INFLUENCE OF A BLEACHING AGENT ON STAINED DIRECT COMPOSITE RESINS.

Caroline Wanjau

A mini-thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science in Dental Sciences in
Restorative Dentistry at the Faculty of Dentistry
University of the Western Cape

Supervisor: Professor Y. I. Osman

November 2008

INFLUENCE OF A BLEACHING AGENT ON STAINED DIRECT COMPOSITE RESINS.

KEY WORDS

Nanocomposite

Discolouration

Bleaching

Tea

Red Wine

ABSTRACT

Despite the phenomenal strides in research of dental resin composites regarding their physical and mechanical properties, discolouration, either intrinsic or extrinsic still remains a major drawback and is one of the main reasons for the replacement of these restorations. Toothbrushing and polishing procedures have been attempted to eliminate extrinsic staining without optimal results. Vital tooth bleaching has over 90% success rates in whitening discoloured teeth and this may be an alternative treatment modality for discoloured composite resins. **Aim:** The aim of this study was to determine whether tooth bleaching agents alter the colour of stained direct composite resins. **Material and Method:** 60 disc shaped specimens (9 x 2mm) of Filtek Supreme XT were prepared. They were randomly divided into 3 groups (n = 20) and exposed to either one of two experimental staining agents, tea or red wine, or artificial saliva (control) continuously over a 7-day period. They were all then bleached with Opalescence Xtra Boost, a chemically activated in-office whitening agent for 3, weekly sessions of a half hour each, broken into 2, fifteen minute cycles. Colour determinations were made using a reflectance spectrophotometer, from baseline, after each day of staining, after the bleaching treatments and after a 1 week rehydration period. The CIE Lab colour space was used and colour changes were monitored using ΔE, that was calculated during intervals between the experimental episodes using L, a and b values. A $\Delta E \geq 3.3$ represented colour changes that were deemed clinically noticeable. Data analysis was carried out using Microsoft excel and a non-parametric test (Wilcoxon Signed Sum Rank Test) with a significance level set at ≤ 0.05 for colour differences that are statistically significant. **Results:** Both staining solutions discoloured the composite resin samples, but red wine produced greater colour changes than tea. After bleaching, the specimens in the tea group reverted to baseline colour with a $\Delta E \leq 3.3$ but those in the red wine group did not revert to baseline values with a $\Delta E \geq 3.3$. **Conclusion:** Filtek Supreme XT, a nanocomposite, is susceptible to discolouration by chromogenic beverages. Red wine produced deeper staining than tea. Opalescence Xtra Boost was effective in removing tea stains but not red wine stains.

DECLARATION

I hereby declare that **Influence of a Bleaching Agent on Stained Direct Composite Resins** is my own work, that it has not been submitted before for any degree or examination in any university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Caroline Wanjau

November 2008

Signed: ……

ACKNOWLEDGMENTS

Professor Y. I. Osman, for his kind words, encouragement, assistance and guidance in completing this thesis. I would also like to thank him for sharing his vast knowledge and imparting his wisdom and experience during the course of the program.

Dr R. Rossouw, for his assistance, kindness and patience in acquiring the right materials to get the research underway and his immeasurable advice and encouragement during the course of this study.

Dr N. Basson, for his kindness and assistance both in terms of advice and in acquiring the mould, staining vials and use of the pH machine.

Dr A. Majeed, for his assistance and patience in the use of the spectrophotometer and in answering all my questions.

Professor Kotze, for his invaluable input in the statistical analysis of this study.

Robyn and Gisela of 3M ESPE, South Africa for generously supplying the composite resin and written material for this study.

Alida of Milners' Dental Suppliers, South Africa, for kindly providing the curing tip and literature needed for this study.

DEDICATION

This is dedicated to my parents, who have been supportive and encouraging to us, their children in all our endeavors. They have made sacrifices to see us through our undertakings and continue to be our greatest advisors. There are no words to thank them.

I would also like to dedicate it to my friends whose encouragement and assistance helped make this thesis a reality.

TABLE OF CONTENTS

TITLE PAGE.....i

KEY WORDS.....ii

ABSTRACT.....iii

DECLARATION.....iv

ACKNOWLEDGMENTS.....v

DEDICATION.....vi

TABLE OF CONTENTS.....vii

LIST OF FIGURES.....x

LIST OF TABLES.....xii

CHAPTER 1 INTRODUCTION.....1

CHAPTER 2 LITERATURE REVIEW.....2

2. 1 INTRODUCTION.....2

2. 2 AESTHETIC DENTISTRY.....2

2. 3 BLEACHING.....3

2. 3. 3. 1 Vital Tooth Bleaching.....7

2. 3. 3. 1. 1 Night Guard Vital Bleaching (NGVB).....7

2. 3. 3. 1. 2 In-office Bleaching.....8

2. 3. 3. 1. 3 Over-the-counter Bleaching Agents (OTC).....9

2. 3. 3. 1. 4 Other Methods.....10

2. 3. 3. 2 Non-vital Tooth Bleaching.....10

2. 3. 3. 2. 1 Walking Bleach Technique.....10

2. 3. 3. 2. 2 Thermocatalytic Technique.....10

2. 3. 3. 2. 3 “Inside-outside” Technique.....11

2. 4 COMPOSITE RESINS.....13

2. 4. 1 History 13
2. 4. 2 Composition 14
2. 4. 2. 1 Organic Matrix 14
2. 4. 2. 2 Inorganic Fillers 15
2. 4. 2. 3 Coupling Agents 17
2. 4. 3 Discolouration of Composites 17
2. 4. 3. 1 Intrinsic Staining 18
2. 4. 3. 2 Extrinsic Staining 20
2. 3. 3. 3 Internalized Staining 22
2. 4. 4 Effect of Bleaching Agents on the Colour of Composite Restorations 22
2. 5 COLOUR AND COLOUR MEASUREMENT 25
2. 5. 1 Colour Perception 25
2. 5. 2 Science of Colour 25
2. 5. 3 Colour Measurement 28
2. 5. 3. 1 Visual Techniques 28
2. 5. 3. 1. 1 Shade Guides 28
2. 5. 3. 1. 2 Stain Indices 28
2. 5. 3. 2 Instrumental Techniques 29
2. 5. 3. 2. 1 Colourimeters 29
2. 5. 3. 2. 2 Spectrophotometers 30
2. 5. 3. 2. 3 Digital Imaging Devices 31
2. 6 CONCLUSION 32
CHAPTER 3 AIMS AND OBJECTIVES 33
3. 1 AIM 33
3. 2 OBJECTIVES 33
3. 3 NULL HYPOTHESIS 33
CHAPTER 4 MATERIALS AND METHODS 34

4. 1 STUDY DESIGN **34**

4. 2 STUDY POPULATION AND SAMPLING **34**

4. 3 MATERIALS **34**

4. 3. 1 Filtek Supreme XT 35

4. 3. 2 Staining Agents 35

4. 3. 3 Opalescence Xtra Boost 37

4. 4 METHODOLOGY **39**

4. 4. 1 Composite Resin Specimen Preparation 40

4. 4. 2 Polymerization Procedure 41

4. 4. 3 Colour Measurement 43

4. 4. 4 Staining Process 44

4. 4. 5 Bleaching Procedure 46

4. 5 Data Analysis **47**

CHAPTER 5 RESULTS **48**

CHAPTER 6 DISCUSSION **61**

CHAPTER 7 CONCLUSIONS AND RECOMMENDATIONS **72**

REFERENCES **74**

APPENDICES **83**

LIST OF FIGURES

Figure 2.1: Nano-sized filler particles in Filtek Supreme XT. 16

Figure 2.2: Munsell Colour System (Derbabian *et al*, 2001)..... 26

Figure 2.3: The CIE Lab colour space (Jarad *et al*, 2005). 27

Figure 4.1 Filtek supreme XT. 35

Figure 4.2: Ketepa Pride Tea from Kenya. 36

Figure 4.3: Simonsvlei Cabernet Sauvignon Merlot, 2006. 36

Figure 4.4: Opalescence Xtra Boost. 38

Figure 4.5: Flowchart of the study. 39

Figure 4.6: Rubber mould. 40

Figure 4.7: Mould with composite sandwiched between 2 glass slabs and 2 polyester strips..... 40

Figure 4.8: Optilux 150 Curing light. 41

Figure 4.9: Visible light intensity meter. 41

Figure 4.10: The curing process. 42

Figure 4.11: Test specimens. 43

Figure 4.12: Konica Minolta Sensing spectrophotometer. 43

Figure 4.13: Colour measurements. 44

Figure 4.14: Development of the tea stain. 45

Figure 4.15: Staining process 45

Figure 4.16: Bleaching process. 46

Figure 5.1: Cumulative mean ΔE values from baseline for Group C. 50

Figure 5.2: Dispersion for Group C 52

Figure 5.3: Cumulative mean ΔE values from baseline for Group T. 53

Figure 5.4: Dispersion for Group T 55

Figure 5.5: Cumulative mean ΔE values from baseline for Group W. 56

Figure 5.6: Dispersion for Group W 58

Figure 5.7: Comparison of mean ΔE values from baseline for the three groups. 59

Figure 5.8: Comparison of sequential mean ΔE for the three groups. 60

LIST OF TABLES

Table 2.1: Studies on Effect of Bleaching Agents on the Colour of Composite Resins..... **24**

Table 4.1: Materials Used in the Study..... **34**

Table 4.2: Composition of Artificial Saliva..... **37**

Table 5.1: Extract from raw data **48**

Table 5.2: Mean ΔE, standard deviation and p values for all groups. **49**

Table 5.3: Mean (standard deviation) values during the staining period for group C.**.** **51**

Table 5.4: Mean (standard deviation) values during the bleaching and rehydration period for Group C**.**..... **51**

Table 5.5: Mean (standard deviation) values during the staining period for group T **54**

Table 5.6: Mean (standard deviation) values during the bleaching and rehydration period for Group T **54**

Table 5.7: Mean (standard deviation) values during the staining period for group W **57**

Table 5.8: Mean (standard deviation) values during the bleaching and rehydration period for Group W..... **57**

Table 5.9: Mean (standard deviation) values of the overall changes for all three groups **58**

CHAPTER 1 INTRODUCTION

Tooth whitening has been a part of aesthetic dentistry since 1898 (Turker and Biskin, 2003), but its popularity has increased dramatically in the last two decades as the public now pursues the 'perfect youthful' smile to complement the benefits reaped from dieting and exercising. Initially, bleaching was carried out by dentists in their offices and was largely considered a preserve for the rich (Burrell, 1997), but now, a large variety of over-the-counter systems are available for anyone who would like to improve their smile. Professionally-staffed tooth whitening centres, whose sole purpose is bleaching teeth, are now being opened in the USA, to meet the growing demand for this service (Sarrett, 2002). The sale of bleaching agents now has a multi-million dollar annual turnover as the craze for whiter, brighter smiles sweeps across the globe (Garber, 1997, Schmidt and Tatum, 2006).

Aesthetic considerations have also extended to restorations, with composite resins being used more frequently, even in the posterior region. They have undergone a great deal of development since their introduction in the 1940s, as regards their physical and chemical properties (Garcia *et al*, 2006). Unfortunately, the discolouration of composite restorations over time, still remains a major drawback (Buchalla *et al*, 2002, Villalta *et al*, 2006) and is one of the main reasons for their replacement (Buchalla *et al*, 2002, Schulze *et al*, 2003, Villalta *et al*, 2006). Bleaching systems have an effect on the tooth colour so it is deduced that composite restorations should also be influenced by these whitening agents (Canay and Cehreli, 2003).

In Kenya and other parts of the world, the treatment of choice for stained composite restorations is their removal and subsequent replacement, which is an invasive procedure. Furthermore, it has been shown that each removal and replacement of a restoration is accompanied by a certain amount of loss of sound dental material, with eventual weakening of the remaining tooth structure (Elderton, 1996).

The aim of this study is to assess the effectiveness of a bleaching agent in removing stains from light polymerized composite resins with a view of using this as an alternative method of returning these discoloured restorations to their former aesthetic levels.

CHAPTER 2 LITERATURE REVIEW

2. 1 INTRODUCTION

There has been a gradual shift in dentistry in the last 25 years, from treating and preventing oral diseases, to aesthetics (Spear and Kokich, 2007). Although patients are still concerned with getting their teeth to a healthy state to carry out their primary masticatory function, they are now also placing great importance on how good their dentitions look (Morley, 1999, Spear and Kokich, 2007). Dental professionals are increasingly being asked to improve the appearance of the individual's smiles even when no real pathology exists in the mouth (Morley, 1999, Spear and Kokich, 2007). This has necessitated a multidisciplinary approach to treatment, with some dentists even suggesting that the treatment planning should begin with aesthetics (Spear and Kokich, 2007). This increased demand for enhanced appearance has led to the exponential growth in the bleaching industry (Garber, 1997, Sarrett, 2002) and the shift to tooth coloured restorations (Sadowsky, 2006). This has been accompanied by major strides in the research into colour measurement and shade matching in prosthodontics (Brewer *et al*, 2004)

The literature review focuses on aesthetic dentistry and society's increasing appetite for it, bleaching procedures, composite resins and colour and colour measurement methods used in dentistry.

2. 2 AESTHETIC DENTISTRY

It is now recognized that beautiful people are perceived to be happier, more outgoing, intelligent and successful compared to their more plain counterparts (Adams and Pang, 2004, Beall, 2007). These conclusions were previously based on overall attractiveness, but studies have shown that a smile alone had a huge impact on what society views as attractive and at the same time, enhanced personality. These individuals have also been found to be treated better in society (Adams and Pang, 2004, Beall, 2007). It is no wonder then that society has increasingly become obsessed with aesthetics whose standards are now media-dictated (Adams and Pang, 2004). The confidence and self-esteem of individuals is now pegged on achieving these sometimes, impossible expectations (Burrell, 1997, Goldstein, 1997, Adams and Pang, 2004).

A beautiful smile is so crucial that it is now accepted as the single most important interactive communication skill an individual has (Joiner, 2004). It is associated with white, well aligned and proportioned teeth (Morley, 1999, Adams and Pang, 2004, Schmidt and Tatum, 2006). The position of the anterior teeth *vis-à-vis* the lip line is also an important consideration in aesthetic dentistry, as is the gingival contour surrounding the teeth (Adams and Pang, 2004, Joiner, 2004, Schmidt and Tatum, 2006, Spear and Kokich, 2007). Older members of society, who today are keeping their natural teeth much longer, are also seeking these same treatment options to fulfill the "youth at all costs" phenomenon (Morley, 1999) or as Burrell (1997) so succinctly put it , the pursuit of "immortality".

Minor aesthetic concerns such as tooth discolouration can be addressed with scaling and polishing (Dahl and Pallesen, 2003, Joiner, 2006), bleaching techniques, micro-abrasion or veneers and crowns (Morley, 1999, Joiner, 2006, Schmidt and Tatum, 2006, Spear and Kokich, 2007). When the position or the contour of the gingiva is less than ideal, periodontal surgery may be indicated (Morley, 1999, Schmidt and Tatum, 2006, Spear and Kokich, 2007). The length and width of teeth may be made more aesthetically pleasing by using veneers or crowns, by crown lengthening procedures or by orthodontic extrusion (Spear and Kokich, 2007). Malaligned teeth are repositioned using orthodontic appliances, and orthognathic surgery is indicated for severe tooth and jaw discrepancies (Schmidt and Tatum, 2006, Spear and Kokich, 2007). There are times when more than one modality of treatment has to be used to achieve the desired aesthetic result (Spear and Kokich, 2007).

Whereas all these modes of treatment are used in aesthetic dentistry, this review focuses mainly on bleaching procedures of discoloured teeth.

2. 3 BLEACHING

Tooth whitening, as a means to improve aesthetics has existed for over a century (Goldstein, 1997, Dahl and Pallesen, 2003, Turker and Biskin, 2003, Suleiman, 2004). It is the most conservative of all the procedures that can be used in treating discoloured teeth (Attin *et al*, 2003, Shethri *et al*, 2003, Kihn, 2007). Bleaching has been declared a safe and efficient method for whitening teeth, with the American Dental Association giving bleaching systems, its "Seal of Approval" (Garber, 1997, Sarrett, 2002, Shethri *et al*, 2003) as has the US Food and Drug Administration (Schmidt and Tatum, 2006).

2. 3. 1 History of Bleaching

Both vital and non-vital teeth may be whitened (Goldstein, 1997, Sarrett 2002, Attin *et al*, 2003, Dahl and Pallesen, 2003, Suleiman, 2004, Tredwin *et al*, 2006).

Truman has been credited with the introduction of bleaching of non-vital teeth which were initially whitened using chlorinated lime around 1850 (Haywood, 1992, Attin *et al*, 2003, Dahl and Pallesen, 2003, Suleiman, 2004). The use of oxalic acid, chlorine and sodium peroxide were employed from 1862 until the turn of the 19th century. Thereafter, sodium hypochlorite, pyrozone, which is a mixture of hydrogen peroxide and ether, and superoxol (30% hydrogen peroxide) were used (Haywood, 1992, Attin *et al*, 2003). The application of light, heat and electric currents to accelerate the bleaching reactions was also described in the early 1900s (Attin *et al*, 2003, Dahl and Pallesen, 2003). A procedure, in which the bleaching agent would be left in the pulp chamber in between dental appointments, was published in 1938 (Attin *et al*, 2003). Back then, a mixture of distilled water and sodium perborate was used (Attin *et al*, 2003). It later came to be called the "walking bleach" technique by Spasser in 1961 when he used a mixture of sodium perborate and water (Attin *et al*, 2003, Dahl and Pallesen, 2003, Suleiman, 2004). Two years later, Nutting and Poe used hydrogen peroxide in place of water to improve tooth whitening, using the same technique (Haywood, 1992, Attin *et al*, 2003, Dahl and Pallesen, 2003, Suleiman, 2004).

The whitening of vital teeth was described as early as 1868, first using oxalic acid and later, pyrozone or hydrogen peroxide. Hydrogen peroxide was the main product being used by the early 1900s with heat or light activation (Haywood, 1992, Suleiman, 2004) in a process known as power bleaching that was reported by Abbot in 1918 (Goldstein, 1997, Joiner, 2006, Buchalla and Attin, 2007). In the 1990s, hydrogen peroxide gels replaced liquids (Suleiman, 2004).

In the late 1960s, Dr. Klusmier, an orthodontist, discovered that carbamide peroxide caused lightening of teeth (Haywood, 1992, Dahl and Pallesen, 2003, Suleiman, 2004, Kihn, 2007). At the time, he was using a 10% carbamide peroxide containing antiseptic on a tray for the treatment of gingivitis on a patient, when he noticed that the teeth whitened. This kicked off the night guard vital bleaching technique which was published in 1989 by Haywood and Heymann (Haywood, 1992, Dahl and Pallesen, 2003, Suleiman, 2004).

Currently, hydrogen peroxide and its compounds, sodium perborate and carbamide peroxide are used for tooth whitening (Dahl and Pallesen, 2003, Kim *et al,* 2004).

2. 3. 2 Chemistry of Bleaching

Hydrogen peroxide is a colorless liquid that is highly soluble in water. It has a bitter taste and is an oxidizing agent. Industrially, it has many uses including bleaching, treatment of water and sewage systems and seed disinfection (Tredwin *et al*, 2006). While hydrogen peroxide has been used for bleaching teeth since the last century, carbamide peroxide has only been available for this indication from 1989. Carbamide peroxide was originally used as an oral antiseptic (Perdigao *et al*, 2004).

The chemical reactions involved in bleaching are not yet fully understood (Joiner, 2006, Kihn, 2007, Suleiman, 2004). Hydrogen peroxide may be used directly or it may be generated from sodium perborate (SP) or carbamide peroxide (CP) for whitening procedures (Dahl and Pallesen, 2003). Sodium perborate is a stable compound when in powder form but readily decomposes in the presence of acid, water or warm air forming metaborate and hydrogen peroxide (Suleiman, 2005a), while carbamide peroxide, an unstable solution, easily dissociates into its constituents, of which only one third is hydrogen peroxide (HP), the remaining two thirds being urea (Turker and Biskin, 2003, Joiner, 2007).

$$Na_2[B_2(O_2)_2(OH)_4] + 2H_2O \longrightarrow 2NaBO_3 + 2H_2O_2$$

(SP) (HP)

$$H_2NCONH_2.H_2O_2 \longrightarrow H_2NCONH_2 + H_2O_2$$

(CP) (urea) (HP)

The urea undergoes further breakdown to yield ammonia (Canay and Cehreli, 2003, Dahl and Pallesen, 2003, Turker and Biskin, 2003), an alkaline which raises the pH of the environment (Dahl and Pallesen, 2003), and carbon dioxide (Canay and Cehreli, 2003, Turker and Biskin, 2003). The alkalinity facilitates the bleaching reaction resulting in more effective tooth whitening than acidic environments (Attin *et al*, 2003, Dahl and Pallesen, 2003).

Chromophores or stains are organic compounds made up of long chains of alternating single and double bonds, with heteroatoms, phenyl and carbonyl rings (Joiner, 2006). Bleaching occurs by splitting these long chains into colourless, diffusible molecules (Attin *et al*, 2003, Dahl and

Pallesen, 2003, Suleiman, 2004, Kihn, 2007) or oxidizing some chemical components in the chromophores (Joiner, 2006). This occurs via free radicals released from hydrogen peroxide which are strong oxidizing agents and include hydroxyl, perhydroxyl anions (Dahl and Pallesen, 2003, Joiner, 2006) superoxide anions, reactive oxygen and hydrogen peroxide anions (Dahl and Pallesen, 2003). These smaller molecules result in a lightening effect of the teeth because they reflect less light (Suleiman, 2004, Kihn, 2007). In alkaline conditions, perhydroxyl anions generally carry out the bleaching process, while the free radicals are more important in neutral and acidic conditions (Joiner, 2006).

$$H_2O_2 \longrightarrow 2HO^{\cdot} \text{ (hydroxyl radicals)}$$

$$HO^{\cdot} + H_2O_2 \longrightarrow H_2O + HO_2^{\cdot} \text{ (perhydroxyl radicals)}$$

$$HO_2^{\cdot} \longleftrightarrow H^+ + O_2^{\cdot} \text{ (superoxide anions)}$$

$$2H_2O_2 \longleftrightarrow 2H_2O + 2\{O\} \longleftrightarrow 2H_2O + O_2$$

(reactive oxygen)

$$H_2O_2 \longleftrightarrow H^+ + HOO^- \text{ (hydrogen peroxide anions)}$$

The cleavage of hydrogen peroxide molecules is accelerated in the presence of heat, light or lasers (Buchalla and Attin, 2007, Kihn, 2007). Heat increases the decomposition of hydrogen peroxide by a factor of 2 for each 10^0C temperature rise (Joiner, 2006, Buchalla and Attin, 2007), but due to the risk of pulpal death, the temperature should not exceed the critical threshold of 5.5^0C (Suleiman, 2005b, Buchalla and Attin, 2007). Light sources on the other hand cause lysis of hydrogen peroxide by two mechanisms (Buchalla and Attin, 2007). First, the light is absorbed by the bleaching agents and some of the energy is converted to heat (Buchalla and Attin, 2007). Secondly, they can cause decomposition of hydrogen peroxide directly by excitation of the whitening agent, photolysis (Joiner, 2006, Buchalla and Attin, 2007). Colourants may be mixed into the formulations of the bleaching agents to increase the absorption of light (Joiner, 2006, Buchalla and Attin, 2007). For example, carotene raises the absorption of blue light while small silica particles, raise the absorption of infra-red light (Buchalla and Attin, 2007).

2. 3. 3 Bleaching Methods

Different methods are used to bleach vital and non-vital teeth and the success of the procedures is influenced by the type of stain (Haywood, 1992, Suleiman, 2005a, Joiner, 2006), the ability of

the whitening agent to access the stain and the number of times it is in contact with the stain (Dahl and Pallesen, 2003).

2. 3. 3. 1 Vital Tooth Bleaching

There are various methods available for the whitening of vital teeth, which vary in type and concentration of the bleaching agent, mode of application, duration and method of activation (Joiner, 2006, Kihn, 2007). Three basic methods are currently used; night-guard or at-home bleaching, in-office or power bleaching and bleaching by means of over-the-counter (OTC) products (Dahl and Pallesen, 2003, Joiner, 2006, Kihn, 2007).

2. 3. 3. 1. 1 Night Guard Vital Bleaching (NGVB)

This method of bleaching renewed the interest of the population in tooth whitening (Haywood, 1992). It was found to be cheaper and safer than the modes employed before 1989 (Haywood, 1992, Suleiman, 2005a). Now, a larger section of the population was able to access this appearance enhancing technique (Haywood, 1992, Burrell, 1997). It is currently the most popular bleaching method available (Perdigao *et al*, 2004, Christensen, 2005, Suleiman, 2005a) and is highly successful (Haywood, 1992, Perdigao *et al*, 2004, Suleiman, 2005a, Kihn, 2007).

Night guard vital bleaching generally utilizes 10% carbamide peroxide, which is equivalent to about 3% hydrogen peroxide (Haywood, 1992). However, products are available with concentrations of carbamide peroxide ranging from 5% to as high as 36% and hydrogen peroxide from 6% to 15% (Kihn, 2007). This is a dentist-prescribed/home applied technique where the patient wears a custom-fabricated tray filled with the bleaching agent for a period of time (Haywood, 1992, Perdigao *et al*, 2004, Suleiman, 2005a, Joiner, 2006, Kihn, 2007). Some advocate for twice daily use for 30 minutes for up to 6 weeks (Kihn, 2007) while others for overnight use for 2 or 3 weeks (Haywood, 1992, Suleiman, 2005a, Joiner, 2006).

