off the enamel using a suction tip, but without using water, whose use would interfere with the bleaching process.

5) Steps 3 & 4 were repeated a total of 3 times.

6) After the final application of gel, the teeth were cleaned and rinsed with water.

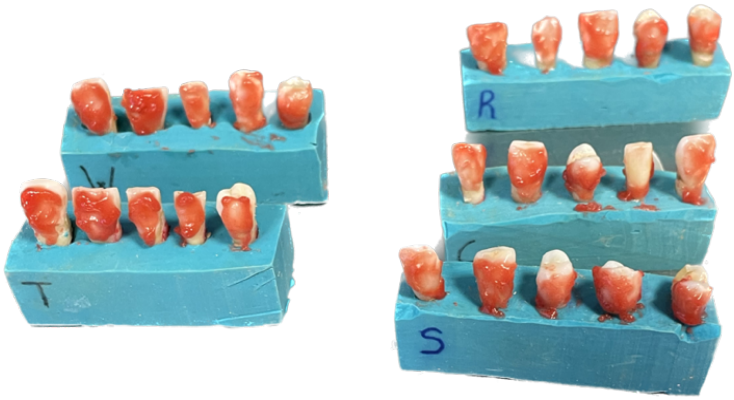


Figure 28. The 5 groups of teeth during the bleaching protocol

Figure 29. Opalescence boost, the commercial bleaching agent used in the bleaching protocol

3.3 DIGITAL IMAGING

To evaluate and measure the colour of the teeth, an image will be taken using a dentalize polarizing filter as seen in figure 30. These filters are commonly used in dentistry to reduce glare and reflections from the tooth surface, which can improve the quality of the photograph as seen in figure 32. It allows for a more accurate colour analysis using L*a*b* values. The images taken were used using a commercially available camera canon eos 700 with a 100mm macro lens as shown in figure 31. The camera settings used always remained the same for all three sets of images. The settings were: shutter speed 1/125, aperture F 8.0, ISO 100 and flash 1/1.

Figure 30. Dentalize polarizing filter used for the digital imaging

Figure 31. Canon eos 700 camera used for digital imaging

Figure 32. Image on the left is taken with a polarizing filter (23).

3.4 IMAGE ANALYSIS WITH PHOTOSHOP

The L*a*b* values of the teeth are measured and analysed using photoshop software. Analyzing the values using this software avoids the inaccuracies and subjective nature that other color measurement techniques incur, such as assessing tooth shade by eye, using guides like the vita chromoscope. A grey card was used in the foreground of each image taken; it acts as a neutral reference point, to allow consistent and repeatable colour balance of every image. The grey card can be seen in figure 33.

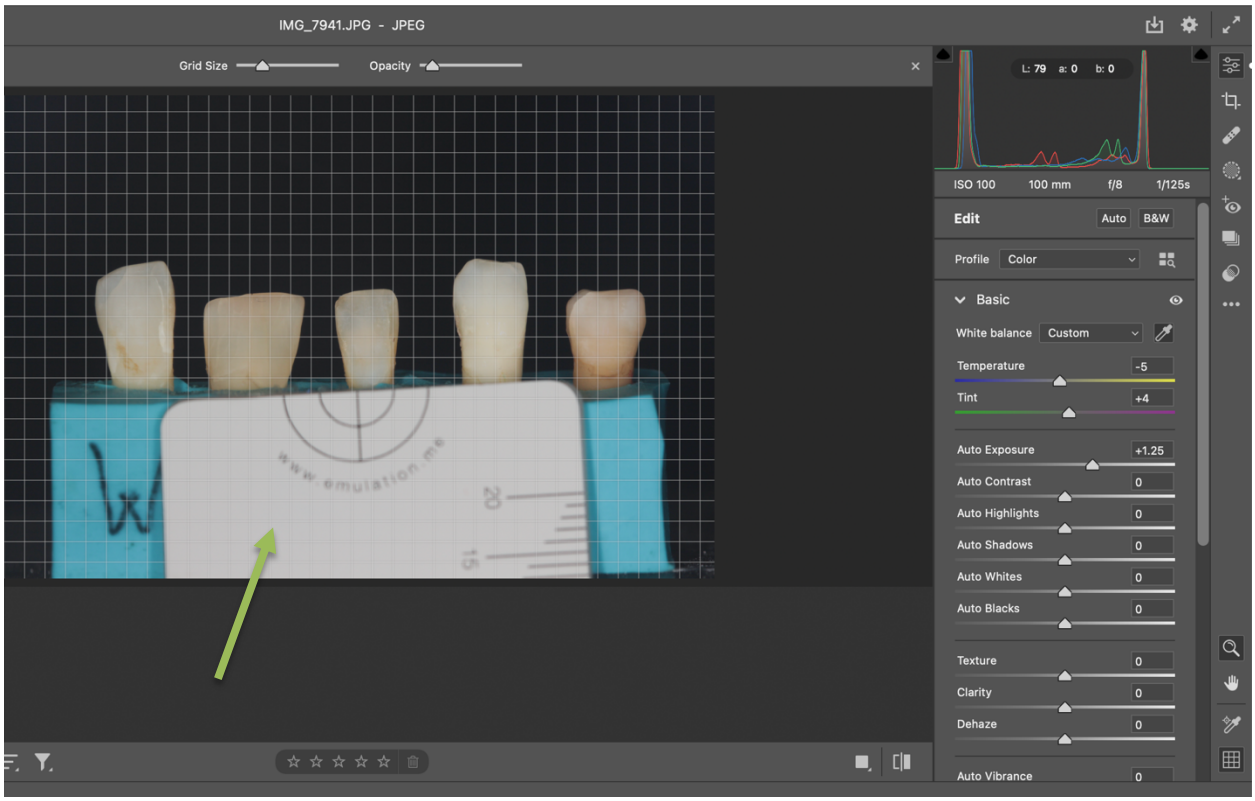


Figure 33. The display of photoshop with the digital image of the control group, the green arrow indicates the grey card

Each image was processed using the same procedure in photoshop, the L*a*b* reference values (79 0 0) from the grey card were used to perfectly balance the colour. The L* value can be increased or decreased by changing the autoexposure value. The a* and b* value can be changed through adjusting the tint and temperature respectively. As seen in figure 35, the temperature has been adjusted to -5, tint to -4 and auto exposure to +1.25, these changes mean the colour can now be analyzed since they are calibrated to the grey card values. The calibrated values can be seen in figure 34.

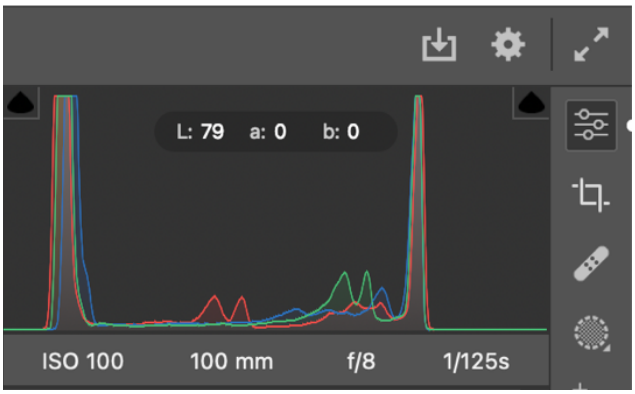


Figure 34. Calibrated L*a*b* values at 79 0 0

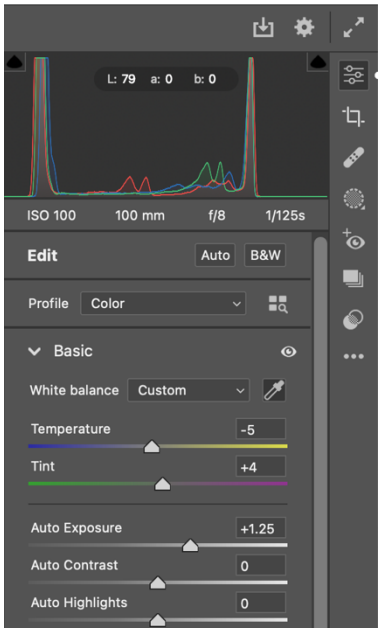


Figure 35. Temperature, tint and autoexposure adjustments to calibrate L*a*b* values

Three L*a*b* measurements of tooth colour were taken. The initial tooth colour was analyzed at T0, before bleaching. The second measurement, T1, was done 1 week post tooth bleaching, to allow for colour stabilization of the crown. The third measurement, T2, was performed after the teeth had been subjected to 14 days of staining. Recording the same point of the crown each time in an accurate and repeatable manner involved superimposing a grid square over the images, which allowed the same exact point to be recorded at T0, T1 and T2. Figure 36 shows the interface used to record the L*a*b* values.

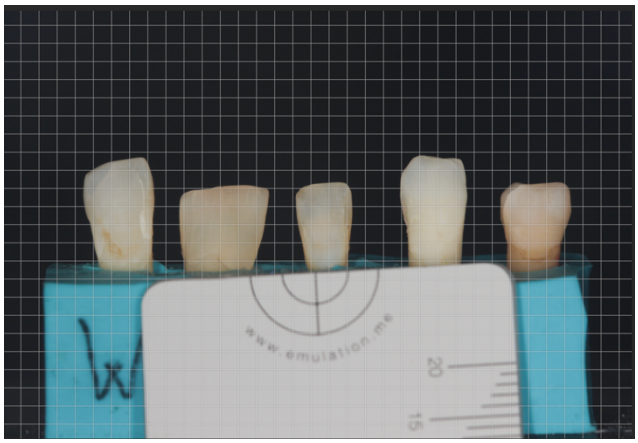


Figure 36. Grid used to precisely measure colour from the same point

Once the L*a*b* values from all groups had been gathered, calculation of the CIED00 and CIE76 delta E values were processed and generated by the José Pereira online calculator as seen in figure 37.

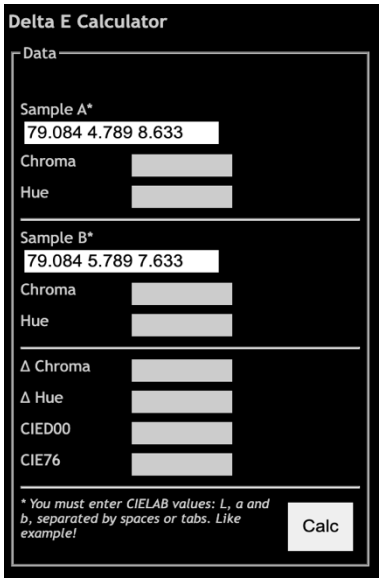
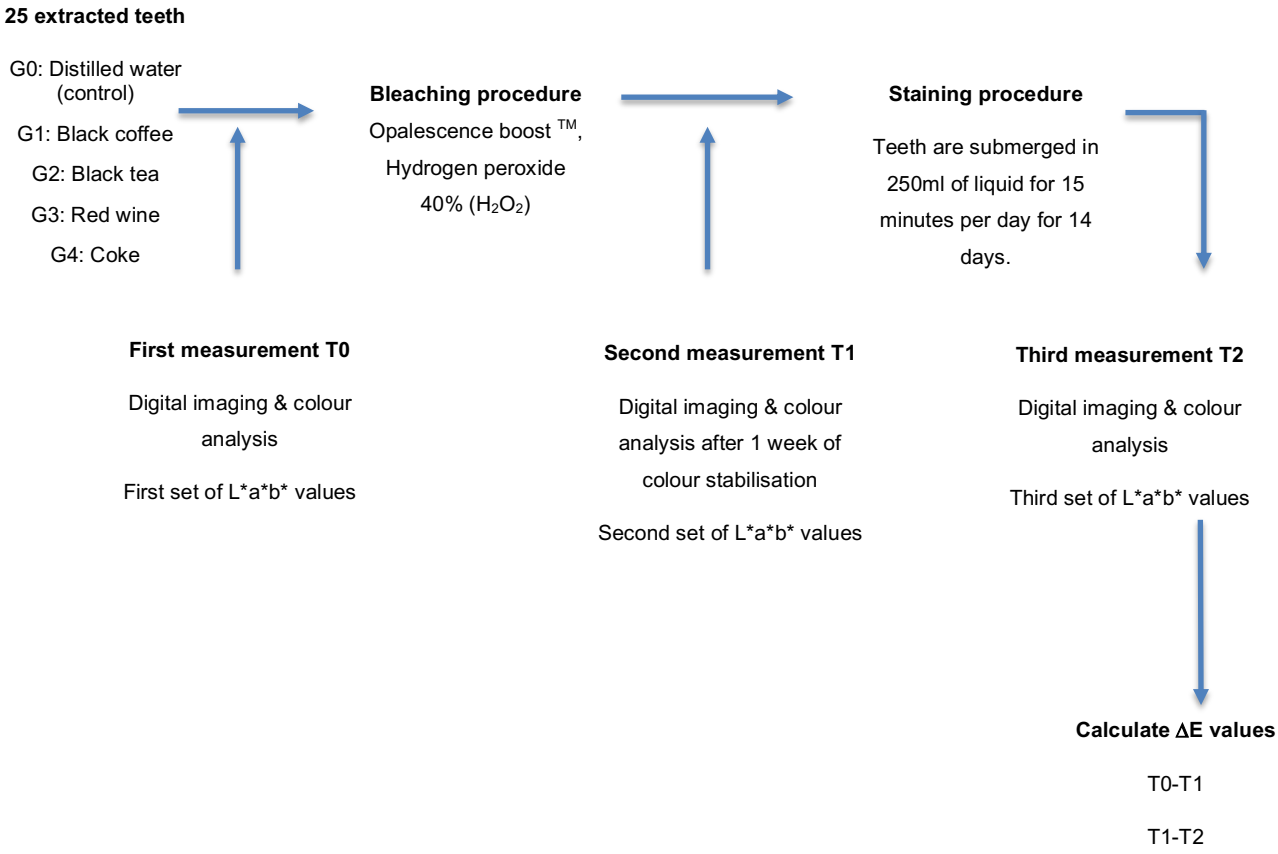