The dental staff is able to monitor the bleaching process, thereby preventing over whitening of the teeth. However, there are times when patients buy carbamide or hydrogen peroxide from shops and continue to bleach their teeth after the process has been discontinued by the dental professional resulting in unnaturally white teeth with surrounding restorations now appearing too dark (Christensen, 2005). Nightguard vital bleaching requires compliance from the patient for

optimal results to be achieved and this is often seen as a disadvantage (Suleiman, 2005a, Kihn, 2007).

2. 3. 3. 1. 2 In-office Bleaching

This method of bleaching is popular with patients who desire quick results, lack compliance for the at-home remedies (Shethri *et al*, 2003, Perdigao *et al*, 2004, Buchalla and Attin, 2007, Kihn, 2007) or simply require one discolored tooth to be whitened (Goldstein, 1997, Sarrett, 2002, Suleiman, 2005b, Buchalla and Attin, 2007). High concentrations of the whitening agents are used (Sarrett, 2002, Dahl and Pallesen, 2003, Suleiman, 2005b, Joiner, 2006, Buchalla and Attin, 2007, Kihn, 2007) ranging from 17% to 50% hydrogen peroxide (Suleiman, 2005b). It therefore necessitates the use of rubber dam isolation or paint-on gingival barriers to protect the soft tissues (Sarrett, 2002, Perdigao *et al*, 2004, Suleiman, 2005b, Joiner, 2006, Buchalla and Attin, 2007, Kihn, 2007).

In-office bleaching, as the name suggests is carried out in the dentist's clinic. It is time consuming because rarely are satisfactory results obtained in just one appointment. The patient has to undergo several appointments with two or more applications of the bleach per session (Perdigao *et al*, 2004, Suleiman, 2005b, Joiner, 2006, Buchalla and Attin, 2007, Kihn, 2007). It is also a costly procedure (Suleiman, 2005b, Kihn, 2007). To reduce the chair time used, and optimize on the final result, in-office bleaching may be used to initiate the whitening process, followed by the patient using nightguard vital bleaching for a few days (Goldstein, 1997, Shethri *et al*, 2003, Suleiman, 2005b, Buchalla and Attin, 2007, Kihn, 2007).

The bleaching process may be accelerated by heat, light or laser (Suleiman, 2005b, Joiner, 2006, Buchalla and Attin, 2007, Kihn, 2007). Light sources include plasma arc light emitting diodes, argon lasers and xenon-halogen lamps (Suleiman, 2005b, Buchalla and Attin, 2007, Kihn, 2007). This has not been found to significantly increase the rate of hydrogen peroxide decomposition due to the fact that only a limited rise in temperature is permitted, so as to spare the pulp from permanent damage (Perdigao *et al*, 2004, Joiner, 2006, Buchalla and Attin, 2007). The clinical studies on the benefits of light activation have been controversial and further testing is required to give a firm statement on whether or not this form of accelerating in-office bleaching is at all necessary (Joiner, 2006, Buchalla and Attin, 2007, Kihn, 2007).

Recently, chemically activated in-office bleaching agents have become available (Goldstein, 1997, Perdigao *et al*, 2004, Suleiman, 2005b). The chemical catalyst is added to the hydrogen peroxide just prior to its use resulting in rapid bleaching. No other form of activation is necessary with these systems (Perdigao *et al*, 2004, Suleiman, 2005b).

Ultrasonically activated bleaching systems have been introduced in the UK, utilizing 6% – 7.5% hydrogen peroxide. It is a quick procedure which only requires two cycles of 5 minutes each to achieve the whitening effect. It is thought that the ultrasonic energy results in the production of more free radicals (Suleiman, 2005b)

2. 3. 3. 1. 3 Over-the-counter Bleaching Agents (OTC)

Whitening strips were introduced in 2000, and like nightguard vital bleaching agents, were widely available to the public. They are self-prescribed, easy to use systems (Gerlach, 2004). Hydrogen peroxide gel is delivered and held against the teeth by flexible polyethylene strips (Sarrett, 2002, Gerlach, 2004, Kihn, 2007). They took the world by storm and within just a few months of production, the distribution of Crest White strips, one of the commercially available products, sold millions of kits (Gerlach, 2004). The concentration of the hydrogen peroxide gel is as low as 5.3% and 6.5% in the original strips (Gerlach, 2004, Perdigao *et al*, 2004), compared to the newer products which contain 14% (Gerlach, 2004). Two whitening strips are used each day, for 30minutes each, over a period of 2 to 3 weeks (Gerlach, 2004, Perdigao *et al*, 2004, Joiner, 2006).The latter products are said to result in faster, safer and more efficient whitening than other professionally prescribed whitening agents with similar or higher concentrations of hydrogen peroxide (Gerlach, 2004).

Little research has been carried out on these products therefore their long term side effects are yet to be established (Sarrett, 2002, Kihn, 2007). They tend to be abused by the population with over-bleaching being the final result at the best (Christensen, 2005) and bleaching undiagnosed pathologies such as caries, at the worst (Sarrett, 2002). There have been reports of damage to enamel with long term use of these products (Sarrett, 2002).

Whitening kits that are supplied with prefabricated trays are also available. These may present problems of ill-fitting trays. Bleaching results with the use of these trays have not been consistent (Kihn, 2007).

2. 3. 3. 1. 4 Other Methods

Other methods of whitening teeth include dentifrices which contain mild abrasives and some also have low concentrations of hydrogen peroxide added (Sarrett, 2002, Kihn, 2007), and paint-on liquids (Perdigao *et al*, 2004, Kihn, 2007). The toothpastes basically remove surface stains from teeth and cannot be used to treat intrinsic discolourations (Sarrett, 2002). The paint-on liquids are currently available as 18% carbamide peroxide or 19% sodium percarbonate systems. As with whitening strips, supporting clinical studies are few, but have so far shown them to be effective (Perdigao *et al*, 2004).

2. 3. 3. 2 Non-vital Tooth Bleaching

Discoloured teeth to be bleached using the non-vital bleaching technique have to be well obturated and have healthy periodontal tissues, to prevent the bleaching material from leaking into the peri-apical region (Attin *et al*, 2003, Dahl and Pallesen, 2003, Suleiman, 2005a, Tredwin *et al*, 2006). Three techniques have been described; the walking bleach, the thermocatalytic and the "inside-outside" techniques (Suleiman, 2005a).

2. 3. 3. 2. 1 Walking Bleach Technique

Either 30% hydrogen peroxide on its own or mixed with sodium perborate or water may be used (Friedman, 1997, Attin *et al*, 2003, Suleiman, 2005a). The bleaching agent is sealed into the pulp chamber and left *in-situ* for up to 2 weeks. The whitening process is repeated until the tooth is satisfactorily whitened (Friedman, 1997, Sarrett, 2002, Attin *et al*, 2003, Dahl and Pallesen, 2003, Suleiman, 2005a, Tredwin *et al*, 2006), but, if no appreciable change is noticed after 3 appointments, another method needs to be employed to treat the discoloured tooth (Dahl and Pallesen, 2003).

2. 3. 3. 2. 2 Thermocatalytic Technique

In this method, 30% to 35% hydrogen peroxide gel is used, with the application of light (Suleiman, 2005a) or heat to accelerate the cleavage of the hydrogen peroxide (Friedman, 1997, Suleiman, 2005a). In between visits, the walking bleach technique is employed (Friedman, 1997, Suleiman, 2005a).

2. 3. 3. 2. 3 "Inside-outside" Technique

Liebenberg described a modification of the walking bleach technique (Liebenberg, 1997, Dahl and Pallesen, 2003) where the patient uses 10% carbamide peroxide in a prepared pulp chamber, which they change on their own every 2 hours until the desired results are obtained. A splint keeps the whitening agent in place in the intracoronal access cavity, and it is also loaded with the bleach for extracoronal whitening (Liebenberg, 1997, Suleiman, 2005a). The access cavity stays open during the treatment (Liebenberg, 1997, Suleiman, 2005a, Dahl and Pallesen, 2003) and the patient is advised not to chew on the tooth until the end of the treatment (Liebenberg, 1997, Suleiman, 2005a). In as much as this method reduces both the concentration and duration of the treatment, it carries the risk of endodontic failure, ingestion of bleaching material and additional discolouration from foods and drinks ingested (Dahl and Pallesen, 2003). Patient compliance and manual dexterity is called upon for this treatment to be successful (Suleiman, 2005a).

The long-term efficacy of bleaching non-vital teeth is low, and after a few years, the procedure has to be repeated and more so when sodium perborate is used instead of hydrogen peroxide (Friedman, 1997).

2. 3. 4 Adverse Effects of Bleaching

Tooth sensitivity, gingival irritation (Dahl and Pallesen, 2003, Suleiman, 2005a, Tredwin *et al*, 2006) and changes to enamel surfaces all occur with vital tooth bleaching, with tooth sensitivity occurring commonly. These adverse reactions are especially apparent with high concentrations of hydrogen and carbamide peroxide (Dahl and Pallesen, 2003, Tredwin *et al*, 2006). It has been suggested that patients with pre-existing tooth sensitivity are more likely to suffer from this side effect (Dahl and Pallesen, 2003) and should be treated with a desensitizing agent prior to bleaching (Suleiman, 2005a). In-office power bleaching is especially implicated in tooth sensitivity (Dahl and Pallesen, 2003, Tredwin *et al*, 2006). It is thought that bleaching agents penetrate enamel and dentine to reach the pulp (Dahl and Pallesen, 2003, Tredwin *et al*, 2006). Patients can undergo treatment for a couple of weeks by wearing a splint with fluoride, prior to bleaching to minimize sensitivity (Suleiman, 2004). Soft tissues should always be protected during the whitening process even when low concentrations of the bleaching agent are used. Whitening strips may therefore not be the best option in this regard (Dahl and Pallesen, 2003).

These symptoms have however been shown to be transient, and disappear when the treatment is discontinued or concluded (Dahl and Pallesen, 2003, Suleiman, 2005a).

The surface hardness and fracture toughness of dentine and enamel has been shown to be reduced, as well as changes in their histological and chemical composition, following bleaching, but these changes are not clinically significant (Attin *et al*, 2004, Joiner, 2007) and have been attributed more to the pH of the whitening agent, than from the agent itself (Suleiman, 2004).

Some patients have reported a metallic taste in the mouth in the morning after using the night guard vital bleaching technique, but this taste disappears 2 hours after removing the trays from the mouth (Suleiman, 2005a).

The use of 30% hydrogen peroxide with the thermocatalytic technique is discouraged because cervical root resorption is a possible adverse effect (Friedman, 1997, Attin *et al*, 2003, Dahl and Pallesen, 2003, Tredwin *et al*, 2006). As such, a sodium perborate and water mixture is preferred and in severe cases of discolouration (Friedman, 1997, Attin *et al*, 2003, Dahl and Pallesen, 2003), 3% hydrogen peroxide may replace the water (Attin *et al*, 2003). The lightening effect with 30% hydrogen peroxide or sodium perborate mixed with 3% hydrogen peroxide or water seems to produce the same results after bleaching (Dahl and Pallesen, 2003, Tredwin *et al*, 2006). Liebenberg also suggested application of catalase to the bleached surfaces immediately after bleaching, rendering the whitening agent inactive, thereby minimizing the risk of cervical root resorption (Liebenberg, 1997).

2. 3. 5 Effects of Bleaching Agents on Restorations

Several *in-vitro* studies have been carried out on the effects of whitening agents on the properties of composite resin restorations. However, results have been conflicting as pertains to surface hardness. Some of the studies show a decrease (Okte *et al*, 2006) and others no change after bleaching (Turker and Biskin, 2003, Schemehorn *et al*, 2004). Surface porosities were shown to be increased after application of bleaching agents (Turker and Biskin, 2003). Bond strength between enamel and composite resins is reduced in the period immediately after bleaching. It is thought to be caused by inhibition of polymerization of the resin by remnants of oxygen in the tooth structure (Spyrides *et al*, 2000, Lai *et al*, 2002, El-din *et al*, 2006). The tags formed in bleached enamel after acid etching are shallow and lack definition (El-din *et al*, 2006). It is recommended that definitive resin based restorations are not placed immediately after bleaching

(Dahl and Pallesen, 2003, Tredwin *et al*, 2006). The change of colour of resin restorations after bleaching will be discussed later in the review.

Bonding of glass ionomers to bleached dentine is also compromised immediately after the whitening process (Spyrides *et al*, 2000). It has also been suggested that bleaching agents dissolve glass ionomer and other cements (Swift et al, 1999) and increase their surface roughness (Turker and Biskin, 2003).

Mercury and silver have been shown to be released from amalgam during bleaching, and this increases with higher concentrations of the whitening agent (Rotstein *et al,* 2000). Oxidation of the material by bleaching agents has been implicated in the "greening" seen at the margins of amalgam restorations after tooth whitening (Haywood, 2002).

2. 4 COMPOSITE RESINS

Composite resins are the most aesthetic direct restorative material currently available (Bayne, 2000, ADA Council on Scientific Affairs, 2003). They have greatly evolved since they were introduced in the 1940s, to not only be used on anterior teeth, but on all classes of cavities (Bayne, 2000, ADA Council on Scientific Affairs, 2003, Garcia *et al*, 2006). Composite resins are versatile materials that now even include packable and flowable composites, for just about every clinical indication (Garcia *et al*, 2006, LeSage, 2007).

2. 4. 1 History

Composite resins were developed in the 1940s to replace the only tooth coloured materials in use then, the silicates and acrylic resins (Anusavice, 2003, Garcia *et al*, 2006). Both these materials underwent significant polymerization shrinkage and had high coefficient of thermal expansion (Anusavice, 2003). Rafael Bowen, in the early 1960s developed the new composite resins with bisphenol A-glycidyl methacrylate (bis-GMA) as the monomer (Anusavice, 2003, Garcia *et al*, 2006). This remains the basis of composite resins to date (Leinfelder, 2001) and it improved on the drawbacks of acrylic resins (Anusavice, 2003, Garcia *et al*, 2006). Since then, composite resins have undergone great improvements (Anusavice, 2003, ADA Council on Scientific Affairs, 2003, Garcia *et al*, 2006) with the production of diverse materials which can be used for a variety of therapeutic situations (Garcia *et al*, 2006). Initially, the composite resins available were chemically cured but in the 1970s, light cured composite resins became available

commercially, thereby improving on the colour stability and eliminating the shortcomings of hand-mixed materials such as proportioning (Garcia *et al*, 2006). During the same period, the world started shifting towards composite restorations, even in the posterior region, as the safety of amalgam continued to be questioned and patients' preference for more aesthetic materials increased (Anusavice, 2003, ADA Council on Scientific Affairs, 2003). By the late 1990s, the number of composite resin restorations placed in the posterior region was greater than amalgam restorations (ADA Council on Scientific Affairs, 2003). Composite resins have been said to be the material most likely to replace amalgam in the future as a restorative material (Bayne, 2000).

2. 4. 2 Composition

Composite resins are basically made up of three main components; the organic matrix, inorganic fillers and silane coupling agents (Anusavice, 2003, ADA Council on Scientific Affairs, 2003, Garcia *et al*, 2006).

2. 4. 2. 1 Organic Matrix

The organic matrix consists of the monomer bis-GMA, with or without urethane dimethacrylate (UDMA) (Anusavice, 2003, Garcia *et al*, 2006, LeSage, 2007). Its aromatic structure increases the compressive strength and stiffness of the material and at the same time reduces the absorption of water (LeSage, 2007). This resin is highly viscous, therefore low molecular weight monomers such as triethylene glycol dimethacrylate (TEGDMA) are added as diluents (Anusavice, 2003, Garcia *et al*, 2006). Their low molecular weight increases polymerization shrinkage of the composite, so they must be used with care (Anusavice, 2003).

An activator-initiator system is also found within the organic matrix, which releases free radicals that facilitate the polymerization reaction (Anusavice, 2003, Garcia *et al*, 2006). Light cured composite resins have camphoroquinone as the initiator using blue light of about 470nm wavelength to release free radicals. Self cured composite resins on the other hand contain an aromatic tertiary amine for this purpose (LeSage, 2007).

An inhibitor within the organic matrix prevents auto-polymerization of the material when it is in storage (Anusavice, 2003, Garcia *et al*, 2006) and ultra-violet light absorbers are included in the organic phase to stabilize the colour of the set restoration over time (Garcia *et al*, 2006).

2. 4. 2. 2 Inorganic Fillers

The filler component is responsible for the physical and mechanical properties of the composite resin. A higher filler load is desirable as this reduces polymerization shrinkage and the coefficient of thermal expansion and improves the aesthetics and strength of the restoration (ADA Council on Scientific Affairs, 2003, Garcia *et al*, 2006). Filler particles are produced by grinding quartz or glass (Anusavice, 2003), and they range in size from 25nm to 100 μm (Anusavice, 2003, Garcia *et al*, 2006). Composites are classified according to the size of the primary filler particles they contain (ADA Council on Scientific Affairs, 2003, Garcia *et al*, 2006), which determine the properties of the materials (Anusavice, 2003, ADA Council on Scientific Affairs, 2003, Garcia *et al*, 2006). There are macrofilled, microfilled, hybrid and nanofilled composites. The majority of composites are hybrid composites and these can be used both anteriorly and posteriorly as restorative materials (Garcia *et al*, 2006). Hybrids using optimal size particles were later developed that have better finish than the conventional hybrids and are as highly filled (Wakefield and Kofford, 2001). Recently, nanocomposites were introduced which give excellent aesthetics and maintain the glossiness of the restorations over time. They also have high filler loads, and are therefore strong and wear away less than hybrids. These are also used in both the anterior and posterior regions of the mouth (Mitra *et al*, 2003, Sadowsky, 2006).

The composite resin used in this study is Filtek Supreme XT. It is a nanofilled material. “Nano” is adopted from the Greek work meaning dwarf (Saravana and Vijayalakshmi, 2006) hence nanotechnology or molecular engineering is the production of materials using tiny structures ranging between 0.1 and 100 nanometers (Mitra *et al*, 2003). This is equivalent to a billionth of a meter (Mitra *et al*, 2003, Saravana and Vijayalakshmi, 2006) or a thousandth of a micrometer (Mitra *et al*, 2003). This technology is currently creating waves in science (Mitra *et al*, 2003, Saravana and Vijayalakshmi, 2006) resulting in materials that are much improved in electrical, chemical, mechanical and optical characteristics (Mitra *et al*, 2003).

Filtek supreme XT was manufactured to combine the aesthetics of microfilled composites, with the strength and durability of hybrid composites (Mitra *et al*, 2003). It was introduced to the market in October 2002 (Baseren, 2004) and contains silica nanoparticles ranging between 5 and 20 nm and loosely bound zirconia/silica nanoclusters measuring 0.6 to 1.4 μm (Baseren, 2004,

Villalta *et al*, 2006). The filler loading of Filtek Supreme XT is 78.5% by weight (Baseren, 2004) due to the packing of nanoparticles between the nanoclusters (3M ESPE, Filtek Supreme Technical Product Profile, 2005). This was supposed to be a truly universal composite restorative material, which at the time no material fulfilled (Mitra *et al*, 2003). The manufacture of its filler particles uses the bottom-up approach, a move away from the top-down technique that was previously used with other composite resins (Mitra *et al*, 2003, Saravana and Vijayalakshmi, 2006). Traditionally, composite resin fillers are produced from larger particles that are milled to smaller ones. This process cannot produce particles of less than 100nm (Mitra *et al*, 2003). Filtek Supreme XT starts off with synthetically manufactured nanofillers of 25nm for the regular shades and 75nm for the translucent shades (3M ESPE, Filtek Supreme Technical Product Profile, 2005) as shown in the scanning electron micrograph in figure 2.1.

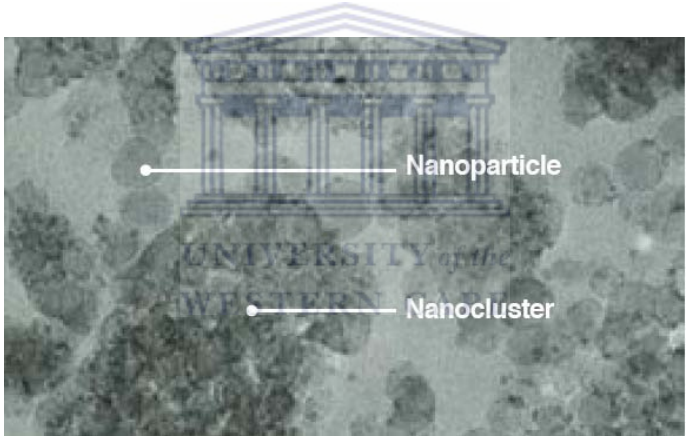


Figure 2.1: Nano-sized filler particles in Filtek Supreme XT (3M ESPE, Filtek Supreme Technical Product Profile, 2005).

This produces a highly aesthetic restorative material which lacks adequate handling and physical properties. 3M ESPE then introduced an innovative way to maximize on their strength and aesthetics by aggregating these particles into nanoclusters (Mitra *et al*, 2003, 3M ESPE, Filtek Supreme Technical Product Profile, 2005, Saravana and Vijayalakshmi, 2006). The nanoparticles fill the interstitial spaces between the nanoclusters, resulting in a highly filled material (Mitra *et al*, 2003, Attar, 2007).

Its organic matrix consists of bis-GMA, bis-EMA (bisphenol A polyethylene glycol diether dimethacrylate), UDMA and small amounts of TEGDMA to reduce the viscosity of the resin (3M ESPE, Filtek Supreme Technical Product Profile, 2005, Villalta *et al*, 2006).

One of the great advantages of Filtek Supreme XT is that it achieves and maintains a high polish. This is enabled by the mode of wear that this restorative material undergoes; individual nanoparticles of 25 nm or less break off their surfaces and not the larger nanoclusters (Mitra *et al*, 2003, 3M ESPE, Filtek Supreme Technical Product Profile, 2005). This results in defects that are smaller than the wavelength of light, hence cannot be detected by the human eye (Mitra *et al*, 2003). It is said to have flexural, compressive and diametral strength and fracture toughness comparable to hybrids due to its high filler loading (Mitra *et al*, 2003, 3M ESPE, Filtek Supreme Technical Product Profile, 2005) and has good handling characteristics (Mitra *et al*, 2003). The nanoparticles are small enough to allow light to go through them resulting in highly translucent materials and when combined with nanoclusters, become opaque resins. They therefore come in a wide range of shades (Mitra *et al*, 2003).

2. 4. 2. 3 Coupling Agents

Coupling agents are used to bond the organic and inorganic phases of composite resins (Anusavice, 2003, Garcia *et al*, 2006, LeSage, 2007). They are mainly organosilanes which coat the filler particles (Anusavice, 2003). The silane group at one end forms an ionic bond with the filler and a methacrylate group at the other end bonds covalently with the organic resin (Anusavice, 2003, Garcia *et al*, 2006). Coupling agents transmit the stresses from the matrix to the filler particles (Anusavice, 2003).

2. 4. 3 Discolouration of Composites

The major developments that have gone into composite resins and bonding systems have resulted in highly aesthetic restorations, but their success has been hampered by discolouration (Patel *et al*, 2004, Villalta *et al*, 2006, Sarac *et al*, 2006), which remains one of the main reasons for their replacement (Buchalla *et al*, 2002, Schulze *et al,* 2003, Villalta *et al*, 2006). Discolouration of composites can be caused by intrinsic and extrinsic factors (Schulze *et al,* 2003, Patel *et al*, 2005, Villalta *et al*, 2006).

2. 4. 3. 1 Intrinsic Staining

Intrinsic staining occurs as a result of changes within the restoration itself (Villalta *et al*, 2006). It is the more important of the two main types of staining because it involves all the layers of the restoration and cannot be eliminated by polishing as most extrinsic stains can (Kolbeck *et al*, 2006). These colour changes could be due to the type of monomer and other constituents of the composite resin, the filler content, degree of polymerization and the effect of moisture and heat on the materials (Buchalla *et al*, 2002, Patel *et al*, 2004, Villalta *et al*, 2006, Sarafianou *et al*, 2007).

Composite resins, in the oral environment absorb water which is thought to occur via the matrix-filler interface (Buchalla *et al*, 2002, Vichi *et al*, 2004, Patel *et al*, 2004, Villalta *et al*, 2006). This causes darkening of these restorations over time (Buchalla *et al*, 2002, Patel *et al*, 2004, Villalta *et al*, 2006, Sarafianou *et al*, 2007), due probably to the difference in the scattering of light as a result of degradation of the silane-coupling agent (Vichi *et al*, 2004, Sarafianou *et al*, 2007). This process has been suggested to be the carrier for extrinsic stains from chromogenic foods and beverages into the restorations (Schulze *et al*, 2003, Bagheri *et al*, 2005). Water too is responsible for the leaching of monomers possibly also resulting in colour changes (Okte *et al*, 2006, Villalta *et al*, 2006, Sarafianou *et al*, 2007). The resins that include hydrophilic monomers such as TEGDMA show higher water sorption with greater colour changes in the restorations, compared to those that do not contain hydrophilic monomers (Imazato *et al*, 1999, Bagheri *et al*, 2005, Sarafianou *et al*, 2007). A 0 to 1% increase of TEGDMA in bis-GMA based composite resins, increases the water sorption by 3 to 6% (Bagheri *et al,* 2005). UDMA is less hydrophilic than both TEGDMA and bis-GMA, therefore products containing more UDMA are less susceptible to discolouration by extrinsic stains than those that do not (Turkun and Turkun, 2004, Bagheri *et al,* 2005). It has also been shown that higher temperatures cause more rapid diffusion of water through the composite resins (Imazato *et al*, 1999, Vichi *et al*, 2004, Sarafianou *et al*, 2007).