Figure 37. Jose Pereira delta E calculator

3.5 FLOW CHART OF THE METHODOLOGY



4. RESULTS

4.1 CONTROL GROUP VALUES

Table 1. L*a*b* values before bleaching, T0

Control group	W1	W2	W3	W4	W5
L* value	80	77	79	84	79
a* value	0	3	1	0	3
b* value	5	13	7	5	7

Table 2. L*a*b* values after bleaching, T1

Control group	W1	W2	W3	W4	W5
L* value	77	74	73	79	71
a* value	0	3	1	0	4
b* value	0	15	8	9	12

Table 3. L*a*b* values after staining, T2

Control group	W1	W2	W3	W4	W5
L* value	71	65	70	76	68
a* value	0	1	1	-1	4
b* value	7	12	9	8	12

The L* values decrease from both T0 to T1 by 6.27% and from T1 to T2 by 6.42%. The a* value increases from T0 to T1 by 14.29%, then decreases from T1 to T2 by 37.5%. The b* value increases from both T0 to T1 by 18.92% and T1 to T2 by 9.09%.

4.2 COFFEE GROUP VALUES

Table 4. L*a*b* values before bleaching, T0

Coffee group	C1	C2	C3	C4	C5
L* value	78	81	81	80	80
a* value	0	1	1	1	3
b* value	6	9	7	7	10

Table 5. L*a*b* values after bleaching, T1

Coffee group	C1	C2	C3	C4	C5
L* value	71	78	72	78	75
a* value	0	1	2	1	3
b* value	8	13	9	7	12

Table 6. L*a*b* values after staining, T2

Coffee group	C1	C2	C3	C4	C5
L* value	63	66	67	58	63
a* value	1	6	4	4	6
b* value	8	14	8	11	16

The L* values decrease from both T0 to T1 by 6.5% and from T1 to T2 by 15.24%. The a* values increase from both T0 to T1 by 16.67% and from T1 to T2 by 200%. The b* values increase from both T0 to T1 by 25.64% and T1 to T2 by 16.33%.

4.3 TEA GROUP VALUES

Table 7. L*a*b* values before bleaching, T0

Tea group	T1	T2	T3	T4	T5
L* value	77	81	77	74	81
a* value	1	0	1	2	0
b* value	8	6	6	10	8

Table 8. L*a*b* values after bleaching, T1

Tea group	T1	T2	T3	T4	T5
L* value	74	76	72	73	81
a* value	1	1	2	1	0
b* value	11	10	10	10	9

Table 9. L*a*b* values after staining, T2

Tea group	T1	T2	T3	T4	T5
L* value	55	49	48	49	59
a* value	5	10	7	6	7
b* value	14	19	13	13	15

The L* values decrease from both T0 to T1 by 3.59% and from T1 to T2 by 30.85%. The a* values increase from both T0 to T1 by 25% and from T1 to T2 by 600%. The b* values increase from both T0 to T1 by 31.58% and from T1 to T2 by 48%.

4.4 COKE GROUP VALUES

Table 10. L*a*b* values before bleaching, T0

Coke group	S1	S2	S3	S4	S5
L* value	78	77	84	84	81
a* value	2	1	-1	-1	1
b* value	9	9	4	4	6

Table 11. L*a*b* values after bleaching, T1

Coke group	S1	S2	S3	S4	S5
L* value	70	73	81	83	77
a* value	1	3	-1	-1	0
b* value	13	14	5	5	6

Table 12. L*a*b* values after staining, T2

Coke group	S1	S2	S3	S4	S5
L* value	57	60	66	67	60
a* value	7	7	8	7	6
b* value	19	18	23	21	19

The L* values decrease from both T0 to T1 by 4.95% and from T1 to T2 by 19.27%. The a* values remain the same from T0 to T1 and increases from T1 to T2 by 1650%. The b* values increase from both T0 to T1 by 34.38% and from T1 to T2 by 132.56%.

4.5 RED WINE GROUP VALUES

Table 13. L*a*b* values before bleaching, T0

Red wine group	R1	R2	R3	R4	R5
L* value	72	77	79	78	76
a* value	3	0	0	2	2
b* value	12	7	6	7	8

Table 14. L*a*b* values after bleaching, T1

Red wine group	R1	R2	R3	R4	R5
L* value	65	71	75	75	75
a* value	2	0	1	1	3
b* value	10	8	8	7	7

Table 15. L*a*b* values after staining, T2

Red wine group	R1	R2	R3	R4	R5
L* value	45	58	62	68	59
a* value	5	2	4	3	5
b* value	9	8	12	7	9

The L* value decreases from both T0 to T1 by 5.5% and T1 to T2 by 19.11%. The a* values remain the same from T0 to T1 and increases from T1 to T2 by 171.43%. The b* values remain the same from T0 to T1 and increases from T1 to T2 by 12.5%.

4.6 DELTA E VALUES CONTROL GROUP

Table 16. Delta E value: before bleaching vs after bleaching

Delta E value	W1	W2	W3	W4	W5
CIED00	4.96	2.52	4.4	4.57	6.85
CIE76	5.83	3.61	6.08	6.4	9.49

Table 17. Delta E value: after bleaching vs after staining

Delta E value	W1	W2	W3	W4	W5
CIED00	7.5	7.59	2.39	2.65	2.33
CIE76	9.22	9.7	3.16	3.32	3

The average CIED00 delta E value for the control group from T0 to T1 is 4.66 and the average CIE76 delta E value is 6.282.