Surface roughness of composite resins increases in wet environments due to the leaching of monomers leaving filler particles standing proud (Schulze *et al*, 2003, Villalta *et al*, 2006). This reduces the glossiness of restorations with time, due to the scattering of light (Schulze *et al*, 2003). The size of the filler particle that a composite resin contains is thought to affect the

stability of its optical properties. Microfilled and nanofilled composite resins are highly polishable and are able to maintain these smooth surfaces over time because small individual filler particles are plucked from their surfaces during wear, resulting in defects that are smaller than the wavelength of light (Mitra *et al*, 2003). It has been suggested that microfilled composites have greater matrix-filler interface area and should therefore discolour more than hybrids, but the opposite was found to be the case by Buchalla *et al* (2002). Vichi *et al* (2004) also found this to be true and concluded that composite resins with larger filler particle size were more susceptible to water sorption, and hence, optical changes.

Composite resins tend to yellow over time and camphoroquinone, the photoinitiator, has been implicated (Seghi *et al,* 1990, Kolbeck *et al*, 2006, Sarafianou *et al*, 2007). Seghi *et al* (1990) and Buchalla *et al* (2002) found that there was a shift in the chroma of composite resins after curing, when the initiator, camphoroquinone, is depleted and is therefore no longer absorbing blue light, but reflecting it. This is accompanied by either an increase or decrease in translucency of the restorations (Lee *et al*, 2005). Ferracane *et al* (1985) showed that ultra-violet irradiation caused yellowing of composite resins. They thought that the chemical activator, initiator, inhibitor and monomer of self-cured composite resins all played a role in this colour change. However, they found that composites yellowed even when they were polymerized under intense heat, without the addition of activators and initiators, suggesting that the resin itself was more important in colour stability than the other materials (Ferracane *et al*, 1985). They hypothesized that vinyl groups within the organic matrix are oxidized giving off coloured peroxides (Ferracane *et al*, 1985, Kolbeck *et al*, 2006). The tertiary aromatic amines, which are the accelerators found in the organic matrix (Schulze *et al*, 2003, Sarafianou *et al*, 2007), too have been implicated in discolouration of composite resins. They are thought to give off photo-reactive chromatic by-products when composite resins are exposed to heat or light (Sarafianou *et al*, 2007). Several other researchers have tried to explain this yellowing of composite resins when exposed to UV light, with some suggesting that residual free radicals from camphoroquinone are remobilized (Kolbeck *et al*, 2006, Sarafianou *et al*, 2007), while others postulating that chemical bonds are broken in UV-light (Kolbeck *et al*, 2006). Kolbeck *et al* (2006) thought unreacted camphoroquinone gave resin-based composites their yellow colour over time. It is notable that all the above theories name the organic matrix as responsible for the colour changes in the composite resins (Kolbeck *et al*, 2006).

The degree of conversion of monomer to polymer is important to the colour stability of composite resins as shown by the higher level of discoloration of self-cured resins compared to light polymerized resins (Schulze *et al*, 2003). This may also be brought about by inadequate curing due to insufficient light output from a curing unit, reduced curing time or depth of cure (Usumez *et al,* 2005). This determines the amount of unreacted components that remain in the resin post-cure (Kolbeck *et al*, 2006). It is said, that composite resins containing 35% and more of unreacted carbon-carbon double bonds, are prone to colour changes (Sarafianou *et al*, 2007) probably by their oxidation within in the polymer, resulting in coloured peroxides (Ferracane *et al*, 1985, Imazato *et al*, 1999, Buchalla *et al*, 2002, Sarafianou *et al*, 2007). Physical properties, including water sorption and colour stability are affected by inadequate curing (Usumez *et al,* 2005). The varied degree of polymerization is a possible explanation for the difference in colour change found by Usumez *et al* (2005) after curing composite resins with different types of curing units and storing them.

2. 4. 3. 2 Extrinsic Staining

Extrinsic staining occurs as a result of adsorption or absorption of chromogenic foods, beverages and other substances taken into the mouth (Villalta *et al*, 2006). Intake of chromogenic beverages such as tea and coffee, as well as habits like tobacco smoking, cause staining of resin-based composites through the matrix-filler interface during the water sorptive process (Patel *et al*, 2004, Villalta *et al*, 2006). Adsorption of stains occurs on the surface of composite resins (Turkun and Turkun, 2004) via the accumulation of chromogens in the salivary pellicle and plaque (Omata *et al*, 2006).

Procedures such as finishing and polishing also affect the colour of composite restorations (Patel *et al*, 2004, Lee *et al*, 2005, Bagheri *et al*, 2005, Sarac *et al*, 2006) and their stainability (Patel *et al*, 2004, Bagheri *et al*, 2005, Sarac *et al*, 2006). Rough surfaces lead to plaque accumulation, secondary caries and staining (Patel *et al*, 2004, Bagheri *et al*, 2005, Sarac *et al*, 2006). This is especially true of composite resins that have larger filler particles. Polishing may result in loss of the resin matrix leaving the larger filler particles protruding from the surface (Bagheri *et al*, 2005, Sarac *et al*, 2006). Composite resins with high gloss finishes are more resistant to extrinsic staining (Mitra *et al*, 2003, Patel *et al*, 2004, Sarac *et al*, 2006).

Composite resins which polymerize under polyester strips have been shown to produce the smoothest finishes, hence lowest surface roughness (Baseren, 2004, Patel *et al*, 2004, Bagheri *et al*, 2005, Sarac *et al*, 2006, Attar, 2007). The oxygen inhibited layer found on the surfaces of resins that have not been cured under polyester strips result in an incompletely polymerized surface layer of resin (Rueggeberg and Margeson, 1990). Its presence on the surface of the composite resins as well as in porosities enhances extrinsic discolouration of the material (Schulze *et al*, 2003). Matrix strips eliminate this layer, resulting in a higher degree of polymerization (Rueggeberg and Margeson, 1990). However, Patel *et al* (2004), in a study on the stainability of filled and unfilled resin based composites, showed the greatest colour change was found in composite resins that had been polymerized under polyester strips, compared to those finished by other means. They thought that even though the oxygen inhibited layer was eliminated, the degree of polymerization beneath the polyester strip was still less than that in the body of the composite resin. They suggested that restorations polymerized under these strips therefore needed to be polished by other means, for longevity and maintenance of aesthetics (Patel *et al*, 2004). Other investigators have also found that composite resins finished off with polyester strips were more susceptible to staining by chromogenic foods and beverages than those finished off by other means and thought the rich resin layer found on their surfaces was responsible for their increased stainability (Setcos *et al*, 1999, Bagheri *et al,* 2005, Okte *et al*, 2006).

Glazes have been used to cover composite resins, giving them a glossy finish. These are unfilled resins that fill-in the defects on the surface of composite restorations created after finishing and polishing procedures (Doray *et al*, 2003, Sarac *et al*, 2006). They serve the duo purpose of increasing resistance of the restoration to abrasion and as well as staining (Sarac *et al*, 2006). However, they have been shown to be removed after tooth brushing, thereby not fulfilling their intended purposes (Doray *et al*, 2003). Recently, a surface sealant for covering composite resin restorations has been introduced. This is a light-cured liquid polish system that polymerizes without leaving an oxygen inhibited layer at the surface (Attar, 2007). It has been shown to confer on the surface of the composite restoration, a surface with Mylar strip-like smoothness (Attar, 2007).

2. 3. 3. 3 Internalized Staining

Discolouration of composite resins may also occur by incorporation of stains into the substance of the material (Turkun and Turkun, 2004, Kolbeck *et al*, 2006, Lee and Powers, 2007). Degradation or changes in the components of composite resins may facilitate this process (Schulze *et al*, 2003, Turkun and Turkun, 2004). Alcohol, for instance, has been shown to soften the organic matrix of these restorations, rendering them susceptible to discolouration (Patel *et al*, 2004, Bagheri *et al*, 2005). This results in the penetration of chromogens into the sub-surface area of the material (Turkun and Turkun, 2004, Kolbeck *et al*, 2006, Lee and Powers, 2007) and is referred to as internalized staining (Kolbeck *et al*, 2006, Lee and Powers, 2007). Unreacted monomer or initiators may readily take up hydrophilic colourants from ingested substances resulting in this type of discolouration (Kolbeck *et al*, 2006).

2. 4. 4 Effect of Bleaching Agents on the Colour of Composite Restorations

It has been hypothesized that tooth whitening agents cause colour changes in composite resins through oxidation by free radicals, of the amine compounds in the organic matrix (Monaghan *et al*, 1992a). Kim *et al* (2004) echoed these sentiments, but went on to postulate that the coupling agent may be degraded with a shift of stain accumulation to the matrix-filler interface. Other authors believe that the bleach undergoes a chemical reaction with the unreacted monomer in the composite resin (Monaghan *et al*, 1992a, Buchalla *et al*, 2002, Rosentritt *et al*, 2005). The monomer composition and type and amount of filler, are thought to influence the degree of lightening that bleaching agents may cause in dental composite resins (Monaghan *et al*, 1992a, Rosentritt *et al*, 2005).

Studies on the effects of bleaching systems on the colour of composite resins have been few (Turkan and Turkan, 2004, Rosentritt *et al*, 2005). Monaghan *et al* (1992a) showed, *in-vitro*, that composite resins lighten in shade after simulated in-office vital bleaching accelerated by heat. They suggested that this may be a way to remove stains from these restorations. In another study, they found the colour changes in composite resins produced by a carbamide peroxide home bleaching kit were so small, that they could only be detected using a Minolta chroma meter (Monaghan *et al,* 1992b). Cullen *et al* (1993) studied the effect of 10% carbamide peroxide and 30% hydrogen peroxide on the tensile strength of composite resins. They exposed the samples to the bleaching agents continuously for a week and observed that the samples lightened

considerably with the 30% hydrogen peroxide. These changes could be readily seen with the naked eye. Canay and Cehreli (2003), published results of a study carried out, *in-vitro*, to compare the effect of 10% hydrogen peroxide and 10% carbamide peroxide on composite resins. They showed that 10% hydrogen peroxide lightened all the composite resins tested, and the changes were noticeable, even to the naked eye, but the changes produced by 10% carbamide peroxide were slight, except in the polyacid modified composite resins. Kim *et al* (2004) evaluated the effect of tooth-whitening strips and films on the colour of microfilled and nanofilled resin composites. They found that the colour changes produced were clinically insignificant. Rosentritt *et al*, (2005) studied the effect of different bleaching systems on the colour, hardness and surface roughness of various composite materials. They included bovine enamel in their experiment as it approximated human enamel in composition and structure. The colour difference by all the whitening agents was greatest on the bovine enamel than on the composite resins tested. They also found that the polyacid modified resins as well as the microfilled composite resins showed the greatest colour changes when investigating the influence of different concentrations of hydrogen peroxide in various bleaching systems, on a variety of composite resin materials. All the composite resins tested showed discolouration when a high concentration of hydrogen peroxide was used. This study also showed that the surface roughness of dental composite resins increased after they were subjected to the various bleaching agents (Rosentritt *et al*, 2005). Yalcin and Gurgan (2005) evaluated the effect of 10% carbamide peroxide and 6.5% hydrogen peroxide on the gloss of a flowable and a packable composite resin and an ormocer and found the gloss to be significantly reduced in all tested samples. They observed that the gloss decrease was indirectly proportional with the size of the filler particles in the composite restorations. Fay *et al* (1999) stained tooth coloured restorative material samples with cranberry juice, tea or chlorhexidine and found that tooth whiteners removed extrinsic stains from the composite resins and the hybrid ionomers, but not from the compomers. Turkun and Turkun (2004) exposed composite samples to tea and coffee then polished and bleached them, effectively removing the surface stains. Similarly, Villalta *et al* (2006) also demonstrated that bleaching agents remove stains from the surface of composite resins. They discoloured their samples with coffee and red wine. Villalta *et al* (2006) suggested that whereas bleaching systems effectively whitened teeth, they did not discolour composite resins, beyond removing their exterior stains. These studies have been summarized in table 2.1.

Table 2.1: Summary of Studies on the Effect of Bleaching Agents on the Colour of Composite Resins.

Study	Materials tested	Stain	Bleaching Agent	Method	Lightened
Monaghan *et al*, 1992a	Composites	No	30% hydrogen peroxide	In-office	Yes
Monaghan *et al*, 1992b	Composites	No	Carbamide peroxide	NGVB	Yes
Cullen *et al*, 1993	Composites	No	10% carbamide peroxide 30% hydrogen peroxide	1 week exposure	No Yes
Canay and Cehreli, 2003	Composites Compomers	No	10% hydrogen peroxide 10% carbamide peroxide	NGVB	Yes
Kim *et al*, 2004	Composites	No	3 & 6.5% hydrogen peroxide 18% carbamide peroxide 19% sodium perborate	OTC	Yes
Rosentritt *et al*, 2005	Composites Compomers Ormocers	No	10 – 16% carbamide peroxide 35% hydrogen peroxide	NGVB In-office	Yes
Fay *et al*, 1999	Composite, Ionomer, Compomer	Yes	10% carbamide peroxide	Paint-on	Yes No
Turkun and Turkun, 2004	Composites	Yes	15% hydrogen peroxide	In-office	Yes
Villalta *et al*, 2006	Composites	Yes	16 and 18% hydrogen peroxide 35% hydrogen peroxide	Paint-on In-office	Yes

2. 5 COLOUR AND COLOUR MEASUREMENT

2. 5. 1 Colour Perception

There are about ten million colours that the human eye can detect (Brook *et al*, 2007) and they are perceived when light falls on objects and is reflected to the eye (Derbabian *et al*, 2001, Brewer *et al*, 2004, Joiner, 2004). Natural light is composed of a mixture of component bands and falls between 380 and 770nm wavelength of the electromagnetic spectrum. Objects contain pigments that absorb some parts of the light spectrum and reflect others, thereby producing the sensation of colour (Brewer *et al*, 2004). The retina of the eye contains sensors, rods and cones that respond to light. The cones which are 1 in every 20 of these sensors are responsible for the perception of colour (Brewer *et al*, 2004). They are of three types; those sensitive to the primary colours of light i.e. red, green and blue wavelength and they send signals to the brain for recognition of the colour of objects (Fondriest, 2003, Brewer *et al*, 2004). The different wavelengths are indistinguishable to the eye, but the dominant or an average wavelength is what is then perceived (Fondriest, 2003). The rods, only register lightness of an object (Brewer *et al*, 2004). The perception of colour is dependent on the light source, the object being viewed and the observer's visual system. All these variables may change giving different descriptions of colour for the same object (Joiner, 2004, Khurana *et al*, 2007).

2. 5. 2 Science of Colour

Colour was described in three dimensions by Albert Munsell (Fondriest, 2003). This allowed a colour to be specifically defined in the colour space or coordinate system (Derbabian *et al*, 2001, Brewer *et al*, 2004). The Munsell System's three attributes are value, hue and chroma (Fondriest, 2003, Brewer *et al*, 2004, Joiner, 2004). The value refers to the lightness or brightness of an object (Derbabian *et al*, 2001, Watts and Addy, 2001, Fondriest, 2003, Brewer *et al*, 2004, Joiner, 2004). This is a measure on the grey scale with black at one extreme and white on the other (Fondriest, 2003). An object with a low value is reflecting little light, such that more light is either being absorbed, scattered or transmitted through it (Fondriest, 2003). Value is considered the most important of the three attributes (Derbabian *et al*, 2001, Khurana *et al*, 2007) as far as shade matching in dentistry is concerned and is the only one that can be measured independently (Derbabian *et al*, 2001). Hue distinguishes one family of colours from another (Derbabian *et al*, 2001, Watts and Addy, 2001, Fondriest, 2003, Joiner, 2004). It is the attribute

by which an object's colour is perceived to be red, green or blue (Watts and Addy, 2001, Joiner, 2004). This is associated with a specific wavelength band (Fondriest, 2003, Brewer *et a*l, 2004). Chroma is the saturation or intensity of hue (Derbabian *et al*, 2001, Watts and Addy, 2001, Fondriest, 2003, Brewer *et al*, 2004). As chroma increases, value decreases (Fondriest, 2003). These attributes are shown in figure 2.2.

Figure 2.2: Munsell Colour System (Derbabian *et al*, 2001).

Miller *et al* suggested that translucency/opacity should be added to Munsell's three-dimensional colour (Watts and Addy, 2001).

The Munsell colour space is one system used for colour assessment (Canay and Cehreli, 2003, Joiner, 2004). In 1931, the Commission Internationale de L'Eclairage (CIE) developed a standard light source and observer which enabled colour to be calculated in digits using the three Munsell attributes (Seghi *et al*, 1990, Canay and Cehreli, 2003, Brewer *et al*, 2004, Joiner, 2004). In 1976, colour space was further defined, CIE Lab, which is based on the premise that all colours occurring in nature are blends of red, blue and green (Derbabian *et al*, 2001, Canay and Cehreli, 2003, Brewer *et al*, 2004, Joiner, 2004). This is the most widely used system for colour assessment (Seghi *et al*, 1990, Derbabian *et al*, 2001, Canay and Cehreli, 2003, Brewer *et al*, 2004, Joiner, 2004). The CIE Lab colour system assesses colour and colour changes in dental materials perceived by the human eye, in three coordinates; L* being the value or lightness of an object, the black-white axis, a* measures the redness of an object in the red-green parameter, while b* measures the yellowness of an object in the yellow-blue coordinate. A positive L* value indicates a bright object, while a positive a* signifies a specimen that is more red, and b*, a more yellow one (Monaghan *et al,* 1992b, Seghi *et al*, 1990, Patel *et al*, 2004, Joiner, 2004, Sarac *et al*, 2006). The CIE Lab colour space is shown in figure 2.3.

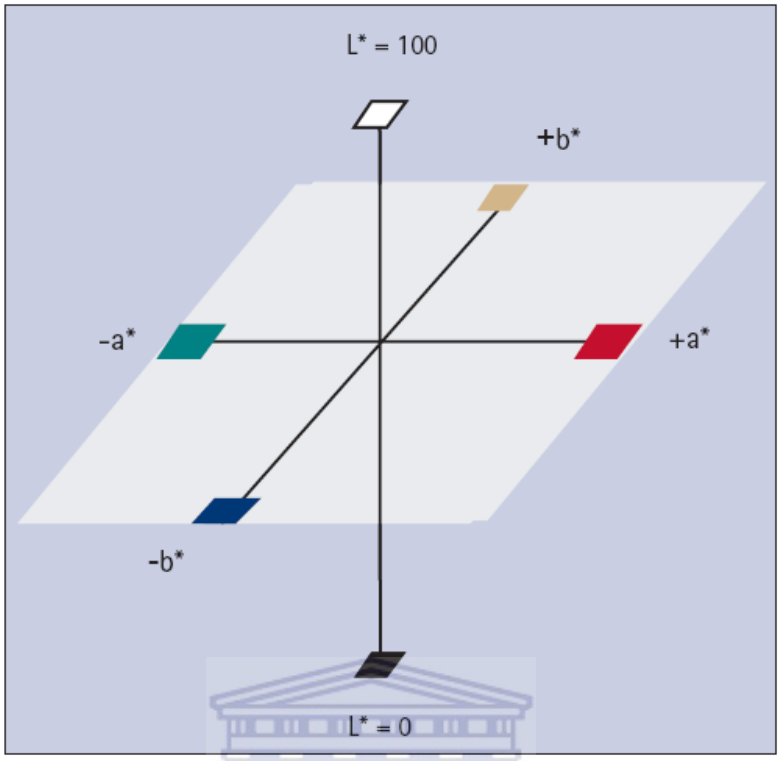


Figure 2.3: The CIE Lab colour space (Jarad *et al*, 2005).

Colour change is denoted as delta E (ΔE) and it expresses the difference between the three coordinates for different objects, or the same object at different times (Sarac *et al*, 2006). Several studies have given ranges of values for which the human eye perceives colour changes; a ΔE value of 0 to 2 units is clinically imperceptible, 2 to 3, just perceptible, 3 to 8, moderately perceptible and greater than 8, markedly perceptible clinically (Sarac *et al*, 2006). Some authors have considered a ΔE of less than 3.3 to be clinically insignificant (Canay and Cehreli, 2003, Kim *et al*, 2004, Guler *et al*, 2005a, Rosentritt *et al*, 2005), while others use a ΔE value of 3.7 as the threshold for clinically acceptable colour changes (Sarac *et al*, 2006).

Other attributes of colour include fluorescence, the absorption of light by an object and its spontaneous emission in a longer wavelength, and opalescence, the ability of an object to appear as one colour when light is reflected from it and another, when light is transmitted through it (Fondriest, 2003).

2. 5. 3 Colour Measurement

Currently, several methods exist for measuring tooth colour and colour changes after bleaching in dentistry, ranging from visual to instrumental assessments (Joiner, 2004, Joiner, 2006, Brook *et al*, 2007).

2. 5. 3. 1 Visual Techniques

2. 5. 3. 1. 1 Shade Guides

This visual method of assessing tooth colour is the most commonly used in dentistry (Joiner, 2004, Joiner, 2006, Brook *et al*, 2007). Shade guides are composed of sets of tooth shaped tabs made either of porcelain (Joiner, 2004, Brook *et al*, 2007) or acrylic (Joiner, 2004) and are supposed to cover the possible range of colour space occupied by natural teeth (Brook *et al*, 2007). Traditional shade guides did not adequately cover the colour space (Brewer *et a*l, 2004, Joiner, 2004, Park *et al*, 2006, Brook *et al*, 2007) as observed by several researchers (Brewer *et a*l, 2004, Brook *et al*, 2007). Furthermore, they were not arranged in a logical manner and covered only 1-dimension of the colour space, namely from light to dark (Brewer *et a*l, 2004, Park *et al*, 2006). More recent shade guides are based on the Munsell colour system (Brewer *et a*l, 2004, Joiner, 2004, Park *et al*, 2006, Brook *et al*, 2007) such as the 3-D Master guide (Brewer *et a*l, 2004, Joiner, 2004, Park *et al*, 2006). It is more reliable in colour measurements since the results are repeatable (Brewer *et a*l, 2004, Joiner, 2004, Park *et al*, 2006).

This method, although subjective, has been employed in several longitudinal tooth whitening studies (Joiner, 2004, Joiner, 2006, Brook *et al*, 2007). It is an easy and quick method to use, but has numerous disadvantages; inter-operator variation exists due to eye fatigue, age, experience, lighting conditions (Joiner, 2004, Joiner, 2006, Brook *et al*, 2007) and physiological conditions such as colour blindness (Joiner, 2004).

2. 5. 3. 1. 2 Stain Indices

These evaluate the staining of teeth and include the Lobene stain index and Murray and Shaw stain index (Macpherson *et al*, 2000, Brook *et al*, 2007). Like the shade guides, they are easy to use and quick but a lot of variability exists between assessors and even with the same assessor at different times. Most of these indices evaluate only the labial and lingual surfaces of the teeth, therefore raising questions to the accuracy of the results (Macpherson *et al*, 2000, Brook *et al*,

2007) because approximal surfaces of a lot of patients pick up extrinsic stains (Macpherson *et al*, 2000). This drawback led Macpherson and others (2000) to develop a stain index that covers the proximal aspect and body of the tooth.

Indices to evaluate intrinsic staining of teeth due to developmental defects also exist and have been used by both researchers and clinicians. However, they are cumbersome and not very accurate (Brook *et al*, 2007).

2. 5. 3. 2 Instrumental Techniques

2. 5. 3. 2. 1 Colourimeters

The first colourimeter to be developed for use in dentistry was the Chromascan in the early 1980s. The demand for this kind of device was initially poor and it proved to be inaccurate and had problems related to its design. Several years later, as the demand for aesthetics increased, newer and more efficient designs were introduced (Brewer *et a*l, 2004). The newer devices measure the colour of objects and express it in terms of CIE Lab colour space (Joiner, 2006).

Colourimeters have filters that correlate with spectral function of the standard observer's eye (Brewer *et a*l, 2004, Joiner, 2004, Khurana *et al*, 2007). Unfortunately, it has not been possible to produce filters that exactly mimic the standard observer functions and they are therefore not 100% accurate (Brewer *et a*l, 2004). Nonetheless, they are precise and quick in measuring colour differences and their performance has been comparable to that of spectrophotometers that are deemed to be superior in colour measurement (Brewer *et a*l, 2004, Joiner, 2004).

Colourimeters must be in contact with the object under study and measure the amount of light reflected off its surface (Brook *et al*, 2007, Khurana *et al*, 2007). They give results using the CIE Lab colour scale (Brook *et al*, 2007). Custom-made jigs should be used to ensure the measuring probe is placed on the same position of the tooth each time, to ensure repeatability (Joiner, 2004).

These instruments have a number of disadvantages; they measure the colour of flat objects and are therefore not useful in assessing colour of tooth surfaces, *in-vivo*, that are mostly curved with surface irregularities (Derbabian *et al*, 2001, Joiner, 2004, Jarad *et al*, 2005, Brook *et al*, 2007, Khurana *et al*, 2007). Small diameter apertures are prone to edge-loss errors, which cause inaccurate readings due to loss of light entering the surface of the object from the margins

(Joiner, 2004, Paravina *et al*, 2007). Only small surfaces can be measured at any one time which does not represent the whole tooth surface (Derbabian *et al*, 2001, Brook *et al*, 2007, Khurana *et al*, 2007). Cross infection is a likely occurrence because these devices must be in contact with the tooth surface while taking the measurement (Brook *et al*, 2007). In addition, the translucency of teeth may also result in inaccurate measurements of tooth colour (Derbabian *et al*, 2001, Brook *et al*, 2007). Due to the difficulty in correcting systematic errors within colourimeters, different instruments give varying readings of the same object. Therefore, comparison of data where more than one colourimeter is used is unreliable (Joiner, 2004). Finally, these instruments are expensive and difficult to use in clinical situations (Brook *et al*, 2007, Khurana *et al*, 2007). In light of these drawbacks, it has been recommended that the use of colourimeters be limited to the detection of small colour differences in objects (Joiner, 2004). Several longitudinal studies measuring the differences of tooth colour during bleaching procedures have utilized colourimeters (Joiner, 2006, Brook *et al*, 2007).

2. 5. 3. 2. 2 Spectrophotometers

Whereas colourimeters use filters to match the standard observer function, spectrophotometers are scanning devices. As such, they are thought to be more accurate than colourimeters (Brewer *et a*l, 2004). They are however similar to colourimeters in most other aspects including their use, mode of readings and shortcomings (Joiner, 2004, Brewer *et al*, 2004). Traditional spectrophotometers have only one photodiode therefore measure one wavelength at a time, being reflected from the object being assessed (Brewer *et a*l, 2004, Joiner, 2004, Khurana *et al*, 2007). The latest designs have incorporated multiple diodes for each wavelength that can then be measured simultaneously. Their main components include a light source, a geometric conditioning system for colour measurement, a light disperser, a detector and software that converts light into signals suitable for assessment (Khurana *et al*, 2007). Spectrophotometers control the external light conditions and give colour measurements using the CIE Lab colour space (Khurana *et al*, 2007). They may be more accurate, but both the traditional and newer designs are slower in measuring colour than colourimeters (Brewer *et a*l, 2004).

Spectrophotometers use standard illuminants when taking colour measurements and these different illuminants affect the results obtained. There are several illuminants that can be used such as Standard illuminant D_{65} which represents daylight (Patel *et al*, 2004, Park *et al*, 2006),

illuminant F2 representing fluorescent light and illuminant A, for incandescent light. Illuminants D_{65} and A are the commonly used illuminants in dentistry (Park *et al*, 2006). Spectrophotometers are very sensitive to both colour changes and surface topography (Lee *et al*, 2002, Sarac *et al*, 2006). They use either one of two geometries; the specular component included (SCI) or the specular component excluded (SCE) (Lee *et al*, 2002, Sarac *et al*, 2006). The condition of the surfaces of materials and teeth determine the amount of light that is scattered or reflected from them (Lee *et al*, 2002). It is difficult to ensure equal surface roughness for teeth or samples being measured (Sarac *et al*, 2006) therefore the SCE geometry, which is more accurate in reflecting irregularities than the SCI geometry, is preferred (Lee *et al*, 2002, Sarac *et al*, 2006).

Spectrophotometers are expensive (Joiner, 2004, Jarad *et al*, 2005, Khurana *et al*, 2007), difficult to use intra-orally (Joiner, 2004, Khurana *et al*, 2007) and require being in contact with objects undergoing measurement (Joiner, 2004, Brook *et al*, 2007). Like colourimeters, they measure flat small surfaces, carry a risk of cross-infection (Brook *et al*, 2007) and their results are affected by the translucency of teeth (Brook *et al*, 2007, Paravina *et al*, 2007). Edge-loss error is also a concern with spectrophotometers (Paravina *et al*, 2007). Both colourimeters and spectrophotometers have been used in longitudinal studies to measure colour changes following tooth bleaching (Brook *et al*, 2007).

2. 5. 3. 2. 3 Digital Imaging Devices

Digital imaging devices are the most recently introduced instruments used for measuring colour in dentistry (Brewer *et a*l, 2004, Jarad *et al*, 2005, Brook *et al*, 2007). These are high resolution digital cameras that are attached to image acquiring software (Brewer *et a*l, 2004, Joiner, 2004, Brook *et al*, 2007). They contain charge-coupled devices with thousands to millions of tiny light sensitive elements which perceive light in its three primary colours (Brewer *et a*l, 2004). Results are given using the CIE Lab colour system or as red, green and blue values (Brook *et al*, 2007). They are being used in shade matching where the images are sent electronically to the dental technician (Jarad *et al*, 2005, Brook *et al*, 2007).

Digital imaging devices have been shown to be more accurate, repeatable and economical compared to spectrophotometers. They do not require contact with objects and have the ability to assess the whole tooth surface (Brook *et al*, 2007). They are easy to use and permanent records of images measured are made (Jarad *et al*, 2005, Brook *et al*, 2007).

2. 6 CONCLUSION

The popularity of tooth whitening as a part of the ever increasing demand for aesthetic dentistry continues to rise unabated (Burrell, 1997). The success rate of vital tooth bleaching is high and it has been declared a safe and non-invasive method of improving the colour of teeth (Suleiman, 2005b, Kihn, 2007). This has been accompanied by the frequent use of composite resins as restorative materials, because of their improved aesthetics (Sadowsky, 2006). Their potential to discolour however mars an otherwise beautiful smile, resulting in frequent replacement of these discoloured restorations (Buchalla *et al*, 2002, Schulze *et al*, 2003, Villalta *et al*, 2006). Most of the studies carried out on the effect of whitening agents on tooth coloured restorative materials show that bleaching systems lighten them and also remove surface stains from the restorations, especially when higher concentrations of hydrogen peroxide are used (Canay and Cehreli, 2003, Rosentritt *et al*, 2005, Villalta *et al*, 2006). The question then, can bleaching be used as a viable method of stain removal from old composite restorations to serve the purposes of restoring aesthetics as well as conserving tooth structure, over a lifetime of a composite resin restoration, is justified.

CHAPTER 3 AIMS AND OBJECTIVES

3. 1 AIM

The aim of this study was to determine whether tooth bleaching agents alter the colour of stained direct composite resins.

3. 2 OBJECTIVES

- To determine the colour after staining of the composite resin specimens.
- To compare the colour after bleaching of the stained samples.

3. 3 NULL HYPOTHESIS

- There is no significant difference in the staining ability of the different agents used in this study.
- There is no significant difference in the colour of the stained composite resins after subjecting them to the bleaching agent.

CHAPTER 4 MATERIALS AND METHODS

4. 1 STUDY DESIGN

This was a quantitative, before-after *in-vitro* study carried out at the Tygerberg Oral Health Research Institute Laboratory.

4. 2 STUDY POPULATION AND SAMPLING

This study was carried out using sixty (60) standardized composite resin specimens. A stratified random sampling method was used to divide the samples into three sub-groups (n = 20), of which two were designated the experimental groups and one the control group.

4. 3 MATERIALS

The materials used in this study are listed in table 4.1.

Table 4.1: Materials Used in the Study.

Material	Function	Company	pH	Lot Number
Filtek supreme XT	Composite Resin	3M ESPE, USA	-	20080403
Tea	Staining Agent	Ketepa Pride, Kenya	5.06	69629720164
Red Wine	Staining Agent	Simonsvlei, Cabernet Sauvignon Merlot, 2006, RSA	3.82	B08019
Artificial Saliva	Control	Tygerberg Oral Health Research Institute Laboratory	-	-
Opalescence Xtra Boost	Bleaching Agent	Ultradent, USA	-	B3J6Z,B33Z5, B379W, B3J6Z

4. 3. 1 Filtek Supreme XT

Filtek Supreme XT is a nanofilled composite resin which is supplied in syringes or capsules. The syringes used in this study are shown in figure 4.1. Body enamel Shade A2, was selected because lighter shades of composite have been shown to discolour more than darker shades (Koksal and Dikbas, 2008).

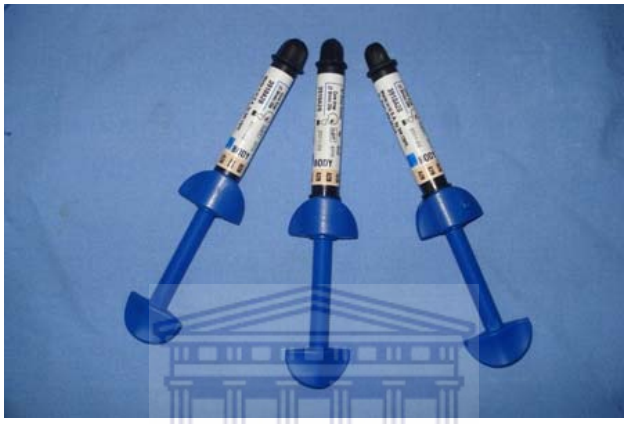


Figure 4.1 Filtek supreme XT.

4. 3. 2 Staining Agents

One of the staining agents was Kenyan tea, Ketepa Pride Ltd. It is 100% very fine granules of tea in filter bags of 2 grams each. It contains vitamin C and E, fluoride, catchins which are anti-oxidants and other flavours. It has no additives, preservatives or artificial colourants. It is made from locally harvested tea leaves and produced by Kenyans, for mainly Kenyans (www.ketepa.com, 24th June 2008). This product is pictured in figure 4.2.

Figure 4.2: Ketepa Pride Tea from Kenya.

A South African red wine was the second staining solution. It was Simonsvlei Cabernet Sauvignon Merlot, 2006. It is made by Simonsvlei International, in CapeTown. This is a medium-bodied, fruity, ruby wine containing 14.43% alcohol (www.ewine.co.za, 24th June 2008). A photograph of the bottle of wine is shown in figure 4.3.

Figure 4.3: Simonsvlei Cabernet Sauvignon Merlot, 2006.

Artificial saliva was the agent used in the control group and was considered under the staining media because it went through all the same tests that tea and red wine did. It was manufactured

at the Tygerberg Oral Health Research Institute Laboratory using the composition that Cipla Medpro, Bellville, South Africa use. Its constituents are shown in table 4.2.

Table 4.2: Composition of Artificial Saliva.

Composition	Concentration (g/l)
Sorbitol	30
Sodium carboxymethylcellulose	10
Potassium chloride	1.2
Sodium chloride	0.844
Potassium dihydrogen phosphate	0.342
Calcium chloride	0.146
Magnesium chloride	0.052

4. 3. 3 Opalescence Xtra Boost

Opalescence Xtra boost is a chemically activated in-office bleaching agent containing 38% hydrogen peroxide. The hydrogen peroxide and the activator are supplied in separate syringes that are only mixed prior to being used. One syringe contains the activator, potassium nitrate and fluoride, while the other has hydrogen peroxide. The syringes are connected and material is pushed from one to the other until it is well mixed, in what the manufacturer calls a syringe-to-syringe jet mixing, before application (Opalescence Xtra Boost Product Profile, 2003). This is shown in figure 4.4.

Figure 4.4: Opalescence Xtra Boost; above, the separate syringes, below, the syringes connected for jet-mixing.

The manufacturer recommends that the resulting gel be left in place for 10 to 15 minutes and be agitated every 5 minutes for good results. If the results obtained are less than satisfactory, this procedure can be repeated for up to 6 cycles in spaced appointments of at least 3 days (Opalescence Xtra Boost Product Profile, 2003).

4. 4 METHODOLOGY

A flow chart of the methodology of this study is shown in figure 4.5.

Figure 4.5: Flowchart of the study.

4. 4. 1 Composite Resin Specimen Preparation

A number of the studies reviewed that tested composite resins, used samples measuring between 9 and 20 mm in diameter. However, the height was constant in all these studies at 2mm so as to cure the material in bulk.

Therefore sixty (60) disc-shaped composite resin specimens were prepared, in a rubber mould measuring 9mm in diameter by 2mm in height shown in figure 4.6. These measurements were confirmed using a digital caliper (Hipex Tools Ltd, China).

Figure 4.6: Rubber mould.

The mould was placed on a glass slab and a transparent polyester strip (Odus universal strips, Odus Technologies, Switzerland) was placed between the mould and the glass slab. Composite resin completely filled the mould. Another polyester strip was then put over the mould, to cover the composite resin and a second glass slab placed over it. The polyester strips were discarded after preparation of each sample. This process is shown in figure 4.7.

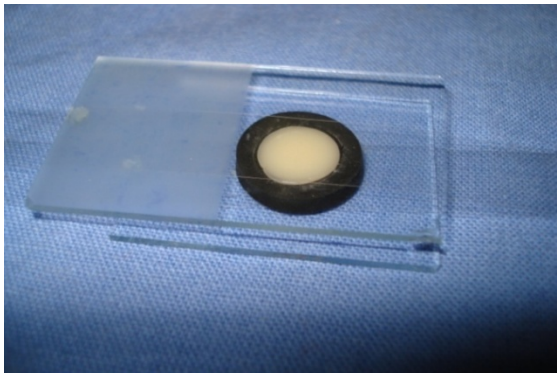


Figure 4.7: Mould with composite sandwiched between 2 glass slabs and 2 polyester strips.

4. 4. 2 Polymerization Procedure

The composite samples were then cured, in bulk i.e. just from one side at a go, using a conventional halogen light polymerizing unit (Optilux 150, Demetron Research Group, Conn., USA) with a light intensity of 440mW/cm^2 for 20 seconds. A photograph of the curing unit is shown in figure 4.8.

Figure 4.8: Optilux 150 Curing light.

The light output was tested using an intensity meter (Cure Rite, Dentsply Caulk, USA) (figure 4.9), before and during the curing process.

Figure 4.9: Visible light intensity meter.

The distance between the curing tip and the composite surface was 1 mm and this was ensured by the presence of the 1 mm upper glass slab placed over the composite resin. The curing tip was in contact with this glass slab during polymerization. The thickness of the glass slab was verified

using digital calipers and only one polymerizing unit was used, to ascertain uniform cure of all the specimens. The curing tip had a diameter of 13 mm that covered the whole composite sample ensuring uniform cure of the whole disc. The curing process is shown in figure 4.10.

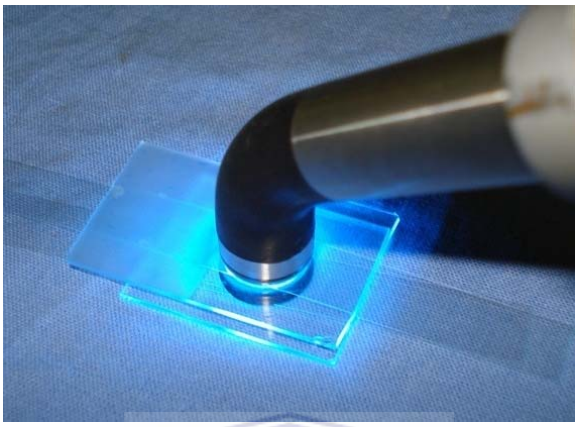


Figure 4.10: The curing process.

The samples were kept dry, at room temperature until all the specimens were prepared. The composite samples were then randomly divided into three sub-groups (n=20). The bottom surface of the samples was marked with the initial C, T or W, depending on which staining agent it would be subjected to, using a waterproof indelible marking pen (Staedtler Permanent, Lumocolor, Germany). Each sample was also numbered from 1 to 20. The top surface was left unmarked and was used for all tests carried out in this study. The specimens were then stored in an incubator (Memmert Schwartbach, Germany), in distilled water for 24 hours, at 37^{o}C, to allow complete polymerization took place and permit the composite resin to imbibe water whose rate is the highest in the first day after polymerization. The specimens are shown in figure 4.11.

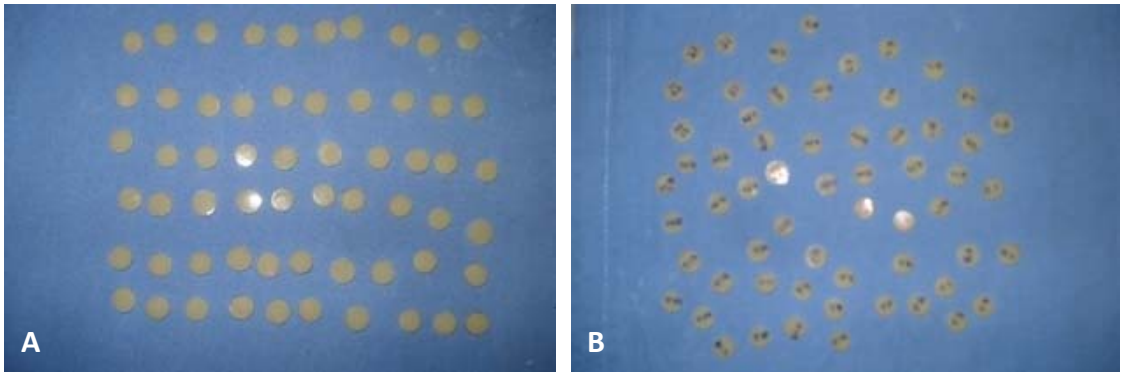


Figure 4.11: A. Top surfaces of the composite resin specimens, **B.** Bottom marked surfaces.

4. 4. 3 Colour Measurement

The colour measurements were taken using the reflectance spectrophotometer (SP CM-2600d Konica Minolta Sensing, Japan) shown in figure 4.12. The aperture of the measuring probe was 8mm and the L*a*b* readings were taken in reflectance mode using standard illuminant D_{65}. The conditions used were S/SCI/100 with a viewing configuration of 10^0. A white background provided by the manufacturer, was used to calibrate the machine each day, before colour measurements were taken. This device measures the colour of objects in numerical terms, and expresses it digitally on its display panel using the CIE Lab colour space.

Figure 4.12: Konica Minolta Sensing spectrophotometer.

The samples were placed in a silicone jig with inner diameter of 9 mm and height of 3 mm. The samples fitted this jig exactly with a 1mm space above them. This is where the spectrophotometer measuring probe was placed; to ensure the same part of all samples was

measured every time, and to eliminate as much external light as possible from falling on the samples, thus influencing the results. This is shown in figure 4.13.

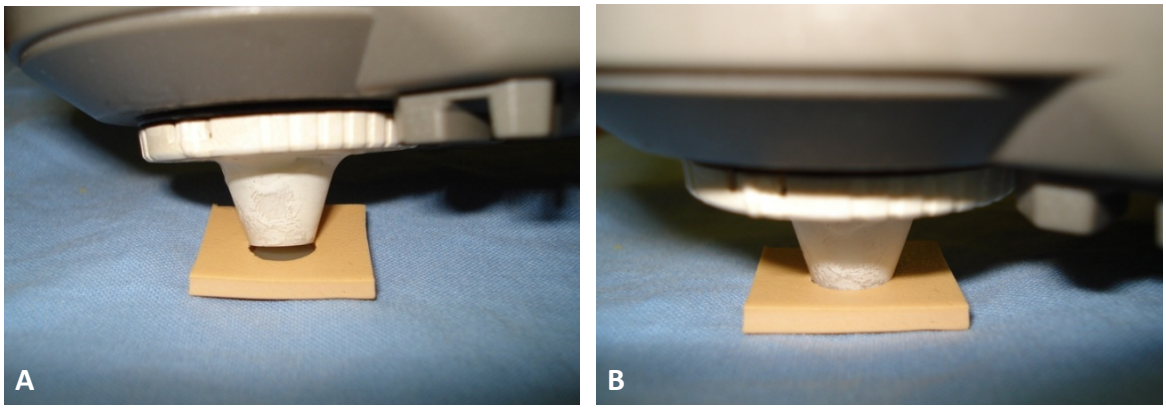


Figure 4.13: Colour measurements. **A.** Measuring probe and sample in silicone jig **B.** Probe fitting in the jig.

Colour change is denoted as ΔE and is calculated with the following formula (Sarac *et al*, 2006):

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

The first colour measurement was taken after the 24 hour incubation period of all the specimens, once they were blotted dry with paper towels. Three readings were taken for each specimen and an average was recorded in an excel spreadsheet. These figures served as the baseline values and were designated time 0.

4. 4. 4 Staining Process

After the 24 hour incubation period, the samples were grouped according to the initial on their lower surfaces. Those marked C served as the control group while the others were the experimental groups; group T and group W. Tea was prepared by immersing one, 2gm teabag into 150 ml of hot distilled water, and boiled for 10 minutes over a Bunsen burner (figure 4.14).

Figure 4.14: Development of the tea stain.

All the samples were completely immersed either in artificial saliva, tea or red wine, respectively for a period of 7 continuous days at 37^{o}C. Each sample was stained in its individual labeled and sealed vial as shown in figure 4.15.

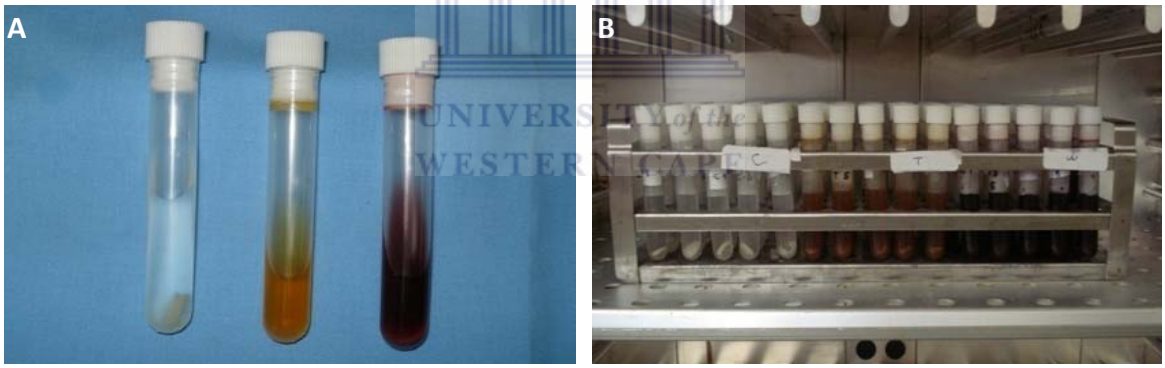


Figure 4.15: A. The specimens in their staining solutions; left, artificial saliva, centre, tea and right, red wine. **B**. The incubated samples during the staining process.

The staining solutions and artificial saliva were renewed every 24 hours; the samples were rinsed under cold running water, blot dried and a colour measurement taken each day to assess the gradient of colour change as the staining process continued. These values were designated 1, 2, 3, 4, 5, 6 and 7 and recorded in the excel spreadsheet. During the staining, the samples were stored in an incubator (Memmert Schwartbach, Germany) at 37^{o}C. The colour change was determined as the difference between one set of values and another. For example, the colour change after staining is the difference between the baseline values and measurements obtained

after staining (day 7) using the equation, where 0 is the baseline colour and 7, the final colour after staining:

$$\Delta E = [(L^*_7 - L^*_0)^2 + (a^*_7 - a^*_0)^2 + (b^*_7 - b^*_0)^2]^{1/2}$$

The samples were then placed in distilled water and stored at 37°C for 24 hours, in an incubator.

4. 4. 5 Bleaching Procedure

All sixty (60) samples were then bleached with Opalescence Xtra Boost. The bleaching agent was mixed according to the manufacturer's instructions. The resulting material was coated onto the top surface of the specimens as shown in figure 4.16. Two 15 minute applications were carried out at room temperature.

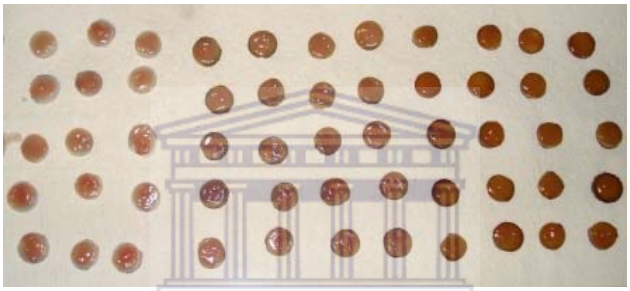


Figure 4.16: The bleaching process.

The bleach was then rinsed off the specimens with running tap water for one minute and blot dried with paper towels in between the 2 applications. A colour measurement was then recorded after 30 minutes of bleaching. This was repeated for 2 more sessions, at weekly intervals giving a total bleaching time of one and a half hours. The specimens were stored in distilled water at 37^{0}C in between sessions. The purpose of repeating the whitening process is based on the clinical situation where most patients undergo multiple applications per session and several appointments of bleaching before the desired results are obtained. At the end of the whitening process, the specimens were placed in distilled water for a week at 37°C, to rehydrate and allow for any rebound effect, before the final colour reading was taken. The readings after the bleaching sessions were designated 8, 9 and 10, and those after the rehydration period, 11. The overall colour change of the specimens could then be calculated as the difference between the final values (11) and the baseline values (0) using the same equation:

$$\Delta E = [(L^*_{11} - L^*_0)^2 + (a^*_{11} - a^*_0)^2 + (b^*_{11} - b^*_0)^2]^{1/2}$$

All values obtained were recorded in an excel spreadsheet and are presented in appendix 1. Appendix 2 shows the changes in the individual L*a*b* and E* for the samples over the experimental period.

4. 5 Data Analysis

The data was analyzed on an excel spreadsheet (Microsoft Office Excel, 2007) where ΔE, means and standard deviations were determined. Graphical methods were used to show the pattern of staining and bleaching. A ΔE value greater than 3.3 units was considered clinically significant (Canay and Cehreli, 2003, Kim *et al*, 2004, Guler *et al*, 2005a, Rosentritt *et al*, 2005). A non-parametric test, the Wilcoxon Signed Rank Sum test was used to analyze the statistical differences in staining and bleaching within and between the groups at various intervals. Paired comparisons which resulted in *p* values below 0.05, were deemed to have differences that are statistically significant while those above this value are not.

CHAPTER 5 RESULTS

The results were tabulated from baseline (day 0) and for each day of the study up to day 11 after the rehydration process. An extract of the type of values in appendix 1 is presented in table 5.1.