The average CIED00 delta E value for the control group from T1 to T2 is 4.49 and the average CIE76 delta E value is 5.68.

4.7 DELTA E VALUES COFFEE GROUP

Table 18. Delta E value: before bleaching vs after staining

Delta E value	C1	C2	C3	C4	C5
CIED00	5.36	3.4	6.74	1.4	3.81
CIE76	7.28	5	9.27	2	5.39

Table 19. Delta E value: after bleaching vs after staining

Delta E value	C1	C2	C3	C4	C5
CIED00	6.57	10.89	4.83	16.46	10.11
CIE76	8.06	13.04	5.48	20.62	13

The average CIED00 delta E value for the control group from T0 to T1 is 4.14 and the average CIE76 delta E value is 5.78.

The average CIED00 delta E value for the control group from T1 to T2 is 9.77 and the average CIE76 delta E value is 12.04.

4.8 DELTA E VALUES COKE GROUP

Table 20. Delta E value: before bleaching vs after bleaching

Delta E value	S1	S2	S3	S4	S5
CIED00	6.7	4.89	2.19	1.07	3.14
CIE76	9	6.71	3.16	1.41	4.12

Table 21. Delta E value: after bleaching vs after staining

Delta E value	S1	S2	S3	S4	S5
CIED00	12.97	11.4	17.99	17.51	16.84
CIE76	15.52	14.18	25.1	24	22.23

The average CIED00 delta E value for the control group from T0 to T1 is 3.60 and the average CIE76 delta E value is 3.92.

The average CIED00 delta E value for the control group from T1 to T2 is 15.34 and the average CIE76 delta E value is 20.21.

4.9 DELTA E VALUES RED WINE

Table 22. Delta E value: before bleaching vs after bleaching

Delta E value	R1	R2	R3	R4	R5
CIED00	5.77	4.49	3.5	2.56	1.81
CIE76	7.35	6.08	4.58	3.16	1.73

Table 23. Delta E value: after bleaching vs after staining

Delta E value	R1	R2	R3	R4	R5
CIED00	19.37	11.11	11.14	5.97	13.09
CIE76	20.25	13.15	13.93	7.28	16.25

The average CIED00 delta E value for the control group from T0 to T1 is 3.62 and the average CIE76 delta E value is 4.58.

The average CIED00 delta E value for the control group from T1 to T2 is 12.14 and the average CIE76 delta E value is 14.17.

4.10 DELTA E VALUES TEA GROUP

Table 24. Delta E value: before bleaching vs after bleaching

Delta E value	T1	T2	T3	T4	T5
CIED00	3.04	4.76	4.82	1.54	0.72
CIE76	4.24	6.48	6.48	1.41	1

Table 25. Delta E value: after bleaching vs after staining

Delta E value	T1	T2	T3	T4	T5
CIED00	16.51	25.09	21.9	21.7	19.15
CIE76	19.65	29.85	24.7	24.7	23.85

The average CIED00 delta E value for the control group from T0 to T1 is 2.98 and the average CIE76 delta E value is 3.92.

The average CIED00 delta E value for the control group from T1 to T2 is 20.87 and the average CIE76 delta E value is 24.55.