Table 5.1: L*, a*, b* values for samples for the tea group (T) from baseline to the 2nd day of staining. ΔE represents the colour change between baseline and each day, while Seq ΔE is the colour change between consecutive days.

	Baseline			Day 1 of staining				Day 2 of staining				
Specimen	L^*_0	a^*_0	b^*_0	L^*_1	a^*_1	b^*_1	**ΔE_1**	L^*_2	a^*_2	b^*_2	**Seq ΔE**	**ΔE_2**
1	60.29	0.32	6.78	56.73	0.81	9.06	4.256	56.83	1.42	12.10	3.102	6.441
2	60.07	0.26	5.92	56.99	0.06	7.28	3.373	55.86	0.22	8.68	1.806	5.034
3	59.82	0.97	7.13	56.98	0.46	7.17	2.886	55.77	0.66	9.12	2.304	4.523
4	59.71	0.53	6.38	57.66	0.19	7.20	2.234	57.14	0.87	9.93	2.861	4.396
5	59.46	0.92	6.82	57.66	0.58	6.91	1.834	57.39	-0.18	6.93	0.807	2.347
6	59.68	0.59	7.59	55.81	1.56	10.54	4.962	55.33	1.06	11.39	1.097	5.795
7	60.53	0.92	7.50	58.07	0.97	8.40	2.620	57.68	0.81	9.80	1.462	3.664
8	59.84	0.24	6.34	57.08	0.39	8.37	3.429	56.09	1.40	11.41	3.353	6.412
9	59.99	0.43	6.62	57.43	0.85	8.65	3.294	54.92	1.30	11.00	3.468	6.756
10	60.16	0.26	6.20	58.11	0.79	8.34	3.010	57.94	0.94	8.71	0.434	3.419
11	59.98	0.57	6.82	58.02	0.46	7.48	2.071	57.52	0.37	8.86	1.471	3.202
12	61.44	1.26	8.19	58.19	1.31	10.51	3.993	56.87	1.12	11.33	1.566	5.547
13	59.88	0.58	6.58	59.19	1.33	7.67	1.492	58.33	0.71	8.32	1.244	2.334
14	60.51	1.23	7.11	58.28	0.77	5.77	2.642	58.09	0.82	7.71	1.950	2.527
15	59.08	0.64	6.42	56.87	0.42	7.13	2.332	56.09	0.55	8.97	2.003	3.931
16	60.32	0.44	6.58	58.41	1.05	8.29	2.635	55.50	0.14	9.09	3.152	5.443
17	60.45	1.16	7.57	57.82	1.17	8.59	2.821	57.38	1.08	9.18	0.741	3.467
18	60.87	0.81	7.16	57.45	0.59	8.24	3.593	57.10	0.56	9.14	0.966	4.266
19	60.64	1.39	7.99	57.88	0.56	8.60	2.946	57.09	1.08	10.37	2.007	4.285
20	60.23	0.48	6.66	57.83	0.45	7.86	2.683	55.83	0.61	9.96	2.904	5.502

The means, standard deviation and *p* values for the effects of staining (difference between day 7 and baseline), the effects of bleaching (difference between day 10 and day 7), the effect of rehydration (difference between day 11 and day 10) and the overall colour change (difference between day 11 and baseline) are listed in table 5.2.

Table 5.2: Mean ΔE, standard deviation (SD) and p values for the three groups at various intervals.

Group	Statistic	ΔE after Staining	ΔE after Bleaching	ΔE after Rehydration	Overall ΔE
Artificial Saliva	Mean	1.040	1.455	0.809	2.306
	SD	0.478	0.753	0.461	0.930
	p	0.009*	0.000*	0.108	0.000*
Kenyan Tea	Mean	7.135	5.518	0.772	2.937
	SD	1.635	1.442	0.355	0.758
	p	0.000*	0.000*	0.062	0.970
Red Wine	Mean	19.349	3.277	1.438	18.852
	SD	1.696	1.194	0.482	2.228
	p	0.000*	0.232	0.970	0.000*

* denotes statistically significant differences.

5. 1 Control Group

Figure 5.1 shows the composite graph of the trend in the colour changes for the control group for the duration of the study. Each point is calculated as the ΔE value between that point and the baseline. The graph of the staining pattern for each of the individual specimens is shown in appendix 3.

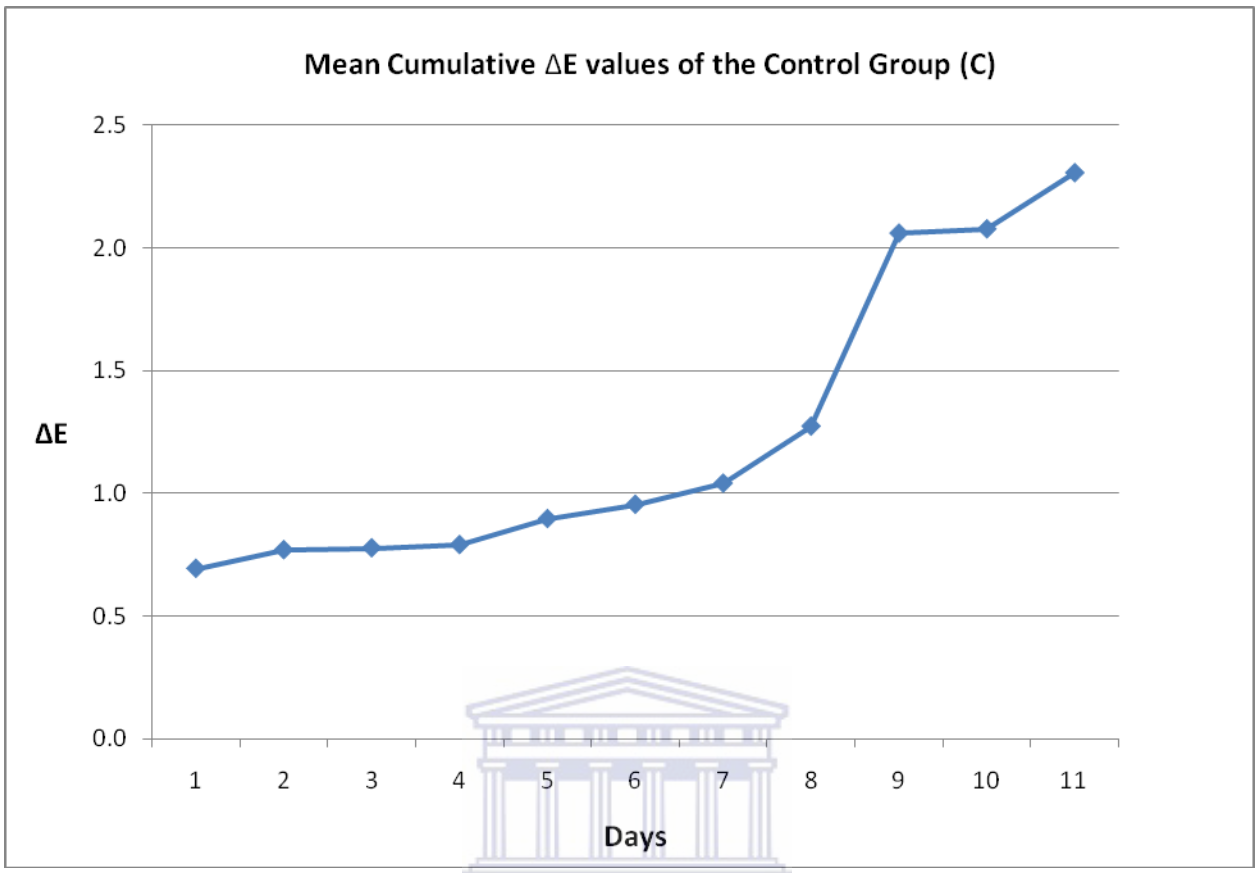


Figure 5.1: Cumulative mean ΔE values from baseline for the control group (C).

Tables 5.3 and 5.4 show the means and standard deviations of the ΔL*, Δa*, Δb* and ΔE values for this group during the staining and bleaching processes. Appendix 2 shows the Δ (L*a*b*) and ΔE* values for each specimen over the duration of the study.

Table 5.3: Mean (standard deviation) values of the cumulative colour changes (ΔL*, Δa*, Δb* and ΔE*) during the 7-day staining period calculated from baseline for the control group (C).

Day	ΔL*	Δa*	Δb*	ΔE*
1	-0.183 (0.435)	0.012 (0. 319)	-0.182 (0.496)	0.693 (0.320)
2	-0.029 (0.492)	0.032 (0.369)	-0.159 (0.625)	0.769 (0.420)
3	0.108 (0.591)	0.115 (0.319)	-0.144 (0.547)	0.776 (0.404)
4	0.117 (0.491)	0.082 (0.323)	-0.431 (0.443)	0.789 (0.321)
5	0.083 (0.594)	0.003 (0.288)	-0.521 (0.554)	0.896 (0.436)
6	0.175 (0.611)	-0.061 (0.275)	0.569 (0.494)	0.954 (0.335)
7	0.165 (0.648)	-0.122 (0.367)	-0.665 (0.550)	1.040 (0.478)

Table 5.4: Mean (standard deviation) values of the cumulative colour changes (ΔL*, Δa*, Δb* and ΔE*) during the 3 bleaching sessions (days 8, 9 and 10) and rehydration period (day 11) calculated from the last day of the staining period (day 7) for the control group (C).

Day	ΔL*	Δa*	Δb*	ΔE*
8	-0.077 (0.550)	-0.089 (0.316)	-0.354 (0.498)	0.775 (0.406)
9	-0.694 (0.509)	-0.442 (0.297)	-1.168 (0.485)	1.471 (0.673)
10	-0.658 (0.516)	-0.425 (0.345)	-1.121 (0.666)	1.455 (0.753)
11	-1.037 (0.667)	-0.508 (0.350)	-1.311 (0.635)	1.822 (0.829)

Figure 5.2 shows a graph of the dispersion of the control group (C).

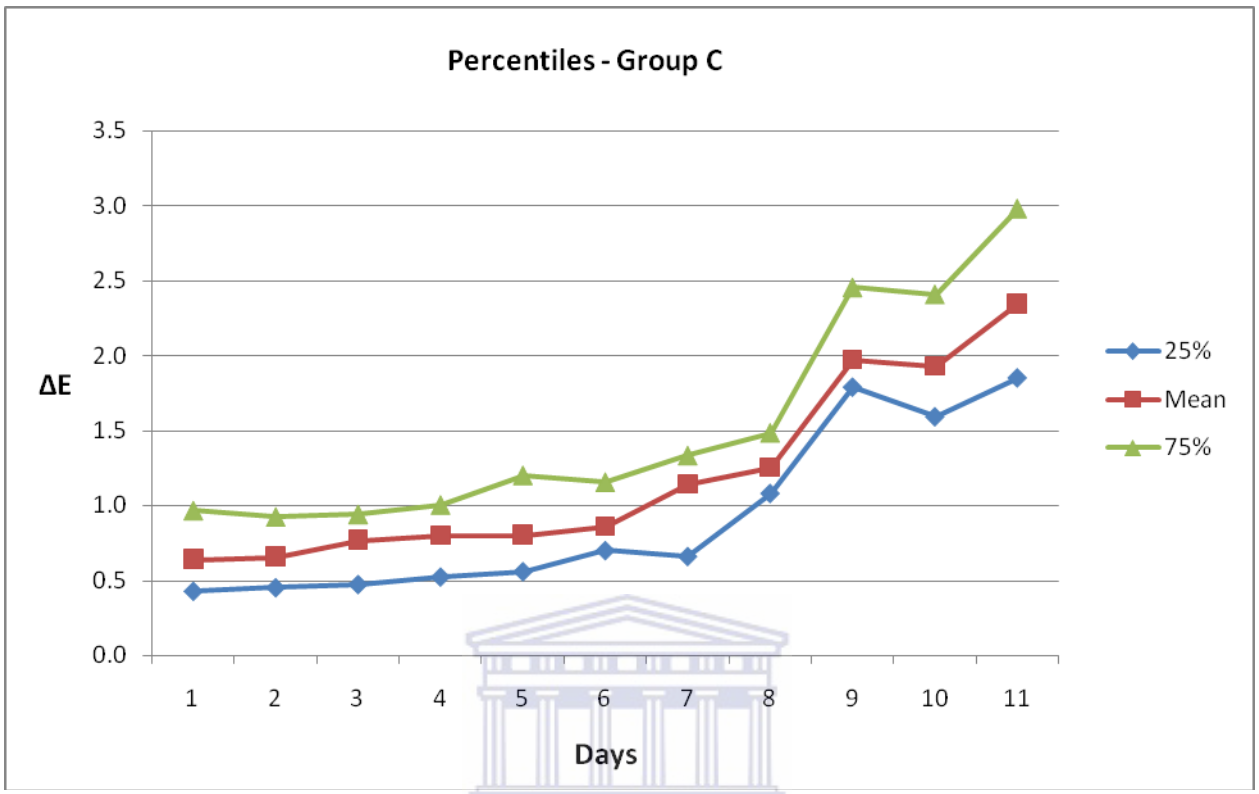


Figure 5.2: Dispersion of the control group over the 11 colour measurements. Day 1 to 7 is the staining process, day 8 to 10, the bleaching period and day 10 and 11, the rehydration period.

5. 2 Tea Group

Figure 5.3 shows graphically, the pattern of discolouration, bleaching and rehydration of the samples in the tea group from the beginning to the end of the study. The graph of the staining pattern for each of the individual specimens is shown in appendix 3.

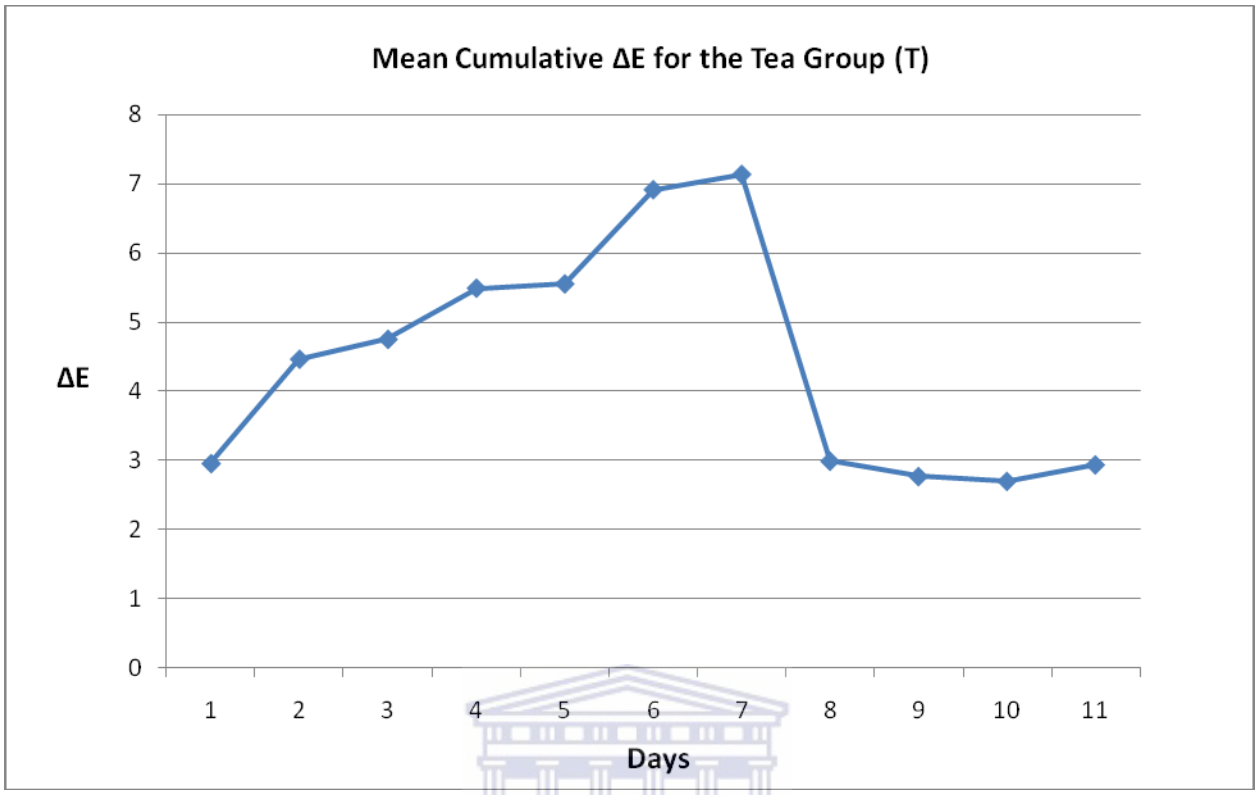


Figure 5.3: Cumulative mean ΔE values from baseline for the tea group (T).

Tables 5.5 and 5.6 list the means and standard deviation of the ΔL*, Δa*, Δb* and ΔE values for Group T for the duration of the study while these values for each individual specimen are shown in appendix 2.

Table 5.5: Mean (standard deviation) values of the cumulative colour changes (ΔL*, Δa*, Δb* and ΔE*) during the 7-day staining period calculated from baseline for the tea group (T).

Day	ΔL*	Δa*	Δb*	ΔE*
1	-2.169 (1.511)	0.039 (0.465)	1.185 (0.979)	2.955 (0.834)
2	-3.410 (1.004)	0.077 (0.554)	2.682 (1.314)	4.464 (1.371)
3	-3.327 (1.110)	0.172 (0.457)	3.299 (1.285)	4.750 (1.573)
4	-3.688 (1.376)	-0.020 (0.448)	3.971 (1.253)	5.490 (1.687)
5	-3.818 (1.471)	-0.218 (0.467)	3.900 (1.394)	5.553 (1.805)
6	-4.754 (1.783)	0.000 (0.490)	4.922 (1.347)	6.914 (2.048)
7	-4.882 (1.564)	-0.032 (0.440)	5.071 (1.209)	7.135 (1.635)

Table 5.6: Mean (standard deviation) values of the cumulative colour changes (ΔL*, Δa*, Δb* and ΔE*) during the 3 bleaching sessions (days 8, 9 and 10) and rehydration period (day 11) calculated from the last day of the staining period (day 7) for the tea group (T).

Day	ΔL*	Δa*	Δb*	ΔE*
8	2.927 (1.561)	-1.336 (0.521)	-3.554 (0.777)	4.930 (1.387)
9	3.058 (1.489)	-1.269 (0.564)	-3.706 (1.027)	5.109 (1.450)
10	2.911 (1.446)	-1.103 (0.546)	-3.942 (1.070)	5.158 (1.442)
11	2.482 (1.338)	-0.978 (0.605)	-3.987 (0.953)	4.936 (1.278)

Figure 5.4 shows the dispersion of the tea group (T).

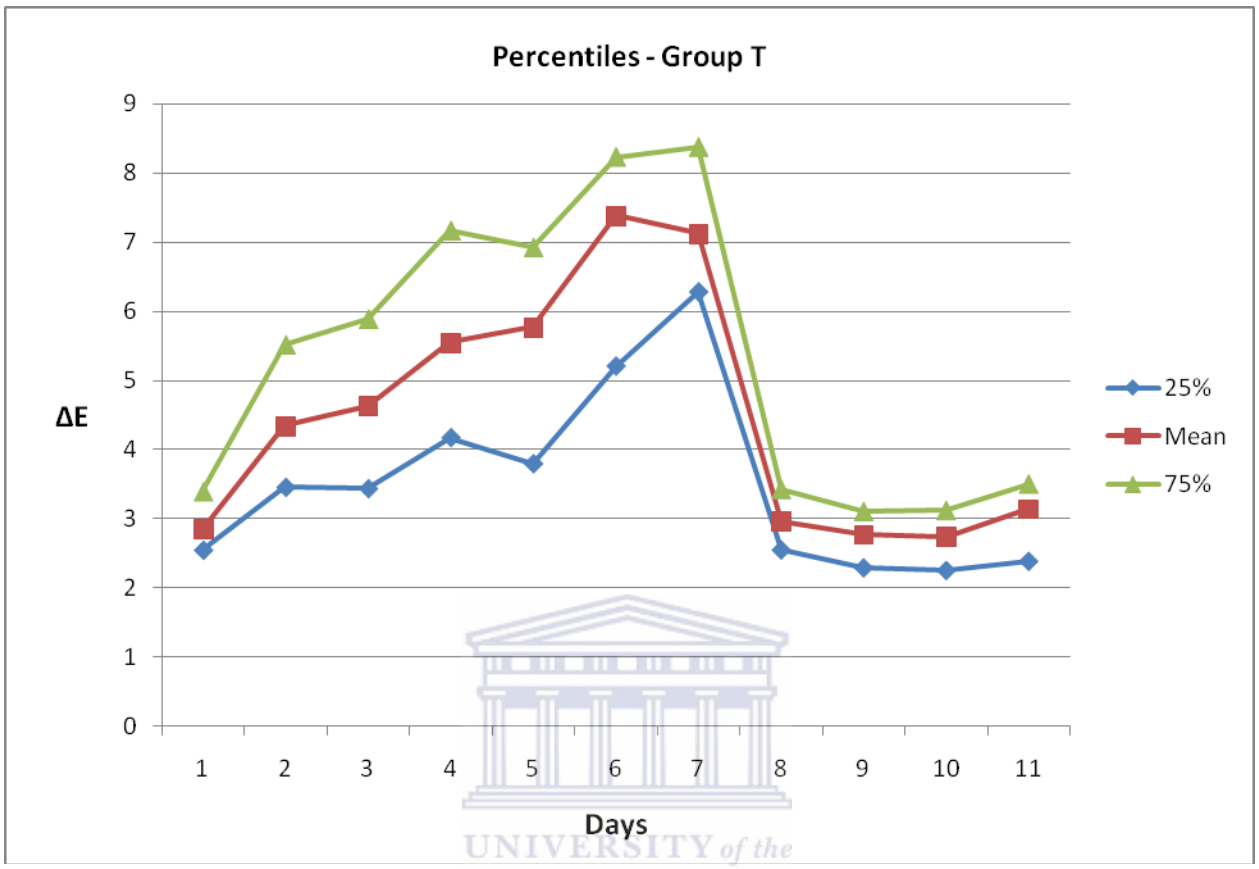


Figure 5.4: Dispersion of the tea group over the 11 colour measurements. Day 1 to 7 is the staining process, day 8 to 10, the bleaching period and day 10 and 11, the rehydration period.

5. 3 Red Wine Group

Figure 5.5 is a composite graph of the trends of the colour changes represented by (ΔE) as they occurred over the study period in the red wine group. The graph of the staining pattern for each of the individual specimens is shown in appendix 3.

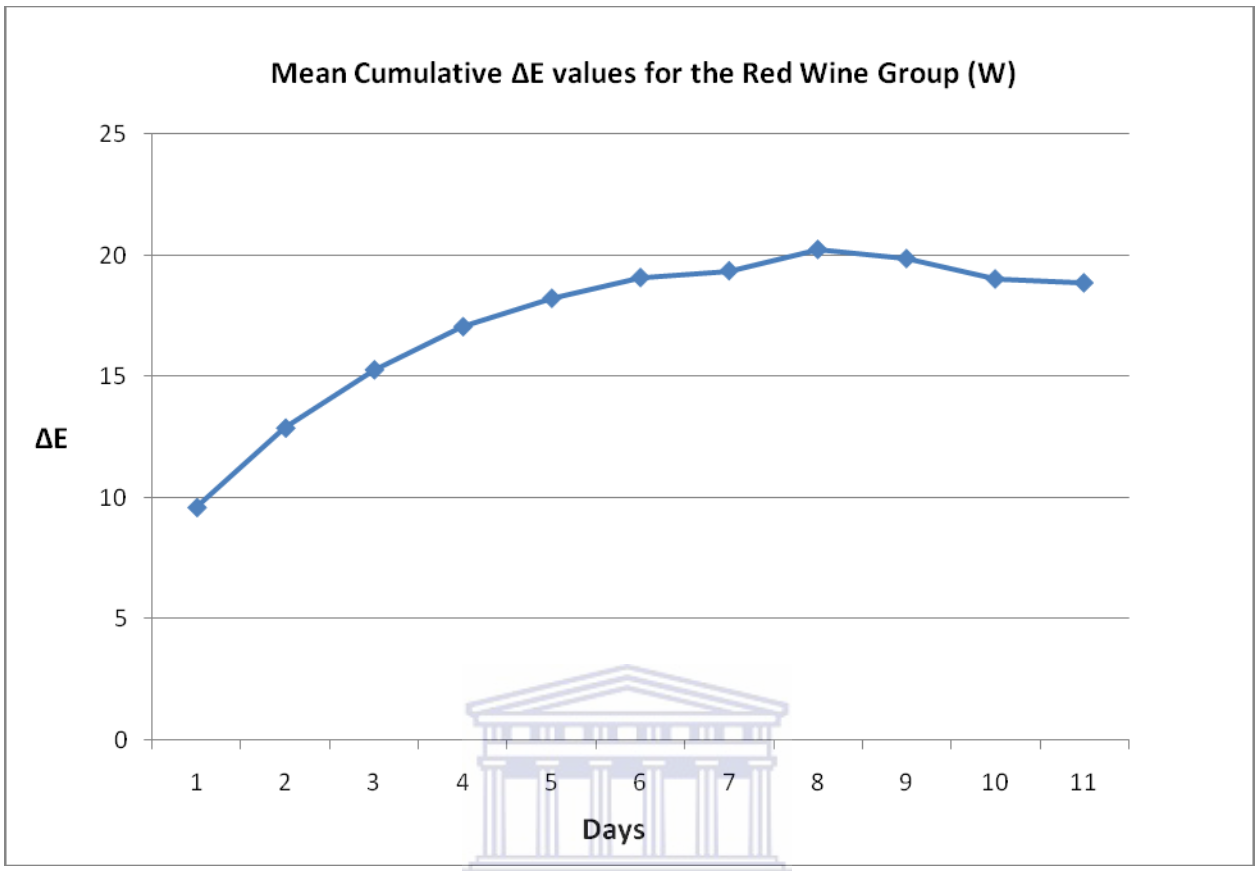


Figure 5.5: Cumulative mean ΔE values from baseline for Group W.

Tables 5.7 and 5.8 list the mean and standard deviation values of the individual ΔL*, Δa*, Δb* and ΔE for the staining and bleaching processes for the red wine group. The Δ (L*a*b*) and ΔE* values for the individual specimens are presented in appendix 2.

Table 5.7: Mean (standard deviation) values of the cumulative colour changes (ΔL*, Δa*, Δb* and ΔE*) during the 7-day staining period calculated from baseline for the red wine group (W).

Day	ΔL*	Δa*	Δb*	ΔE*
1	-4.533 (1.124)	-0.972 (0.541)	8.201 (3.137)	9.588 (2.838)
2	-5.864 (1.291)	-0.627 (0.681)	11.335 (2.792)	12.863 (2.760)
3	-6.507 (1.541)	-0.164 (0.948)	13.700 (2.556)	15.266 (2.575)
4	-7.494 (1.784)	0.547 (1.186)	15.204 (2.080)	17.044 (2.426)
5	-8.264 (2.163)	1.124 (1.488)	16.052 (1.489)	18.214 (2.083)
6	-8.685 (2.098)	1.606 (1.595)	16.751 (1.273)	19.068 (1.817)
7	-9.036 (1.868)	1.915 (1.479)	16.877 (1.295)	19.349 (1.696)

Table 5.8: Mean (standard deviation) values of the cumulative colour changes (ΔL*, Δa*, Δb* and ΔE*) during the 3 treatment sessions (days 8, 9 and 10) with the bleaching agent and rehydration (day 11) calculated from the end of the staining period (day 7) for the red wine group (W).

Day	ΔL*	Δa*	Δb*	ΔE*
8	1.817 (0.785)	-1.221 (0.625)	1.905 (1.616)	3.268 (1.112)
9	2.308 (0.827)	-0.720 (0.680)	1.705 (1.656)	3.342 (1.158)
10	2.486 (1.248)	-0.731 (0.741)	0.876 (1.659)	3.277 (1.194)
11	1.966 (0.921)	0.087 (0.552)	0.431 (1.594)	2.597 (0.929)

Figure 5.6 shows a graph of the dispersion of the red wine group.

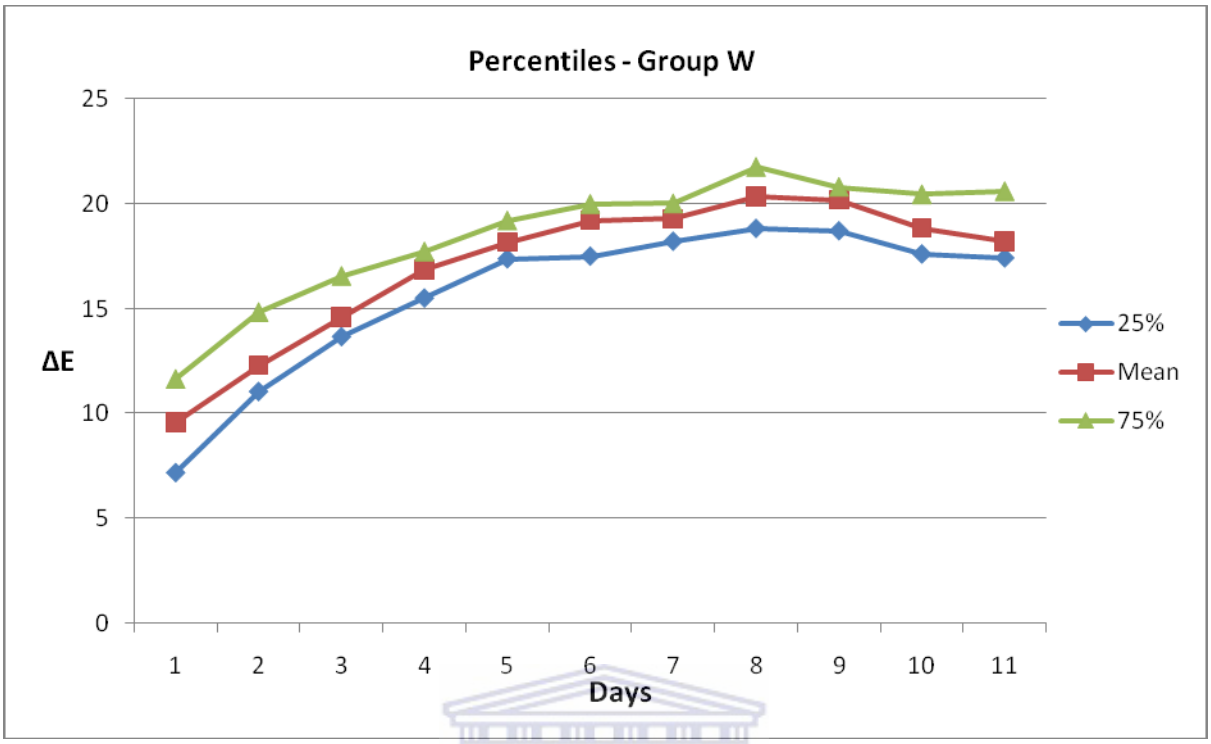


Figure 5.6: Dispersion of the red wine group over the 11 colour measurements. Day 1 to 7 is the staining process, day 8 to 10, the bleaching period and day 10 and 11, the rehydration period.

5. 4 Overall Colour Change

Table 5.9 presents the overall changes of the individual components of the colour space for all the groups at the end of the study.

Table 5.9: Mean (standard deviation) values of the overall colour changes (ΔL*, Δa*, Δb* and ΔE*) at the end of the study for all three groups (difference between day 11 and baseline values).

Group	ΔL*	Δa*	Δb*	ΔE*
Control	-0.925 (0.827)	-0.705 (0.465)	-2.065 (0.790)	2.345 (1.130)
Kenyan Tea	-2.590 (0.680)	-0.975 (0.378)	0.820 (1.553)	3.142 (1.109)
Red Wine	-6.675 (2.458)	1.720 (0.955)	16.940 (2.288)	18.227 (3.162)

5. 5 Inter-Group Comparison

Figure 5.7 shows the composite graph of the cumulative mean changes reflected by a ΔE during the study period for the three groups as calculated from the baseline, while figure 5.8 shows the sequential distances from one colour measurement to the next.

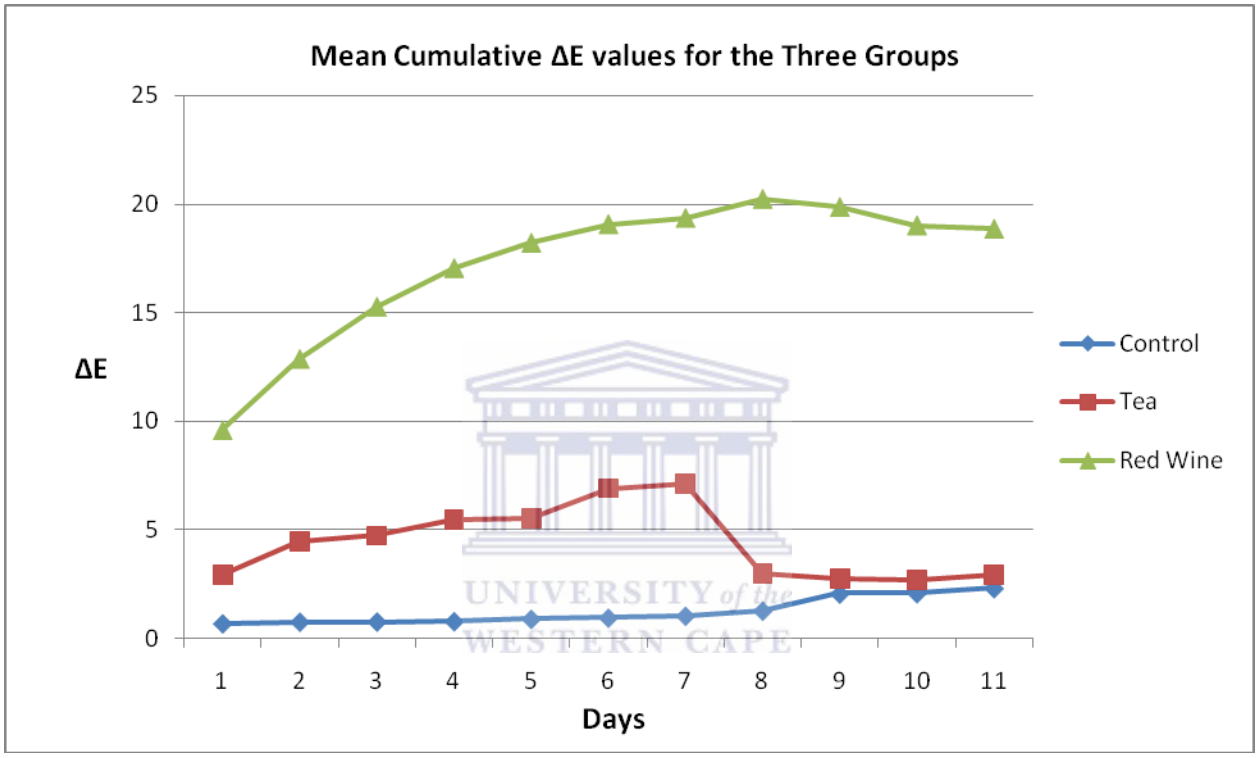


Figure 5.7: Comparison of mean ΔE values from baseline for the three groups.

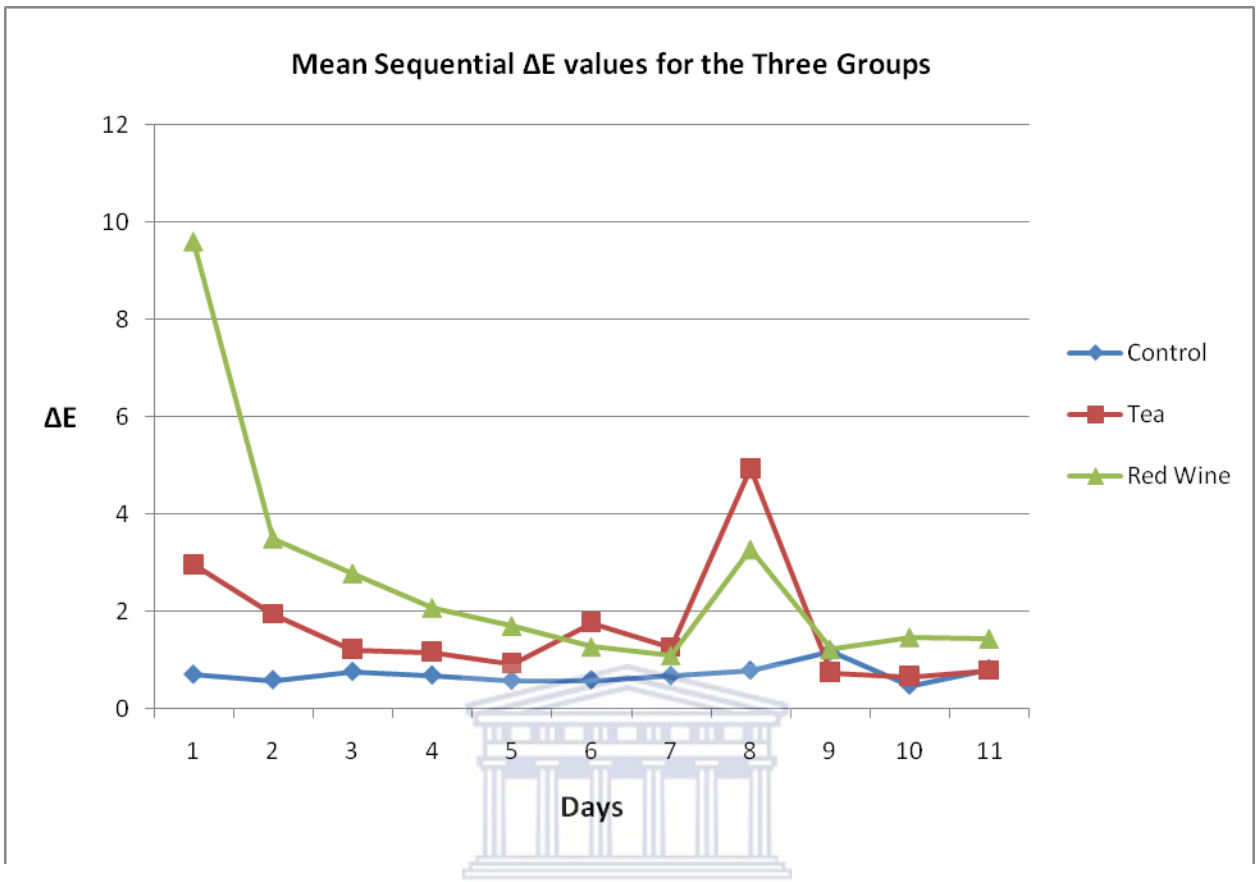


Figure 5.8: Comparison of sequential mean ΔE for the three groups.

CHAPTER 6 DISCUSSION

The discolouration of composite resins is a major cause for concern as aesthetics becomes the focus in the lives of many individuals. Amongst the methods that are used to restore the colour of stained composite resin restorations are abrasive dentifrices and polishing procedures (Turkan and Turkan, 2004). Both modalities involve loss or wear of the restoration (Turkan and Turkan, 2004). Another option lies in replacement of the restoration which is destructive to tooth structure (Elderton, 1996). Bleaching is both a non-invasive and safe way of treating discoloured teeth (Garber, 1997, Sarrett, 2002, Shethri *et al*, 2003, Schmidt and Tatum, 2006) and has a success rate of over 90% (Suleiman, 2005a). This study aimed to determine whether bleaching agents are as successful in altering the colour of stained direct composite resins as they are on teeth.

The samples in the control and the tea group reverted to their baseline colour at the end of the study period but those in the red wine group did not (table 5.2). The mean ΔE observed was 2.306 and 2.937 for the control and the tea group respectively. Statistically, the control group showed a significant difference ($p < 0.5$) even though clinically the changes were imperceptible. The tea group did not show a statistically significant difference ($p > 0.5$) at the end of the study period. After the staining process, the specimens in all three groups had discoloured to some degree reflected by a ΔE ranging from 1.040 to 19.349. The control group showed the smallest ΔE of 1.040 and this was not clinically perceptible as the threshold value is a ΔE of 3.3. However, the difference is statistically significant ($p < 0.5$). The tea and the red wine group also showed statistically significant differences ($p < 0.5$) after the staining period. The colour change was clinically perceptible, with the samples in the red wine group staining more intensely than those in the tea group. The mean ΔE value was 7.135 and 19.349 for the tea and the red wine group respectively and is greater than the threshold value making the colour change clinically perceptible. The bleaching agent had little effect on the samples in the control and the red wine group with a mean ΔE value of less than 3.3 in both groups and therefore, the difference in colour is not clinically perceptible. Statistically, the control group showed significant differences ($p < 0.5$) despite the clinically imperceptible changes in colour. In the red wine group, the difference in colour was not statistically significant ($p > 0.5$) and there was no clinically

perceptible change in colour. This was not the case with the specimens in the tea group which had a mean ΔE value of 5.518 which implied that the difference in colour was clinically evident and also statistically significant ($p < 0.5$). All three groups showed some changes in the ΔE value after the rehydration period. The control group had a mean ΔE value of 0.809 while the tea group had a ΔE value of 0.772. The red wine group showed the greatest ΔE after rehydration compared to the other two groups with a value of 1.438. Statistically, all three groups did not show significant differences ($p > 0.5$) during this period. These figures are represented in table 5.2.

6. 1 Staining process

One of the null hypotheses of this study was rejected as there was a statistically significant difference between the staining ability of the discolouring solutions used. In this study, red wine stained the composite resin samples markedly more than tea as represented by the composite ΔE graph in figure 5.7. The samples stained in red wine had a mean ΔE value of 19.349 at the end of the staining period (day 7) while those stained in tea had a ΔE value of 7.135 at the end of the staining process. The change in colour in both groups was therefore also clinically noticeable. This change in colour of all the specimens in both tea and red wine was also found by other researchers who used various beverages, some of which were also used in this study to stain composite resins (Um and Ruyter, 1991, Fay *et al*, 1999, Turkan and Turkan, 2004, Omata *et al*, 2006, Villalta *et al,* 2006). The b* and L* components of the CIE Lab colour space were most affected by the tea stain with the colour progressively shifting towards yellow, reflected by a Δb* of 5.071 and all the samples darkening with a ΔL* value of -4.882. Movement along the a* axis was minimal throughout the staining period. These findings are represented in table 5.5.

In the red wine group, the samples darkened reflected by a ΔL* value of -9.036 and there was also a considerable shift towards yellow reflected by a Δb* value of 16.877 (table 5.7). This dimension of colour was the most affected by the red wine. Changes along the red-green axis (a*) was mainly towards red but these changes were only minor reflected by a Δa* value of 1.915. Both the L* and b* components stabilized on the 5th day of the 7-day staining process with only small changes observed in these values thereafter. These figures are shown in table 5.7.

Alcohol has been shown to cause softening of the organic matrix and degradation of the surfaces of composite resins (Strober *et al*, 2001, Patel *et al,* 2004, Bagheri *et al*, 2005, Okte *et al,* 2006, Omata *et al*, 2006, Villalta *et al*, 2006) and more so in Bis-GMA and UDMA-based materials

(Okte *et al,* 2006). The resulting rough surface is more prone to pick up extrinsic stains than a smoother surface (Strober *et al*, 2001, Patel *et al,* 2004, Bagheri *et al*, 2005, Villalta *et al*, 2006). The red wine used in this study contained 14.43% alcohol and this may partly explain why it stained the specimens more deeply than tea. The pH of the red wine was 3.82 in this study which was more acidic than the tea which had a pH value of 5.06. Solutions with a low pH value have an adverse effect on the surfaces of composite resins akin to that of alcohol (Strober *et al*, 2001, Bagheri *et al*, 2005, Villalta *et al*, 2006). Therefore, the combination of alcohol content and acidity of the red wine may have caused degradation of the surfaces of the samples, rendering them more susceptible to discolouration than the tea samples.

Both tea and red wine contain polyphenols, but of different kinds and at varying levels, which are responsible for their colouring effect. Polyphenols are by-products of plant metabolism and are involved in plant pigmentation among other functions (Bravo, 1998). Red wine contains tannins in abundance, which are known to cause discolouration of teeth (Macpherson *et al*, 2000) while the colourants found in tea are theoflavins and theorubigins (Bravo, 1998, Suleiman *et al*, 2003). Tannins must have been responsible for the deep discolouration that red wine caused on the composite resin samples. Theoflavins are yellow (Suleiman *et al,* 2003) and are responsible for the positive change in the b* coordinate of the colour space of the samples during the staining process. Theorubigins which are red (Suleiman *et al,* 2003), seemed not to have played a major role in the discolouration of the specimens because the movement along the a* axis was minimal. The different polyphenols found in tea and red wine may play a role in the difference in the staining ability of the two beverages. Omata and others (2006) also found that red wine caused severe discolouration of composite resins compared to tea and coffee, as did Villalta *et al* (2006) when comparing red wine to coffee.

The samples in the experimental groups discoloured the most on the first day of immersion into the staining media reflected by the largest ΔE values for any day of the study (figure 5.8 and appendix 6). This finding is consistent with that found by Um and Ruyter (1991) and Turkan and Turkan (2004).

In this study, the trend in discolouration for the samples in the tea group during the staining period was a general increase in ΔE values as shown in the graph in figure 5.3. The greatest ΔE computed occurred on the first day of the staining period reflected by a ΔE value of 2.955 shown

in the table of sequential colour changes in appendix 5. After this, the samples went on to discolour more deeply, but each day, the colour change from the previous day was less marked until the 5th day when there was a significant increase in the staining intensity reflected by a ΔE of 1.763, compared to the previous days (figure 5.3 and appendix 5). The samples in the red wine group also discoloured the most on the first day of immersion in the staining agent reflected by a ΔE value of 9.588 reflected in appendix 5. The discolouration gradually deepened, but, was less intense with each passing day (figure 5.5 and appendix 5). After the 5th day, the trend in discolouration plateaued out as shown in the graph in figure 5.5.

The samples in the control group, after the staining period showed differences in colour that were clinically insignificant and this was reflected by a ΔE of 1.040. The colour of the samples was almost constant throughout this period with little dispersion of the values (figures 5.1 and 5.2). The presence of the hydrophilic monomers bis-GMA and TEGDMA in Filtek Supreme XT, probably contributed to the slight discolouration observed in the samples in this group. The effects of water sorption on the colour of the composite resin specimens was demonstrated by Imazato *et al* (1999) when the specimens in their control group discoloured over time during storage in distilled water. They tested the water sorption and colour stability of composite resins containing an antibacterial monomer by storing the samples in distilled water at 37^0C and 60^0C. The samples in their control group incubated at 37^0C were markedly discoloured as reflected by a ΔE of almost 7 units in the first week of their experiment which is much higher than that found in this study over the same duration. In the present study, the samples in the control group were stored in artificial saliva during the staining process. The degree of conversion of monomer to polymer during polymerization is also important in the colour stability of composite resins (Schulze *et al*, 2003). Miletic and Santini (2008) showed that the degree of conversion was higher when resin-based composites were stored in artificial saliva than in distilled water. This may have rendered the samples in this study less susceptible to intrinsic colour changes, hence their colour remaining stable at the end of the staining period. Sarafianou *et al*, (2007) found that water sorption in composite resins primarily affected the L* component of the colour space. However, in this study, storage of the samples in artificial saliva produced greater colour changes in the a* axis with a shift towards green, and in the b* coordinate, with movement towards blue than in the L* coordinate. The b* component was the most affected of the three coordinates during the staining period reflected by a Δb* of -0.665 compared to Δa* of -0.122 and ΔL* of

0.165. Higher temperatures also cause a more rapid diffusion of water through composite resins affecting their colour stability (Imazato *et al*, 1999, Vichi *et al*, 2004, Sarafianou *et al*, 2007). The samples in the present study were incubated at 37^0C.

The staining of the samples within the groups was variable, especially in the tea and the control group despite the preparation protocol being kept standard for all specimens. This is reflected in the graphs in appendix 6 of the sequential ΔE values of the individual samples for the duration of the study. The dispersion of the ΔE values in the control group is small. Only on the 5th and 7th days of the staining period did the dispersion increase but not significantly (figure 5.2). There was a wide dispersion of the means during the staining period for the tea group from the 2nd to the 7th day, with a peak in dispersion on day 5 (figure 5.4). These values ranged from 0.834 on day 1 to 2.048 on day 6 as seen from the cumulative ΔE values table in appendix 4. The red wine group did not show as much dispersion of the ΔE values during the staining period as the tea group. This is reflected by a narrow dispersion of ΔE values in this group especially after the 3rd day of staining (figure 5.6). This group also showed the least variability of all three groups in the study. The variability in ΔE values was also a finding made by Strober *et al* (2001) and Lee and Powers (2007) and is probably due to the slight differences in surface morphology of the samples. To achieve identical surfaces between the samples and even from one point of the same sample to another is difficult and the differences are recognized by the spectrophotometer (Sarac *et al*, 2006). Incorporation of porosities may occur, scratches may be found on some specimens and the degree of conversion may not be the same in all samples (Sarac *et al*, 2006). In this study, the specimens were formed by curing the composite resin against polyester strips with no subsequent polishing. Polyester strips produce the smoothest surface possible (Baseren, 2004, Patel *et al*, 2004, Bagheri *et al*, 2005, Sarac *et al*, 2006, Attar, 2007) and eliminate the oxygen-inhibited layer which is responsible for the uncured material at the surface (Rueggeberg and Margeson, 1990). However, it has been shown that the layer under the matrix has a lower degree of conversion compared to the bulk of the composite resin which is not exposed to oxygen at all prior and during curing (Patel *et al,* 2004). This surface layer is resin-rich (Setcos *et al*, 1999, Patel *et al,* 2004, Attar, 2007) and this component of composite resin has been shown to be most responsible for the colour changes within these materials (Ferracane *et al*, 1985, Kolbeck *et al*, 2006). This layer is softer and prone to wear and can result in diminished aesthetics (Setcos *et al*, 1999, Baseren, 2004, Patel *et al,* 2004). It may also contain voids (Setcos *et al*, 1999) which may

partly explain the variability of the colour changes as reflected by a varied ΔE and different degrees of staining within the groups. It is therefore suggested that this layer be removed by polishing, for the longevity of composite restorations (Baseren, 2004, Patel *et al,* 2004). The voids may have been avoided by placing a weight over the polyester strips instead of the glass slide. However, this weight would have to be transparent to prevent interference with the polymerization of the composite resin samples through the weight. This should be done in future studies.

Spectrophotometers use either the specular component included (SCI) geometry or the specular component excluded (SCE) geometry (Lee *et al*, 2002, Sarac *et al*, 2006). The SCE geometry is better suited to measure colour differences when surface variations may exist because it takes these differences into account, giving more accurate colour readings (Lee *et al*, 2002, Sarac *et al,* 2006). The SCI geometry was used in this study and probably resulted in the intra-group differences observed in the colour measurements of the samples. The effects of edge-loss error have also been identified as a source of variability of results from spectrophotometry (Paravina *et al*, 2007). In this study, the circumference of the samples fitted exactly into a prepared silicon jig but the height and shape did not match the conical measuring probe precisely, resulting in some reflected light being lost around the margins. This may also have played a role in the variability of the colour readings within the groups during the study.