4.11 GRAPHICAL REPRESENTATION OF THE DATA

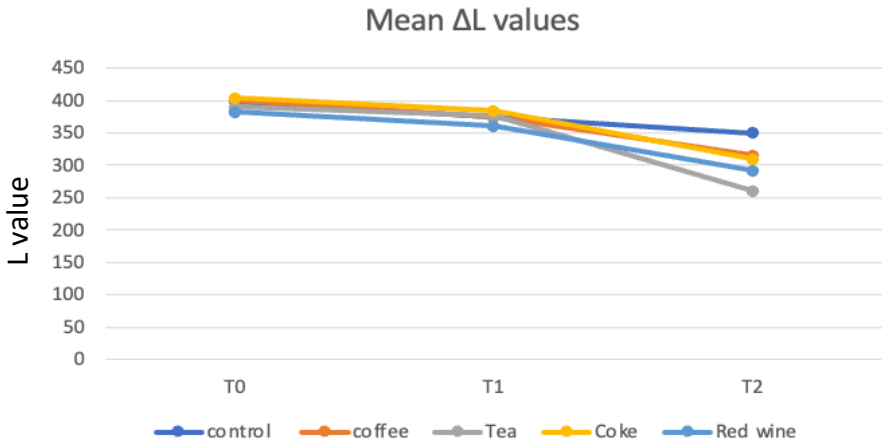


Figure 38. Mean delta L* values of all the groups from T0-T2

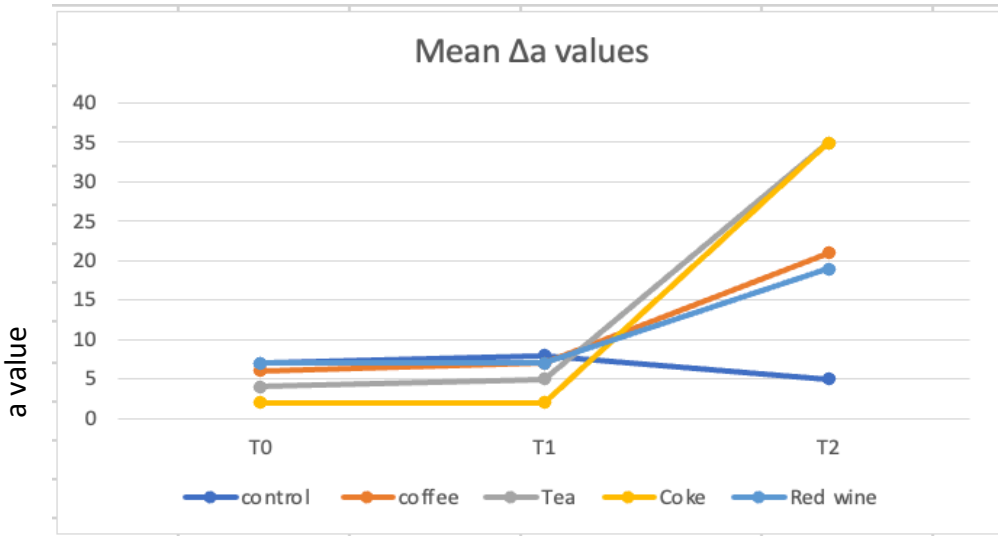


Figure 39. Mean delta a* values of all the groups from T0-T2

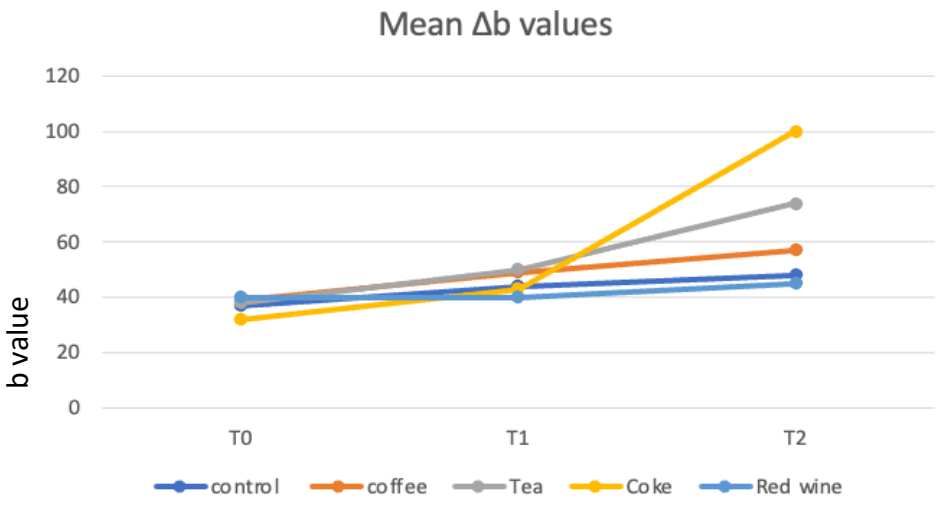


Figure 40. Mean delta b* values of all the teeth from T0-T2

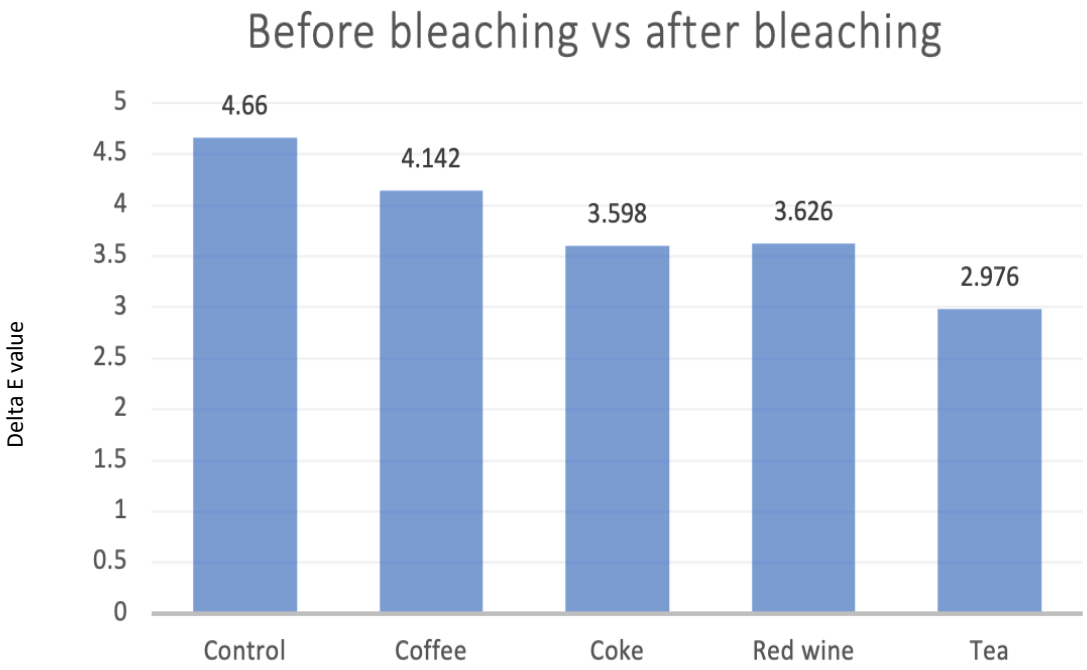


Figure 41. CIED00 Delta E values before bleaching vs after bleaching