6. 2 Bleaching Process

The other null hypotheses of this study was also disproven by the finding that there was a statistically significant difference in the colour of the stained samples in the tea group, but not in the red wine group based on the ΔE values after subjecting the specimens to the bleaching agent (table 5.2 and figure 5.8). The mean ΔE value was 3.277 for the red wine group and 5.518 for the tea samples for the period between staining and bleaching and with the threshold at 3.3, the difference was clinically significant for the tea group but not for the red wine group. The beginning of the whitening stage in the tea group was marked by a significant change in colour reflected by a ΔE value of 4.930 as the samples approached the baseline colour. The greatest ΔE value was observed on the first day of bleaching. The graph in figure 5.3 shows this trend. After the first bleaching treatment some samples had a ΔE value of over 7.0 units as shown on the table of sequential colour changes in appendix 5. All L*a*b* values changed with all the

specimens lightening, and the chroma (a* and b* values) shifting towards the green and blue directions. b* representing the blue-yellow axis changed the most reflecting a value of -3.554 and a* representing the green-red coordinate, the least with a value of -1.336. A ΔL* value of 2.927 was observed after the bleaching cycle (table 5.6). After this initial bleaching treatment, the samples did not whiten much more reflected by a ΔE that remained in the 5.1 region during the subsequent bleaching cycles. During this time, the samples remained largely unchanged as far as lightness and the red-green dimension were concerned. Any changes observed after the first whitening treatment were attributed to the b* axis, with a small shift towards blue. These figures are represented in table 5.6.

The red wine group too showed the greatest colour change after the first bleaching treatment, but the effect was less than that observed for the tea group (figure 5.8), as reflected by a mean ΔE of 3.268 (table 5.8). On the second day and third days of treatment with the bleaching agent, the samples further changed colour following the same pattern as the first day, but the differences were small, as reflected by the ΔE value of 3.342 and 3.277 respectively. The L* component increased, lightening the specimens to a small extent. However, there was a movement towards red as reflected by a higher a* value and the samples became more blue as reflected by a decreased b* value. The samples did not change colour markedly after the initial bleaching session with a ΔE value of less than half a unit after the second and third whitening cycles. The rehydration period produced an unremarkable colour change but, individual components within the L*a*b* system, showed a mild rebound with the samples darkening slightly and a* and b* coordinates continued towards red and blue, respectively. These findings are presented in table 5.8. This minor rebound is reflected by a ΔE of 1.438 between day 10 and 11, shown in appendix 5.

Tooth bleaching has very high success rates but this study showed that tooth whitening agents are not as effective on stained composites, as they are on discoloured teeth. This conclusion was supported by Villalta *et al* (2006). The colour of the samples in the tea group returned to clinically acceptable levels with a ΔE value of 2.937 that is close to the baseline after the bleaching process, but not to the original colour. This finding is consistent with Turkun and Turkun (2004). All the samples reverted to colours close to but not exactly baseline values in their study. In the present study, the bleaching agent was ineffective in removing the stains from the samples in the red wine group leaving the specimens severely discoloured at the end of the

bleaching process with a ΔE value of 18.852. Although both, teeth according to Watts and Addy (2001) and composite resins according to Turkun and Turkun (2004) and Bagheri *et al* (2005), discolour by absorption and adsorption of chromogenic foods and beverages, the two have different structures and composition and therefore the outcome of bleaching on them may also be different (Kim *et al*, 2004). Rosentritt *et al*, (2005) showed that whitening systems have a significantly greater effect on enamel than on composite resins. Teeth become whiter due to the ease of movement of free radicals released from hydrogen peroxide into enamel and dentine (Suleiman, 2004, Villalta *et al,* 2006) via enamel micropores which communicate directly with dentinal tubules resulting in desirable whitening effects (Suleiman *et al,* 2003). The structure of teeth is well ordered probably facilitating this movement, however, the structure of composite resins, is a highly cross-linked polymer (Anusavice, 2003, Miletic and Santini, 2008) which may impede movement of free radicals into the structure of the composite resin resulting in less effective bleaching. Another possible explanation for the difference in bleaching between teeth and composite resins is that chromogens may combine chemically with unreacted monomers in the composite resin (Monaghan *et al*, 1992a, Buchalla *et al*, 2002, Rosentritt *et al*, 2005) and are therefore not readily available for free radical oxidation. Kim *et al* (2004) postulated that the coupling agent in composite resin may also be degraded by bleaching agents with a shift of stain accumulation to the matrix-filler interface. This suggests that the chromogens may remain in this interface and do not diffuse out of the material, as occurs with teeth, thereby leaving some residual staining within the composite resin.

Although not part of the study, a sample from both the tea and the red wine group was ground on an abrasive paper along the outer circumference to assess, under a microscope, the depth of stain penetration, if any. The tea stains were only superficial while the red wine discolouration was evident in the subsurface region. Tea contains high polarity yellow colourants which stain composite resins by adsorption onto their surfaces (Um and Ruyter, 1991, Bagheri *et al,* 2005). These are easily removed by toothbrushing (Um and Ruyter, 1991, Bagheri *et al,* 2005). Turkun and Turkun (2004) showed that both polishing systems and bleaching agents removed tea and coffee stains from composite resins but that bleaching was the more effective method. Red wine on the other hand probably stains by absorption and adsorption due to the changes it confers on the surfaces of the composite resin and the degradation of the matrix-filler interface. Um and Ruyter (1991) stained composite resin samples with tea and coffee and attempted to clean them

by brushing once discolouration had occurred. Using this method, the majority of the tea stains were removed but not the coffee stains. They postulated that colourants in coffee were compatible with components in the organic matrix of composite resins and caused discolouration by both adsorption and absorption. This may also have occurred with the samples discoloured by red wine in the present study. These chromogens may therefore not have been accessible for oxidation by the free radicals. Scanning electron microscopy pictures before and after the staining period should be included in future studies to ascertain this postulation. This may explain the lightening effect with the bleaching treatment in the tea group compared to the red wine group whose samples remained highly stained even after three applications of the whitening agent.

Other studies that assessed the effects of bleaching agents on the colour of composite resins found them to be lightened by the whitening systems (Monaghan *et al*, 1991a, Monaghan *et al*, 1991b, Canay and Cehreli, 2003, Kim *et al*, 2004, Rosentritt *et al*, 2005). In this study the converse was found in the control group; the changes recorded after the three bleaching sessions are reflected in table 5.4. Of the three tri-stimulus attributes, Δb* was the highest at the end of the third and final whitening procedure with a shift towards blue. ΔL* and Δa* were minor with the specimens darkening and moving towards green. All three components in the colour space decreased after the rehydration phase; however, the ΔE was only 0.809 which is not clinically perceptible (appendix 5). The previous studies stored the specimens in distilled water for a few hours between the bleaching cycles (Fay *et al*, 1999, Villalta *et al*, 2006) while in this study, the specimens were stored for 1 week intervals in distilled water, between treatments. Composite resin tends to absorb water in the oral environment (Buchalla *et al*, 2002, Vichi *et al*, 2004, Patel *et al*, 2004, Villalta *et al*, 2006) which causes darkening of these restorations over time (Buchalla *et al*, 2002, Patel *et al*, 2004, Villalta *et al*, 2006, Sarafianou *et al*, 2007). Water sorption, due to the longer period the samples were stored in distilled water may have played a significant role in the discolouration of the specimens in the control group in this study compared to previous research.

This study used Opalescence Xtra Boost as the whitening agent which is an in-office, chemically activated bleaching agent. The duration of application of in-office whitening systems is generally much shorter than that of other vital tooth bleaching methods (Kim *et al,* 2004). Other studies that successfully removed extrinsic stains from discoloured composite resins used longer

protocols with paint-on whitening systems (Fay *et al*, 1999, Villalta *et al,* 2006). Zekonis *et al* (2003) showed *in-vivo* that at-home bleaching agents were significantly more effective than in-office whitening systems, despite the higher concentration of the latter. Contact time may therefore be a factor in the difference in whitening between previous studies and the present study. Zekonis *et al* (2003) went on to suggest that in-office bleaching agents may need to be applied for longer periods to achieve the same results as at-home products, however, the readings in this study showed little change in colour after the first bleaching session in both experimental groups and by extrapolation, it can be assumed that further treatment would not have produced a dramatic improvement of the discoloured specimens. Turkun and Turkun (2004) used an in-office bleaching agent in their research, with their samples being effectively whitened. But, it must be noted, that their staining agents, coffee and tea, did not severely discolour their samples. Additionally, Omata and others (2006) showed that red wine produces greater discolouration of composite samples, than either tea or coffee.

The effects of rehydration were also measured in this study. The lack of significant change between the final bleaching treatment and the rehydration period in all three groups shows that the rebound effect is minimal. Kugel *et al*, (2006) showed *in-vivo* that Opalescence Xtra Boost did not produce a significant rebound effect after bleaching compared to another in-office bleaching agent. Also, bleaching agents that contain glycerin as the carrier for the active constituents draw out water from dentine, resulting in dehydration of teeth following whitening (Betke *et al*, 2006). Manufacturers are now incorporating water into their bleaching products to counteract the effects of dehydration (Suleiman, 2005b). Composite resins may not contain much water in their structures to render them susceptible to the dehydrating effects of tooth whitening systems as teeth do, which may explain the clinically insignificant changes during the rehydration period. Secondly, from the results of the study of Kugel *et al* (2006), Opalescence Xtra Boost does not seem to cause dehydration.

6. 3 Limitations of the Study

This was an *in-vitro* study and therefore the staining process was exaggerated. In life, restorations are regularly cleaned through tooth brushing and exposure to staining foods and beverages is not continuous (Bagheri *et al,* 2005). It is important though to note that staining of composites is still a problem even with newer nanofilled composites with different monomers

and filler particle size. However, this study tested only Filtek Supreme XT; therefore there was no comparison of the stainability of other tooth coloured materials.

This study concentrated on the development and removal of extrinsic stains from composite resins. Kolbeck *et al* (2006) stated that intrinsic discolouration was more important because it involved all the layers of the material, whereas extrinsic staining is confined to the surface and subsurface regions of composite resins. Future studies need to investigate the factors relating to intrinsic staining.

In tooth bleaching, at-home products have been shown to produce better results than in-office systems (Kugel *et al*, 2006). Combination treatment, where in-office agents start the whitening process and night-guard vital bleaching then follows gives very good results (Goldstein, 1997, Shethri *et al*, 2003, Suleiman, 2005b, Buchalla and Attin, 2007, Kihn, 2007). In this study, only an in-office bleaching technique was used. Therefore, the effectiveness of other methods such as combination therapy or at-home therapy was not evaluated.

CHAPTER7 CONCLUSIONS AND RECOMMENDATIONS

7. 1 Conclusions

This *in-vitro* study assessed the ability of an in-office bleaching agent in removing stains from discoloured composite resin specimens. The first null hypothesis of this study was rejected because tea and red wine stained composite resin samples to differing intensities as measured by a reflectance spectrophotometer. The second null hypothesis was also rejected as the tea group specimens reverted to baseline colour, after bleaching but the red wine samples remained deeply stained. The following conclusions can be drawn from this study:

- Composite resins discolour in the presence of beverages with colourants. All the samples immersed in either tea or red wine discoloured markedly.
- Red wine discolours composite resins more than tea. There was a statistically significant difference in the staining ability of the two beverages.
- Opalescence Xtra Boost is effective against extrinsic tea stains on composite resin samples and returns stained samples to a colour value just below what could be clinically perceptible as a different colour.
- Opalescence Xtra Boost is ineffective against discolouration caused by red wine on composite resin samples. All the samples in the red wine group were still severely stained at the end of the bleaching process.
- Opalescence Xtra Boost does not dehydrate composite resins and therefore a rebound effect of the bleaching process does not occur.

From this study, it can be seen that extrinsic staining of composite resins is still a problem even with materials manufactured using innovative technology. The composite resin tested was a nanofilled material but was still susceptible to staining. However, this was an *in-vitro* study and *in-vivo* studies need to be carried out to ascertain the conclusions of this study.

7. 2 Recommendations

The results obtained in this study are based on an *in-vitro* experiment and long term *in-vivo* studies are required to verify these results. Further research is needed in the area of composite

resin discoloration which has been a major concern for the aesthetics of these tooth-coloured materials. Until this is accomplished, it is recommended that all composite resin restorations should be polished to increase their resistance to extrinsic staining.

Bleaching agents seem to be successful in removing some extrinsic surface stains from composite resins but research for alternative methods that are safe and non-invasive for the treatment of discoloured composite restorations is required especially concerning internalized and intrinsic staining.

REFERENCES

3M ESPE, Filtek Supreme Technical Product Profile, 2005

Attar N., 2007. The Effect of Finishing and Polishing Procedures on the Surface Roughness of Composite Resin Materials. *J Contemp Dent Prac*, 8: 27 – 35.

ADA Council on Scientific Affairs, 2003. Direct and Indirect Restorative Materials. *JADA*, 134: 463 – 472.

Adams T. C., Pang P. K., 2004. Lasers in Aesthetic Dentistry. *Dent Clin N Am*, 48: 833 – 860.

Anusavice K. J., 2003. *Phillips Science of Dental Materials,* 11th Edition, Philadelphia: Saunders.

Attin T., Hanning C., Wiegand A., Attin R., 2004. Effect of Bleaching on Restorative Materials and Restorations – A Systematic Review. *Dent Mater*, 20: 852 – 861.

Attin T., Paque F., Ajam F., Lennon A. M., 2003. Review of the Current Status of Tooth Whitening with the Walking Bleach Technique. *Int Endo J*, 36: 313 – 329.

Bagheri R., Burrow M. F., Tyas M., 2005. Influence of Food-Simulating Solutions and Surface Finish on Susceptibility to Staining of Aesthetic Restorative Materials. *J Dent*, 33: 389 – 398.

Baseren M., 2004. Surface Roughness of Nanofill and Nanohybrid Composite Resin and Ormocer-Based Tooth Colored Restorative Materials after Several Finishing and Polishing Procedures. *J Biomater Appl*, 19: 121 – 134.

Bayne S. C. 2000. Our Future in Restorative Dental Materials. *J Esthet Dent*, 12: 175 – 182.

Beall A. E., 2007. Can a New Smile Make You Look More Intelligent and Successful? *Dent Clin N Am*, 51: 289 – 297.

Betke H., Kahler E., Reitz A., Hartmann G., Lennon A., Attin T., 2006. Influence of Bleaching Agents and Desensitizing Varnishes on the Water Content of Dentin. *Oper Dent*, 31: 536 – 542.

Bravo L., 1998. Polyphenols: Chemistry, Dietary Sources, Metabolism and Nutritional Significance. *Nutrition Rev*, 56: 317 – 333.

Brewer J. D., Wee A., Seghi R., 2004. Advances in Color Matching. *Dent Clin N Am*, 48: 341 – 358.

Brook A. H., Smith R. N., Lath D. J., 2007. The Clinical Measurement of Tooth Colour and Stain. *Int Dent J,* 57: 324 – 330.

Buchalla W., Attin T., 2007. External Bleaching Therapy with Activation by Heat, Light or Laser – A Systematic Review. *Dent Mater*, 23: 586 – 596.

Buchalla W., Attin T., Hilgers R. D., Hellwig E., 2002. The Effect of Water Storage and Light Exposure on the Color and Translucency of a Hybrid and a Microfilled Composite. *J Prosthet Dent*, 87: 264 – 270.

Burrell K., 1997. ADA Supports Vital Tooth Bleaching- But Look for the Seal. *JADA*, 128: 3S-5S.

Canay S., Cehreli M. C., 2003. The Effect of Current Bleaching Agents on the Color of Light-Polymerized Composites in Vitro. *J Prosthet Dent*, 89: 474 – 478.

Christensen G. J., 2005. Are Snow-white Teeth Really so Desirable? *JADA*, 136: 933 – 935.

Cullen D. R., Nelson J. A., Sandrik J. L., 1993. Peroxide Bleaches: Effect on Tensile Strength of Composite Resins. *J Prosthet Dent*, 69; 247 – 249.

Dahl J. E, Pallesen U., 2003. Tooth Bleaching – A Critical Review of the Biological Aspects. *Crit Rev Oral Biol Med*, 14: 292-304.

Derbabian K., Marzola R., Donovan T., Arcidiacono A., 2001. The Science of Communicating the Art of Esthetic Dentistry. Part III: Precise Shade Communication. *J Esthet Restor Dent*, 13: 154 – 162.

Doray P. A., Eldiwany M. S., Powers J. M., 2003. Effect of Resin Surface Sealers on Improvement of Stain Resistance for a Composite Provisional Material. *J Esthet Rest Dent*, 15; 244 – 250.

El-din A. K. N., Miller B. H., Griggs J. A., Wakefield C., 2006. Immediate Bonding to Bleached Enamel. *Oper Dent*, 31: 106 – 114.

Elderton R. J., 1996. Treating Restorative Dentistry to Health. *Brit Dent J*. 181: 220 – 225.

Fay R-M., Servos T., Powers J. M., 1999. Color of Restorative Materials after Staining and Bleaching. *Oper Dent*, 24: 292 -296.

Ferracane J. L., Moser J. B., Greener E. H., 1985. Ultraviolet Light-Induced Yellowing of Dental Restorative Resins. *J Prosthet Dent*, 54: 483 – 487.

Fondriest J., 2003. Shade Matching in Restorative Dentistry: The Science and Strategies. *Int J Perio Restor Dent*, 23: 467 – 479.

Friedman S., 1997. Internal Bleaching: Long-term Outcomes and Complications. *JADA*, 128: 51S – 55S.

Garber D. A., 1997. Dentist Monitored Bleaching: A Discussion of Combination and Laser Bleaching. *JADA,* 128: 26S-39S.

Garcia A. H., Lozano M. A. M., Vila J. C., Escribano A. B., Galve P. F., 2006. Composite Resins. A Review of the Materials and Clinical Indications. *Med Oral Patol Oral Cir Bucal,* 11: E215 – 220.

Gerlach R. W., 2004. Whitening Paradigms Revisited: Introduction of a Thin and Concentrated Hydrogen Peroxide Gel Technology for Professional Tooth Whitening. *Comp Cont Edu Dent*, 25: 4 – 8.

Goldstein R. E., 1997. In-Office Bleaching, Where We Came From, Where We Are Today. *JADA* 128: 11S-15S.

Goldstein R. E., Garber D., A. 1995. *Complete Dental Bleaching*. Chicago: Quintessence

Guler A. U., Kurt S., Kulunk T., 2005a. Effects of Various Finishing Procedures on the Staining of Provisional Restorative Materials. *J Prosthet Dent,* 93: 453 – 458.

Guler A. U., Yilmaz F., Kulunk T., Guler E., Kurt S. 2005b, Effects of Different Drinks on Stainability of Resin Composite Provisional Restorative Materials. *J Prosthet Dent*, 94: 118 – 124.

Haywood V. B., 1992. History, Safety and Effectiveness of Current Bleaching Techniques and Applications of the Nightguard Vital Bleaching Technique. *Quint Int*, 23: 471 – 488.

Haywood V. B., 2002. Greening of the Tooth-Amalgam Interface during Extended 10% Carbamide Peroxide Bleaching of Tetracycline-Stained Teeth: A Case Report. *J Esthet Rest Dent*, 14: 12 – 17.

http://www.ewine.co.za

http://www.ketepa.com

Imazato S., Tarumi H., Kato S., Ebisu S., 1999. Water Sorption and Colour Stability of Composites Containing the Antibacterial Monomer MDPB. *J Dent,* 27: 270 – 283.

Jarad F. D., Russell M. D., Moss B. W., 2005. The Use of Digital Imaging for Colour Matching and Communication in Restorative Dentistry. *Brit Dent J*, 199: 43 – 49.

Joiner A. 2004, Tooth Colour: A Review of the Literature. *J Dent,* 32: 3 – 12.

Joiner A., 2006. The Bleaching of Teeth: A Review of the Literature. *J Dent*, 34: 412 – 419.

Joiner A., 2007. Review of the Effects of Peroxide on Enamel and Dentine Properties. *J Dent*, 35: 889 – 896.

Khurana R., Tredwin C. J., Weisbloom M., Moles D. R., 2007. A Clinical Evaluation of the Individual Repeatability of Three Commercially Available Colour Measuring Devices. *Brit Dent J*, 203: 675 – 680.

Kihn P. W., 2007. Vital Tooth Whitening. *Dent Clin N Am*, 51: 319 – 331.

Kim J-H., Lee Y-K., Lim B-S., Rhee S-H., Yang H-C., 2004. Effect of Tooth-Whitening Strips and Films in Changes in Color and Surface Roughness of Resin Composites. *Clin Oral Invest,* 8: 118-122.

Kolbeck C., Rosentritt M., Lang R., Handel G., 2006. Discoloration of Facing and Restorative Composites by UV-Irradiation and Staining Food. *Dent Mater*, 22: 63 – 68.

Koksal T., Dikbas I., 2008. Colour Stability of Different Denture Teeth Materials against Various Staining Agents. *Dent Mater J*, 27: 139 – 144.

Kugel G., Papathanasiou A., Williams A. J., Anderson C., Ferreira S., 2006. Clinical Evaluation of Chemical and Light-Activated Tooth Whitening Systems. *Comp Cont Ed Dent*, 27: 54 – 62.

Lai S. C. N., Tay F. R., Cheung G. S. P., Mak Y. F., Carvalho R. M., Wei S. H. Y., Toledano M., Osorio R., Pashley D. H., 2002. Reversal of Compromised Bonding in Bleached Enamel. *J Dent Res,* 81: 477 – 481.

Lee Y-K., Lim B-S, Kim C-W., 2002. Effect of Surface Conditions on the Color of Dental Resin Composites. *J Biomed Mater Res (Appl Biomater)*, 63: 657 – 663.

Lee Y-K., Lim B-S., Rhee H-C., Yang H-C., Powers J. M., 2005. Color and Translucency of A2 Shade Resin Composites after Curing, Polishing and Thermocycling. *Oper Dent*, 30: 436 – 442.

Lee Y-K., Powers J. M., 2007. Combined Effect of Staining Substances on the Discoloration of Esthetic Class V Dental Restorative Material . *J Mater Sci: Mater Med* , 18: 165 - 170.

Leinfelder K. F., 2001. Dentine adhesives for the Twenty First Century. *Dent Clin N Am,* 46: 1 - 6.

LeSage B., 2007. Aesthetic Anterior Composite Restorations: A Guide to Direct Placement. *Dent Clin N Am*, 51: 359 – 378.

Liebenberg W. H., 1997. Intracoronal Lightening of Discolored Pulpless Teeth: A Modified Walking Bleach Technique. *Quint Int*, 28: 771 – 777.

Macpherson L. M. D., Stephen K. W., Joiner A., Schafer F., Huntington E., 2000. Comparison of a Conventional and Modified Tooth Stain Index. *J Clin Perio*, 27: 854 – 859.

Miletic V. J., Santini A., 2008. Remaining Unreacted Methacrylate Groups in Resin-Based Composites with Respect to Sample Preparation and Storing Conditions Using Micro-Raman Spectroscopy. *J Biomed Mater Res Part B: Appl Biomater.* Available from: http://www3.interscience.wiley.com [Accessed 1 September 2008].

Mitra S. B., Wu D., Holmes B. N., 2003. An Application of Nanotechnology in Advanced Dental Materials. *JADA*, 134: 1382 – 1390.

Monaghan P., Trowbridge T., Lautenschlager E., 1992a. Composite Resin Color Change after Vital Tooth Bleaching. *J Prosthet Dent,* 67: 778 – 781.

Monaghan P., Lim E., Lautenschlager E., 1992b. Effects of Home Bleaching Preparations on Composite Resin Color. *J Prosthet Dent,* 68: 575 – 578.

Morley J., 1999. The Role of Cosmetic Dentistry in Restoring a Youthful Appearance. *JADA,* 130: 1166 – 1172.

Okte Z., Villalta P., Garcia-Godoy F., Lu H., Powers J. M., 2006. Surface Hardness of Resin Composites after Staining and Bleaching. *Oper Dent*, 31: 623 – 628.

Omata Y., Uno S., Nakaoki Y., Tanaka T., Sano H., Yoshida S., Sidhu S. K., 2006. Staining of Hybrid Composites with Coffee, Oolong Tea or Red Wine. *Dent Mater J*, 25: 125 – 131.

Opalescence Xtra Boost Product Profile, 2003

Paravina R. D., Majkic G., Imai F. H., Powers J. M., 2007. Optimization of Tooth Colour and Shade Guide Design. *J Prosth*, 16: 269 – 276.

Park J-H., Lee Y-K., Lim B-S., 2006. Influence of Illuminants on the Colour Distribution of Shade Guides. *J Prosthet Dent*, 96: 402 – 411.

Patel S. B., Gordan V. V., Barrett A. A., Shen C., 2004. The Effect of Surface Finishing and Storage Solutions on the Color Stability of Resin-Based Composites. *JADA,* 135: 587 – 594.

Perdigao J., Baratieri L. N., Arcari G. M., 2004. Contemporary Trends and Techniques in Tooth Whitening: A Review. *Prac Proced Aesthet Dent*, 16: 185 – 192.

Rosentritt M., Lang R., Plein T., Behr M., Handel G., 2005. Discoloration of Restorative Materials after Bleaching Application. *Quint Int,* 36: 33-39.