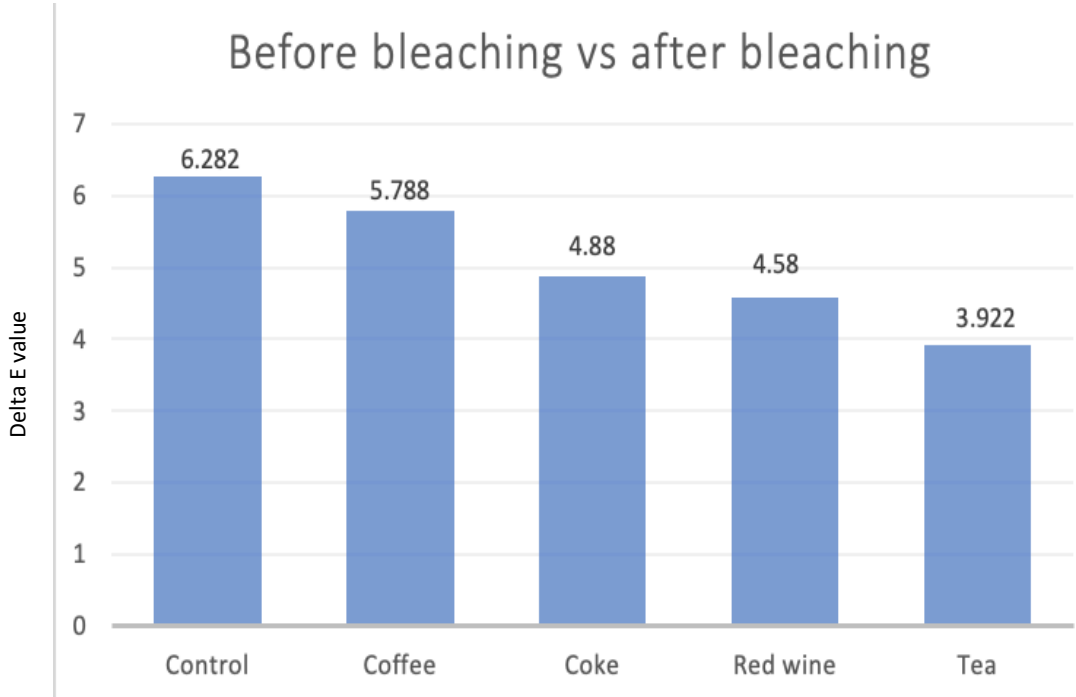


Figure 42. CIE76 Delta E values before bleaching vs after bleaching

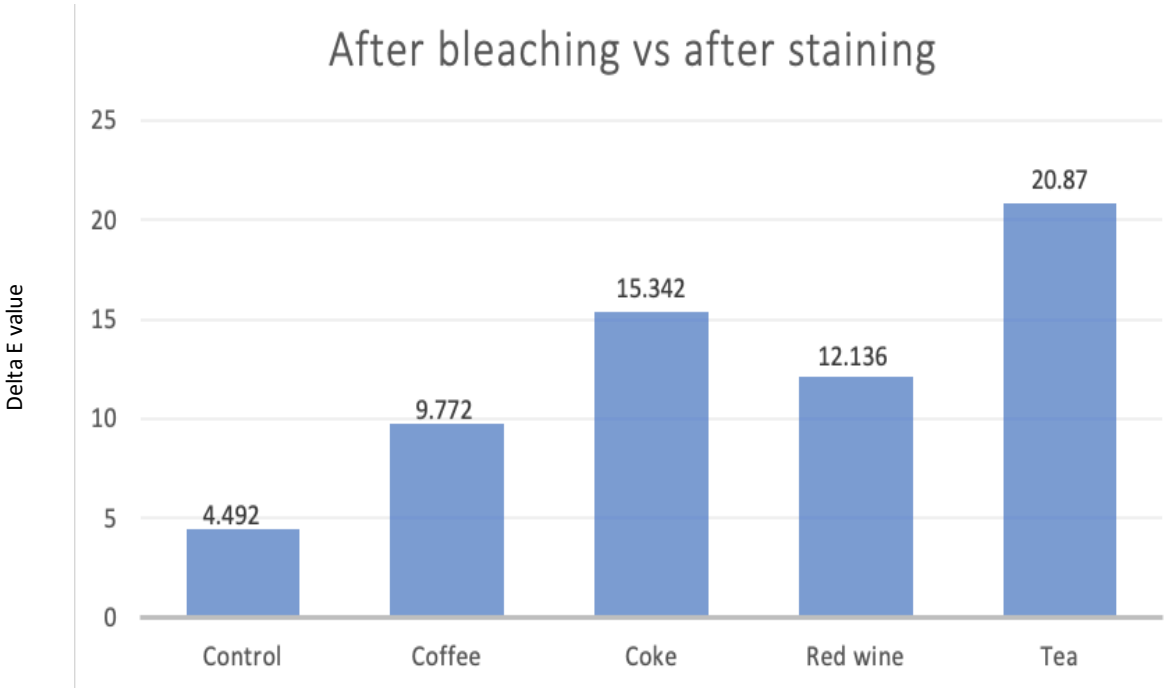


Figure 43. CIED00 Delta E after bleaching vs after staining

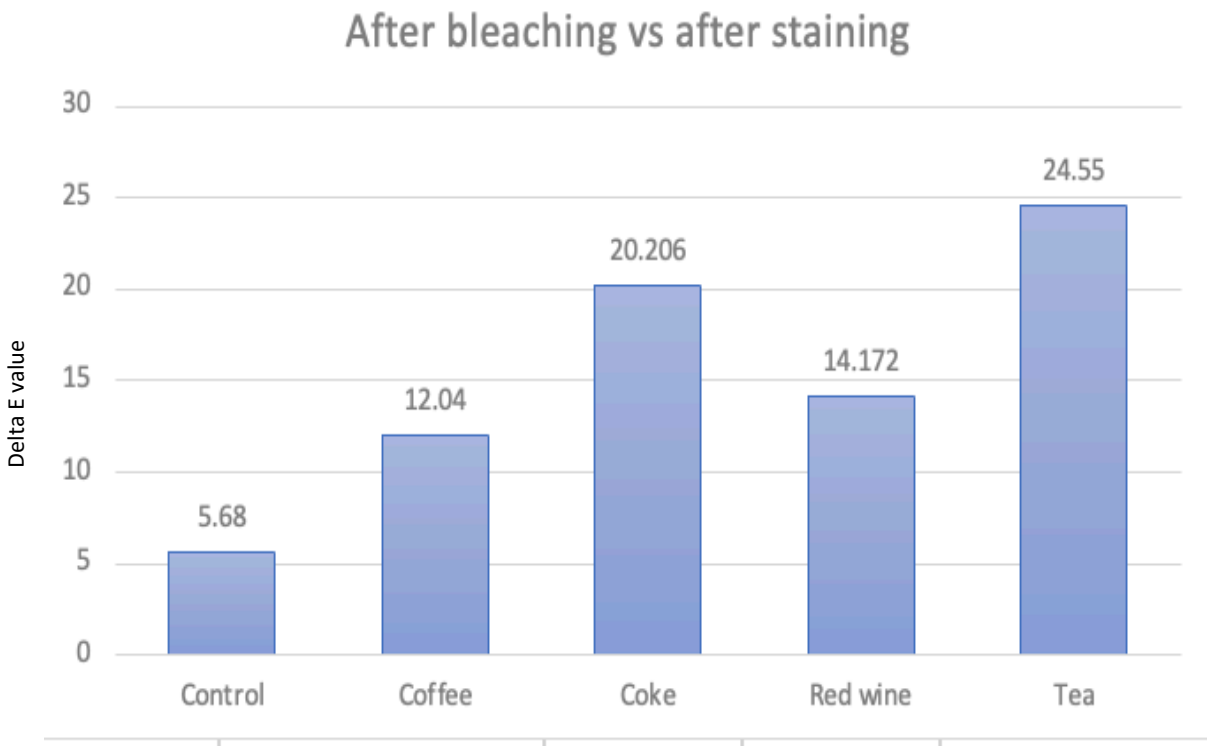


Figure 44. CIE76 Delta E values after bleaching vs after staining

4.12 IMAGES OF THE GROUPS AT T0,T1,T2

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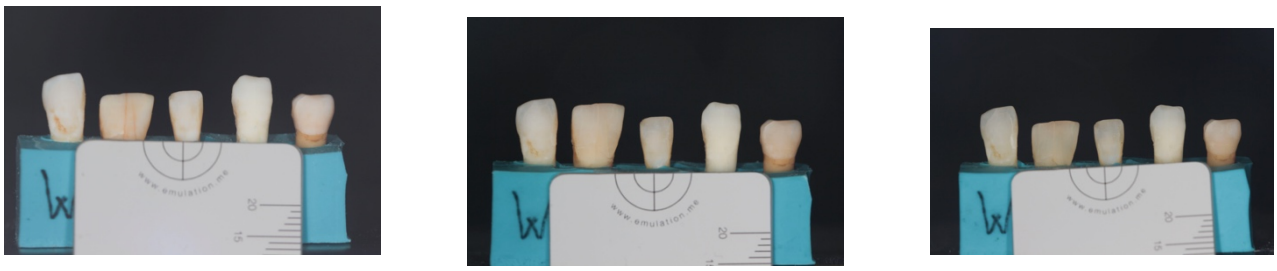

Figure 45. T0-T1-T2 from left to right: Control group

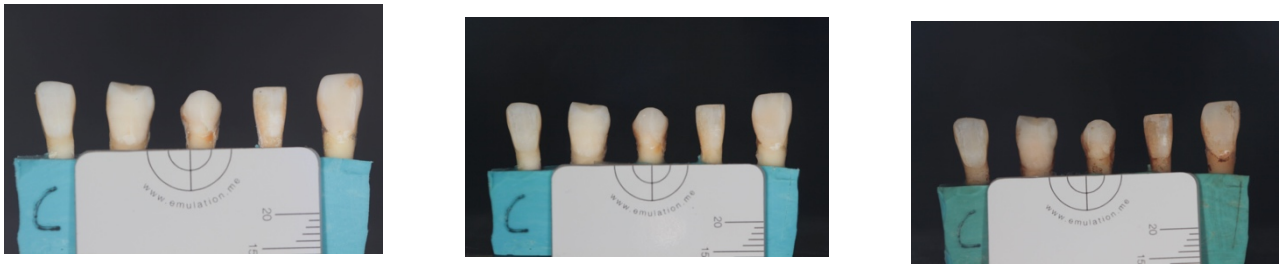

Figure 46. T0-T1-T2 from left to right: Coffee group

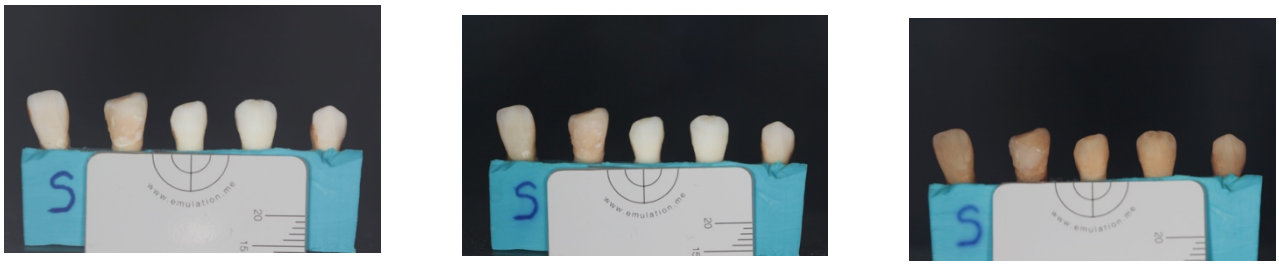

Figure 47. T0-T1-T2 from left to right: Coke group

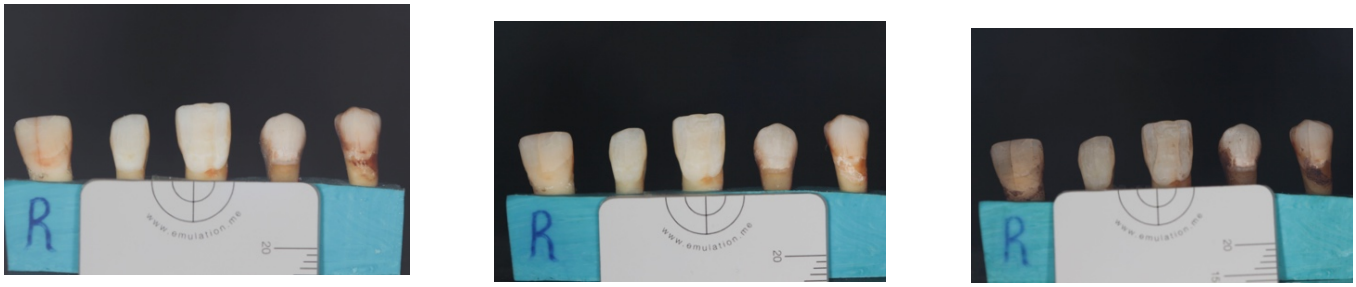

Figure 48. T0-T1-T2 from left to right: Red wine group

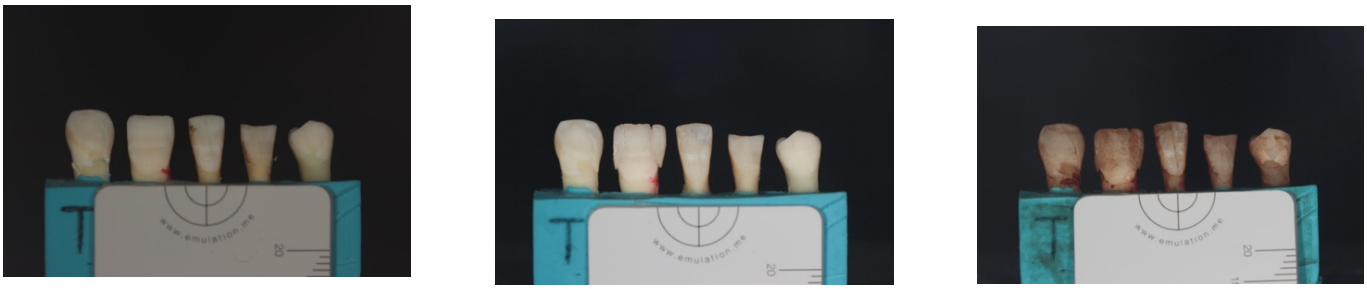

Figure 49. T0-T1-T2 from left to right: Tea group

5. DISCUSSION

In order to perform this type of study on a bigger scale, an evaluation study like this one is necessary to assess its feasibility. The null hypothesis of this study stated that consuming different staining beverages after undergoing in office bleaching, with hydrogen peroxide 40%, does not result in a significant discoloration of the enamel in comparison with the control group via assessment of delta E values. Delta E values range on a scale from 0-100, the human eye is unable to perceive delta E values of less than 1.0. Delta E of 1-2 is perceptible from close observation, 2-10 can be perceived from a glance, 11-49 describes colours which are more similar than opposite each other and 100 represents absolute colour opponents (17)With this in mind, the delta E calculator used to process the L*a*b* values generated two delta E values: CIED00 and CIE76. Both sets of delta E values are recognised by the CIE and were developed to quantify colour change, with the CIED00 being a more updated version of the CIE76 (52) The CIED00 is considered to be a more sensitive measurement of delta E since it includes additional parameters within its colour change analysis.

The L*a*b* values of the teeth before and after bleaching were used to obtain the first set of delta E values. The CIECD00 values ranged from 2.98-4.66 and the CIE76 values ranged from 3.92-6.28. These delta E values suggest that hydrogen peroxide bleaching of enamel has a perceivable change in colour when compared to the baseline colour. As current knowledge of the market indicates, patients request in office bleaching due to its minimally invasive ability to brighten teeth (37), which is quantified by the lightness of the colour, L* value. The L* values analysed in the results of this study, however, indicate that the L* value of the teeth diminishes across all the groups after being bleached, as seen in figure 38. Decreasing L* values have been reported in other studies of this kind, with one explanation being hydrogen peroxide's ability to change the enamel's micromorphology (53). The increased surface roughness and loss of aprismatic enamel was reported in the other studies was confirmed through scanning electron microscopy which revealed that use of low pH bleaching agents, such as the hydrogen peroxide, causes the most micromorphological changes in the enamel (38). Loss of a smooth, uniform surface leads to less light being reflected and refracted and a

subsequent lower brightness value, which lends a possible explanation behind the results observed.

Use of non-vital teeth for the purpose of this study could also influence the diminishing brightness values observed after bleaching. The teeth were suspended in distilled water for the entire duration of the experiment, only being removed to bleach, stain or take photos of them. However, within a vivo setting, the oral cavity contains saliva which possesses remineralizing capabilities. The calcium and phosphate found in the saliva allows the tooth to restrengthen itself and replenish any lost minerals (38). Distilled water has been essentially stripped of all its minerals and ions; therefore, it is impossible for the teeth to remineralize.

In parallel with decreasing L* values, post bleaching, the a* and b* values increased. We observe an increase in the mean Δa* of all groups, suggesting all the teeth have become redder in colour after bleaching, with the mean Δb* of all groups increasing too, implying they have become more yellow in colour. As previously discussed, the reduction of the aprismatic layer of enamel would expose the colour of the underlying dentin more vividly, with the dentin being comprised of yellow-red tones (54)

The colour of the teeth which were bleached, T1, was compared with the colour of the teeth post staining, T2. The CIED00 values ranged from 4.49-20.87 and the CIE76 values ranged from 5.68-20.21. These sets of values describe a marked difference in the colour of the enamel after being exposed to the staining beverages. The a* and b* values of these teeth increase during staining, contributing to the high delta E values obtained. Based upon the findings of this study, we can reject the null hypothesis and accept the alternative hypothesis, since all groups of teeth proved a significant discoloration compared to the control group, who maintained the lowest delta E value when T1 and T2 are compared.

The main objective of this pilot study was to assess the feasibility of performing a larger scale research study which evaluates staining beverages after in office bleaching. The findings of the study indicates that in office bleaching with hydrogen

peroxide and staining with coffee, red wine, coke and tea all causes a perceivable change in the colour of teeth. However, this study did have various limitations, which are essential to acknowledge. Establishing the correct amount of time an individual would have a beverage suspended in their mouth was hard to replicate. A time period of 15 minutes per day for 14 days was determined to be the most appropriate length of time for this pilot study, to ensure repeatable results. However, a subsequent research study based upon this pilot study should take into consideration that hot drinks are generally consumed over a shorter period compared to cold drinks; a factor which was unaccounted for in this study. Another limitation would be the sample size of this study, with only 25 teeth being used, a larger research study would require a much higher number of participants to improve the precision and statistical power of the results obtained. A final limitation of the study is the inability to account for a patient's hygiene habits. The lack of artificial saliva, tooth brushing, and motions of the tongue and cheeks mean the results should be interpreted with caution as these factors could significantly affect how much discolouration the teeth suffer after consuming staining beverages.

When assessing the negative impact of the staining beverages on the results of in office bleaching, we must consider the results obtained after bleaching. When we graphically observe the decrease in L* values, like in figure… we can see a much sharper decrease in the brightness of the enamel when we compare T0-T1 with T1-T2. The a* and b* values of all groups increased significantly from T1-T2, indicating the red and yellow tints of the enamel have become much more pronounced. When comparing the results obtained after staining, with desires of the patient after tooth bleaching, we can conclude that all groups of staining beverages have a negative impact upon the results of in office bleaching.

When comparing the potencies of the staining beverages studied, we can determine that coffee had the lowest staining ability, followed by red wine, coke and tea, in ascending order of staining potency. The delta E values observed were 12.04, 14.172, 20.206 and 24.55 respectively. Many sources of literature suggest that the lower the pH of the solution, the more surface destruction and demineralization it causes (55). This theory feeds into what Dahl and Pallensen suggested, which was that a higher surface roughness leads to an enamel more susceptible to staining (2) The pH of the

solutions used varied, with most sources (56) agreeing that distilled water has a neutral pH of 7.0, coffee with a pH of 5.0, tea with a pH of 5.2, red wine with a pH of 3.3 and coke with a pH of 2.4.

To determine the efficacy of in office bleaching at increasing the value of the teeth, we consider the L* values. These values suffered a decrease across all groups, with the possible reasons being mentioned previously. Based upon these findings, it could be said that in office bleaching has a low efficacy at increasing value, however it has efficacy at altering the colour of the teeth, as observed in figure 38.

6. CONCLUSIONS

Regarding the feasibility of performing a larger scale research study which evaluates staining beverages after in office bleaching, we would have to account for the limitations of the pilot study, such as small sample size, reliable staining times considering the specific beverage used and the lack of hygiene measures which influence extrinsic stain removal. However, the bleaching, digital imaging and analysis all followed an objective and repeatable methodology which could be used in a scaled-up version of this study.

1. Based upon the L*a*b* values analyzed in this study; it can be said that consumption of staining beverages has a negative impact upon the results of in office bleaching.

2. The beverage with the strongest staining potency was tea, it presented a ΔE value of 24.55, coke with a ΔE of 20.206, red wine with a ΔE of 14.172 and coffee with a ΔE of 12.04.

3. In office bleaching with hydrogen peroxide, 40%, appears to lower the L* values in comparison with the L* baseline values. This reduction of L* implies the tooth has lost brightness.

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