Rotstein I., Dogan H., Avron Y., Shemesh H., Steinberg D., 2000. Mercury Release from Dental Amalgam after Treatment With 10% Carbamide Peroxide In Vitro. *Oral Surg Oral Med Oral Path*, 89: 216 – 219.

Rueggeberg F. A., Margeson D. H., 1990. The Effect of Oxygen Inhibition on an Unfilled/Filled Composite System. *J Dent Res*, 69: 1652 – 1658.

Sadowsky S. J., 2006. An Overview of Treatment Considerations for Esthetic Restorations: A Review of the Literature. *J Prosthet Dent*, 96: 433- 442.

Sarac D., Sarac Y. S., Kulunk S., Ural C., Kulunk T., 2006. The Effect of Polishing Techniques on the Surface Roughness and Color Change of Composite Resins. *J Prosthet Dent,* 96: 33 – 40.

Saravana K. R., Vijayalakshmi R., 2006. Nanotechnology in Dentistry. *Ind J Dent Res*, 17: 62 – 65.

Sarafianou A., Iosifidou S., Papadopoulos T., Eliades G., 2007. Color Stability and Degree of Cure of Direct Composite Restoratives after Accelerated Aging. *Oper Dent*, 32: 406 – 411.

Sarrett D. C., 2002. Tooth whitening today. *JADA*, 133: 1535-1538.

Schemehorn B., Gonzalez-Cabezas C., Joiner A., 2004. A SEM Evaluation of a 6% Hydrogen Peroxide Tooth Whitening Gel on Dental Materials In Vitro. *J Dent*, 32 (Suppl 1): 35 – 39.

Schmidt C. J., Tatum S. A., 2006. Cosmetic Dentistry. *Curr Opin Otolaryngo Head Neck Surg*, 14: 254 – 259.

Schulze K. A., Marshall S. J., Gansky S. A., Marshall G. W., 2003. Color Stability and Hardness in Dental Composites after Accelerated Aging. *Dent Mater*, 19: 612 – 619.

Seghi R. R., Gritz M. D., Kim J., 1990. Colorimetric Changes in Composites Resulting from Visible-Light-Initiated Polymerization. *Dent Mater*, 6: 133 – 137.

Setcos J. C., Tarim B., Suzuki S., 1999. Surface Finish Produced on Resin Composites by New Polishing Systems. *Quint Int,* 30: 169 – 173.

Shethri S. A., Matis B. A., Cochran M. A., Zekonis R., Stropes M., 2003. Clinical Evaluation of Two In-Office Bleaching Products. *Oper Dent*, 28: 488 – 495.

Spear F. M., Kokich V. G., 2007. A Multidisciplinary Approach to Esthetic Dentistry. *Dent Clin N Am*, 51: 487 – 505.

Spyrides G.M., Perdigao J, Pagani C., Araujo M. A., Spyrides S. M., 2000. Effect of Whitening Agents on Dentin Bonding. *J Esthet Dent*, 12: 264 – 270.

Suleiman M., 2004. An Overview of Bleaching Techniques: 1. History, Chemistry, Safety and Legal Aspects. *Dent Update*, 31: 608 – 616.

Suleiman M., 2005a. An Overview of Bleaching Techniques: 2. Night Guard Vital Bleaching and Non-Vital Bleaching. *Dent Update*, 32: 39 – 46.

Suleiman M., 2005b. An Overview of Bleaching Techniques: 3. In–Surgery or Power Bleaching. *Dent Update*, 31: 101 – 108.

Suleiman M., Addy M., Rees J. S., 2003. Development and Evaluation of a Method In-Vitro to Study the Effectiveness of Tooth Bleaching. *J Dent*, 31: 415- 422.

Strober T., Gilde H., Lenz P., 2001. Color Stability of Highly Filled Composite Resin Materials for Facings. *Dent Mater*, 17: 87 – 94.

Swift E. J., May K. N., Wilder A. D., Heymann H. 0., Bayne S., 1999. Two-Year Clinical Evaluation of Tooth Whitening Using an At-Home Bleaching System. *J Esthet Dent,* 11: 36 – 42.

Turker S. B., Biskin T., 2003. Effect of Three Bleaching Agents on the Surface Properties of Three Different Esthetic Restorative Materials. *J Prosthet Dent*, 89: 466-73.

Turkun L. S., Turkun M., 2004. Effect of Bleaching and Repolishing Procedures on Coffee and Tea Stain Removal from Three Anterior Composite Veneering Materials. *J Esthet Rest Dent*, 16: 290 -301.

Tredwin C. J., Naik S., Lewis N. J., Scully C. B. E., 2006. Hydrogen Peroxide Tooth-Whitening (Bleaching) Products: Review of Adverse Effects and Safety Issues. *Brit Dent J*, 200: 371 – 376.

Um C. M., Ruyter I. E., 1991. Staining of Resin-Based Veneering Materials with Coffee and Tea. *Quint Int*, 22: 377 – 386.

Usumez A., Ozturk N., Ozturk B., 2005. Two-year Color Changes of Light-cured Composites: Influence of Different Light-curing Units. *Oper Dent*, 30: 655 – 660.

Vichi A., Ferrari M., Davidson C. L., 2004. Color and Opacity Variations in Three Different Resin-Based Composite Products after Water Aging. *Dent Mater*, 20: 530 – 534.

Villalta P., Lu H., Okte Z., Garcia-Godoy F., Powers J. M., 2006. Effects of Staining and Bleaching on Color Change of Dental Composite Resins. *J Prosthet Dent,* 95: 137-42.

Wakefield C. W., Kofford K. R., 2001. Advances in Restorative Materials. *Dent Clin N Am,* 46: 7 – 26.

Watts A., Addy M., 2001. Tooth Discolouration and Staining: A Review of the Literature. *Brit Dent J,* 190: 309 – 316.

Yalcin F., Gurgan S., 2005. Effect of Two Different Bleaching Regimens on the Gloss of Tooth Colored Restorative Materials. *Dent Mater*, 21: 464 – 468.

Zekonis R., Matis B. A., Cochran M. A., Shetri S. A., Eckert G. J., Carlson T. J., 2003. Clinical Evaluation of In-Office and At-Home Bleaching Treatments. *Oper Dent*, 28: 114 – 121.

APPENDICES

APPENDIX 1 – RAW DATA FOR ALL THREE GROUPS

GROUP C

	Baseline			Day 1 of staining				Day 2 of staining				
Specimen	L^*_0	a^*_0	b^*_0	L^*_1	a^*_1	b^*_1	**ΔE_1**	L^*_2	a^*_2	b^*_2	**Seq ΔE**	**ΔE_2**
1	59.31	0.81	6.71	59.43	1.36	7.08	0.674	59.63	1.24	7.00	0.247	0.609
2	59.57	0.92	7.18	60.02	0.90	6.79	0.596	59.70	0.53	6.38	0.638	0.899
3	60.87	1.07	7.58	61.96	1.24	7.34	0.308	60.94	1.41	7.69	0.390	0.364
4	60.25	0.91	7.04	59.70	0.87	7.11	0.556	59.81	0.90	6.92	0.222	0.456
5	59.42	0.48	6.65	60.10	0.86	6.85	0.804	60.43	1.26	7.53	0.855	1.550
6	59.82	0.56	6.77	59.78	0.61	6.55	0.229	60.37	1.07	7.40	1.132	0.980
7	59.29	0.76	6.86	59.37	0.52	7.23	0.448	59.31	0.74	7.54	0.385	0.681
8	60.08	0.86	7.12	60.33	0.86	6.85	0.368	60.18	0.87	6.73	0.192	0.403
9	60.47	0.91	7.26	59.65	1.23	7.71	0.989	59.67	0.95	7.21	0.573	0.803
10	59.59	0.92	7.42	59.53	0.69	6.71	0.749	59.95	0.67	6.83	0.437	0.735
11	59.83	0.80	7.84	58.85	0.89	7.36	1.095	60.07	1.19	8.15	1.484	0.553
12	59.84	0.63	6.73	59.61	0.70	6.59	0.278	59.61	0.35	5.90	0.774	0.906
13	61.48	1.34	8.03	60.93	0.77	7.12	1.206	60.47	0.67	6.84	0.548	1.699
14	60.24	0.93	7.5	59.85	1.24	7.84	0.603	60.05	0.94	7.51	0.489	0.191
15	60.18	1.04	7.42	59.85	0.60	6.34	1.212	59.56	0.48	6.18	0.352	1.495
16	60.18	1.01	8.11	59.85	0.87	7.67	0.568	60.67	1.12	7.72	0.859	0.636
17	59.99	0.31	6.34	60.17	0.94	7.04	0.959	60.19	0.70	6.33	0.750	0.438
18	60.99	1.16	7.71	60.39	1.12	7.36	0.696	60.85	1.24	7.46	0.486	0.297
19	60.16	0.96	7.49	59.49	0.70	6.59	1.152	59.57	0.71	6.65	0.100	1.057
20	59.33	0.86	7.03	59.37	0.50	7.02	0.362	59.28	0.84	7.65	0.722	0.622

Seq ΔE = ΔE over consecutive days, ΔE_x = ΔE each day from baseline.

GROUP C

Day 3 of staining					Day 4 of staining					Day 5 of staining				
L^*_3	a^*_3	b^*_3	**Seq ΔE**	**ΔE_3**	L^*_4	a^*_4	b^*_4	**Seq ΔE**	**ΔE_4**	L^*_5	a^*_5	b^*_5	**Seq ΔE**	**ΔE_5**
59.75	1.17	6.69	0.340	0.569	60.32	1.72	7.02	0.858	1.394	59.42	1.20	6.75	1.074	0.407
60.75	1.42	7.61	1.846	1.352	59.87	0.97	6.42	1.547	0.819	59.67	0.74	6.34	0.315	0.865
59.94	1.06	7.15	1.189	1.025	60.71	1.23	7.09	0.791	0.540	60.80	1.14	6.91	0.220	0.677
59.62	0.94	6.31	0.640	0.965	60.04	0.78	6.42	0.463	0.667	59.75	0.94	7.02	0.685	0.501
60.21	1.30	7.65	0.254	1.515	60.31	1.14	6.80	0.871	1.118	59.83	0.59	6.01	1.076	0.768
59.34	0.57	6.35	1.554	0.638	59.70	0.89	6.44	0.490	0.482	59.72	0.77	6.24	0.234	0.579
59.92	0.82	7.33	0.650	0.788	60.21	0.77	7.22	0.314	0.988	60.86	0.88	7.19	0.660	1.609
60.26	0.93	6.83	0.141	0.348	60.06	0.95	6.68	0.251	0.450	60.21	0.99	6.76	0.175	0.404
60.48	1.03	7.13	0.818	0.177	60.11	0.98	6.91	0.433	0.507	60.27	1.03	7.06	0.225	0.307
59.76	0.72	6.67	0.253	0.795	59.69	0.76	6.57	0.128	0.871	59.66	0.87	6.64	0.134	0.785
60.66	1.19	7.67	0.761	0.933	59.75	0.98	7.49	0.951	0.402	60.36	1.27	7.74	0.720	0.715
60.21	1.12	7.43	1.815	0.931	59.45	0.65	6.37	1.386	0.531	59.70	0.42	5.71	0.742	1.051
60.68	0.74	6.66	0.285	1.696	60.93	0.9	6.72	0.303	1.487	61.16	0.91	6.89	0.286	1.260
59.88	1.00	7.44	0.193	0.372	60.41	1.02	7.14	0.609	0.408	60.95	1.26	7.74	0.842	0.819
59.77	0.78	6.85	0.764	0.749	60.5	0.92	6.62	0.778	0.870	59.49	0.57	6.07	1.202	1.587
59.95	1.19	7.76	0.724	0.456	60.76	1.07	7.23	0.975	1.056	60.20	0.94	6.93	0.648	1.182
59.92	0.74	6.54	0.344	0.479	60.10	0.82	6.43	0.226	0.529	59.91	0.70	6.27	0.276	0.404
61.22	1.23	7.58	0.389	0.273	60.59	1.00	6.99	0.893	0.839	60.21	0.94	7.31	0.500	0.904
60.61	0.91	7.05	1.132	0.631	59.66	0.74	6.59	1.069	1.053	59.75	0.44	5.84	0.813	1.778
60.12	0.67	7.22	0.959	0.834	60.06	0.59	7.02	0.224	0.778	60.63	0.71	6.96	0.586	1.310

GROUP C

Day 6 of staining					Day 7 of staining					1st bleaching - 8				
L^*_6	a^*_6	b^*_6	**Seq ΔE**	**ΔE_6**	L^*_7	a^*_7	b^*_7	**Seq ΔE**	**ΔE_7**	L^*_8	a^*_8	b^*_8	**Seq ΔE**	**ΔE_8**
59.94	1.15	6.53	0.567	0.738	60.43	1.18	6.80	0.560	1.183	59.64	1.11	6.4	0.888	0.543
60.31	0.89	6.38	0.659	1.090	59.07	0.47	5.67	1.489	1.653	59.54	0.57	5.92	0.542	1.308
60.73	0.89	6.74	0.310	0.870	60.62	0.47	6.65	0.443	1.135	60.94	0.89	6.55	0.537	1.048
60.06	0.72	6.54	0.612	0.568	60.20	0.86	7.06	0.556	0.073	60.61	0.96	6.46	0.734	0.684
59.89	0.54	5.95	0.098	0.845	60.52	0.99	6.92	1.241	1.242	60.02	0.45	5.69	1.433	1.132
60.26	1.00	6.82	0.825	0.624	60.34	0.99	6.63	0.206	0.689	59.84	0.34	5.68	1.255	1.112
60.93	0.85	7.38	0.205	1.723	60.58	0.64	6.66	0.828	1.311	60.60	0.58	6.66	0.063	1.337
59.76	0.68	6.52	0.597	0.703	59.49	0.50	6.22	0.442	1.135	60.27	0.38	5.75	0.919	1.464
60.38	0.78	6.59	0.544	0.688	60.47	1.11	7.12	0.631	0.244	60.41	0.87	6.61	0.567	0.654
60.32	0.86	6.75	0.669	0.993	60.47	0.89	6.68	0.168	1.150	59.34	0.72	6.18	1.247	1.281
60.10	0.84	6.66	1.191	1.211	59.85	0.63	6.27	0.509	1.579	60.11	0.69	6.32	0.271	1.549
59.92	0.48	5.92	0.218	0.836	59.91	0.67	6.16	0.371	0.576	59.46	0.37	5.6	0.779	1.220
61.10	0.84	6.73	0.185	1.444	60.76	0.47	6.73	0.502	1.722	60.99	0.76	6.37	0.516	1.825
59.92	0.74	6.82	1.476	0.775	59.46	0.44	6.44	0.668	1.404	59.82	0.69	6.52	0.446	1.093
60.14	0.60	6.43	0.744	1.084	59.79	0.60	5.91	0.627	1.620	59.61	0.51	5.63	0.345	1.952
59.50	0.78	7.07	0.732	1.264	60.32	1.19	7.59	1.054	0.568	60.84	0.84	6.82	0.993	1.459
60.13	0.90	6.6	0.444	0.660	60.18	0.54	5.94	0.753	0.499	60.68	1.07	6.86	1.174	1.151
60.79	1.20	7.34	0.636	0.422	60.72	0.92	7.08	0.388	0.726	60.33	0.65	6.39	0.837	1.561
59.72	0.61	6.50	0.682	1.139	60.68	0.71	6.67	0.980	1.003	59.65	0.16	5.66	1.544	2.061
60.71	0.67	7.14	0.201	1.397	60.33	0.54	6.29	0.940	1.285	59.94	0.42	6.35	0.412	1.014

GROUP C

2nd bleaching - 9					3rd bleaching - 10					Rehydration - 11				
L^*_9	a^*_9	b^*_9	**Seq ΔE**	**ΔE_9**	L^*_{10}	a^*_{10}	b^*_{10}	**Seq ΔE**	**ΔE_{10}**	L^*_{11}	a^*_{11}	b^*_{11}	**Seq ΔE**	**ΔE_{11}**
59.28	0.85	5.61	0.906	1.101	60.50	1.33	6.54	1.607	1.310	58.92	0.83	5.48	1.967	1.291
58.55	0.09	4.85	1.535	2.675	58.72	0.45	5.23	0.550	2.179	59.14	0.24	5.02	0.514	2.305
60.05	0.46	5.71	1.297	2.131	60.02	0.55	5.75	0.103	2.084	59.81	0.43	5.74	0.242	2.218
59.70	0.50	5.50	1.400	1.686	59.79	0.48	5.56	0.110	1.608	60.12	0.96	6.35	0.982	0.704
59.48	0.08	4.74	1.154	1.952	59.42	0.10	4.80	0.087	1.889	59.07	0.07	4.83	0.353	1.898
59.41	0.19	5.03	0.794	1.826	59.42	0.02	4.84	0.255	2.044	58.84	0.00	4.78	0.583	2.288
59.81	0.27	5.63	1.335	1.422	59.97	0.37	5.81	0.261	1.310	59.07	0.33	5.83	0.901	1.138
59.36	0.36	5.45	0.958	1.886	59.55	0.05	4.92	0.643	2.404	59.38	0.14	4.89	0.195	2.446
59.75	0.35	5.60	1.314	1.894	59.61	0.46	5.71	0.209	1.829	59.28	0.14	5.17	0.709	2.525
58.75	0.19	5.07	1.364	2.600	58.68	0.10	4.99	0.139	2.721	58.18	0.00	4.67	0.602	3.224
59.87	0.51	5.84	0.566	2.021	59.87	0.59	6.66	0.824	1.199	58.72	0.11	5.79	1.520	2.431
58.35	-0.18	4.55	1.624	2.762	58.51	-0.25	4.24	0.356	2.957	59.06	0.19	5.06	1.081	1.895
59.92	0.07	5.08	1.812	3.571	59.45	-0.06	4.75	0.589	4.104	59.64	0.13	4.97	0.347	3.770
59.46	0.42	5.84	0.815	1.904	59.46	0.36	5.95	0.125	1.826	59.37	0.15	5.42	0.577	2.386
59.46	0.09	4.87	0.881	2.815	59.06	0.10	4.62	0.472	3.159	58.05	-0.10	4.31	1.077	3.941
59.28	0.37	6.00	1.824	2.382	59.80	0.30	5.82	0.555	2.427	58.95	0.08	5.16	1.098	3.329
59.72	0.35	5.50	1.814	0.883	59.94	0.18	5.14	0.455	1.208	59.73	0.57	5.89	0.871	0.581
60.69	0.87	6.50	0.436	1.280	59.95	0.60	6.14	0.866	1.965	59.23	0.26	5.36	1.115	3.071
59.36	0.07	5.40	0.400	2.408	59.59	0.46	5.89	0.667	1.771	58.96	0.05	4.95	1.204	2.953
60.06	0.06	5.36	1.060	1.990	59.72	0.13	5.72	0.500	1.550	59.94	0.09	5.61	0.249	1.727

GROUP T

	Baseline			Day 1 of staining				Day 2 of staining				
Specimen	L^*_0	a^*_0	b^*_0	L^*_1	a^*_1	b^*_1	**ΔE_1**	L^*_2	a^*_2	b^*_2	**Seq ΔE**	**ΔE_2**
1	60.29	0.32	6.78	56.73	0.81	9.06	4.256	56.83	1.42	12.10	3.102	6.441
2	60.07	0.26	5.92	56.99	0.06	7.28	3.373	55.86	0.22	8.68	1.806	5.034
3	59.82	0.97	7.13	56.98	0.46	7.17	2.886	55.77	0.66	9.12	2.304	4.523
4	59.71	0.53	6.38	57.66	0.19	7.20	2.234	57.14	0.87	9.93	2.861	4.396
5	59.46	0.92	6.82	57.66	0.58	6.91	1.834	57.39	-0.18	6.93	0.807	2.347
6	59.68	0.59	7.59	55.81	1.56	10.54	4.962	55.33	1.06	11.39	1.097	5.795
7	60.53	0.92	7.50	58.07	0.97	8.40	2.620	57.68	0.81	9.80	1.462	3.664
8	59.84	0.24	6.34	57.08	0.39	8.37	3.429	56.09	1.40	11.41	3.353	6.412
9	59.99	0.43	6.62	57.43	0.85	8.65	3.294	54.92	1.30	11.00	3.468	6.756
10	60.16	0.26	6.20	58.11	0.79	8.34	3.010	57.94	0.94	8.71	0.434	3.419
11	59.98	0.57	6.82	58.02	0.46	7.48	2.071	57.52	0.37	8.86	1.471	3.202
12	61.44	1.26	8.19	58.19	1.31	10.51	3.993	56.87	1.12	11.33	1.566	5.547
13	59.88	0.58	6.58	59.19	1.33	7.67	1.492	58.33	0.71	8.32	1.244	2.334
14	60.51	1.23	7.11	58.28	0.77	5.77	2.642	58.09	0.82	7.71	1.950	2.527
15	59.08	0.64	6.42	56.87	0.42	7.13	2.332	56.09	0.55	8.97	2.003	3.931
16	60.32	0.44	6.58	58.41	1.05	8.29	2.635	55.50	0.14	9.09	3.152	5.443
17	60.45	1.16	7.57	57.82	1.17	8.59	2.821	57.38	1.08	9.18	0.741	3.467
18	60.87	0.81	7.16	57.45	0.59	8.24	3.593	57.10	0.56	9.14	0.966	4.266
19	60.64	1.39	7.99	57.88	0.56	8.60	2.946	57.09	1.08	10.37	2.007	4.285
20	60.23	0.48	6.66	57.83	0.45	7.86	2.683	55.83	0.61	9.96	2.904	5.502

GROUP T

Day 3 of staining					Day 4 of staining					Day 5 of staining				
L^*_3	a^*_3	b^*_3	**Seq ΔE**	**ΔE_3**	L^*_4	a^*_4	b^*_4	**Seq ΔE**	**ΔE_4**	L^*_5	a^*_5	b^*_5	**Seq ΔE**	**ΔE_5**
55.24	1.22	12.52	1.657	7.698	55.73	0.81	12.55	0.640	7.371	54.7	0.85	13.33	1.293	8.627
57.88	0.35	7.78	2.215	2.875	58.53	-0.08	7.92	0.792	2.547	58.33	-0.08	7.91	0.200	2.665
55.33	0.57	10.01	0.997	5.349	55.39	0.67	10.75	0.749	5.729	55.45	0.58	11.18	0.443	5.971
56.40	1.21	11.16	1.475	5.854	55.57	0.92	12.20	1.362	7.153	55.01	0.76	12.08	0.595	7.391
56.94	0.66	9.16	2.425	3.449	55.99	0.73	10.90	1.984	5.359	56.21	0.11	10.57	0.736	5.028
54.83	1.12	11.71	0.597	6.386	53.52	1.09	12.77	1.685	8.064	54.09	1.11	10.57	2.273	6.356
57.37	0.91	10.13	0.464	4.111	58.05	0.56	9.85	0.814	3.435	57.98	0.29	10.02	0.327	3.640
54.99	1.31	11.95	1.229	7.493	55.02	1.05	12.34	0.470	7.739	54.61	0.52	12.04	0.734	7.741
55.61	1.23	11.57	0.898	6.658	55.3	1.11	12.19	0.703	7.313	54.38	0.76	12.19	0.984	7.912
58.35	0.49	8.33	0.718	2.805	58.26	0.55	9.63	1.304	3.932	57.63	0.76	12.19	2.645	6.522
57.88	0.67	9.49	0.785	3.398	58.00	0.75	10.58	1.099	4.253	57.94	0.16	9.94	0.873	3.750
57.88	0.97	11.78	1.116	5.064	56.65	0.65	12.38	1.405	6.393	57.43	0.74	12.07	0.844	5.604
58.46	0.87	9.39	1.090	3.162	57.17	0.74	10.89	1.983	5.094	58.12	0.52	9.96	1.348	3.811
58.41	0.60	7.90	0.432	2.330	58.10	0.43	8.75	0.921	3.023	58.12	0.50	8.83	0.108	3.034
56.32	0.95	9.38	0.617	4.059	56.98	0.47	9.22	0.832	3.504	57.36	0.25	8.63	0.735	2.827
57.67	0.59	9.38	2.235	3.858	57.48	0.45	9.91	0.580	4.377	56.26	-0.33	10.14	1.466	5.454
56.83	1.21	9.95	0.955	4.333	54.37	0.99	11.45	2.890	7.215	54.47	0.99	11.31	0.172	7.055
56.59	0.89	10.13	1.162	5.210	56.31	0.37	10.77	0.871	5.833	57.21	0.23	10.31	1.020	4.864
56.55	0.95	12.33	2.037	5.980	56.72	0.85	11.50	0.853	5.289	56.10	0.61	11.73	0.703	5.934
56.88	0.67	10.28	1.099	4.936	56.06	0.49	11.22	1.260	6.179	55.20	0.32	11.35	0.886	6.879

GROUP T

Day 6 of staining					Day 7 of staining					1st bleaching –8				
L^*_6	a^*_6	b^*_6	**Seq ΔE**	**ΔE_6**	L^*_7	a^*_7	b^*_7	**Seq ΔE**	**ΔE_7**	L^*_8	a^*_8	b^*_8	**Seq ΔE**	**ΔE_8**
54.34	1.42	14.75	1.572	10.007	54.62	0.68	13.67	1.339	8.930	58.68	-0.50	10.06	5.560	3.745
57.89	0.23	9.44	1.622	4.140	58.04	0.00	9.52	0.286	4.141	58.03	-0.64	7.20	2.407	2.571
53.21	0.66	11.97	2.377	8.198	54.72	0.39	10.96	1.837	6.404	57.58	-0.89	7.66	4.551	2.959
53.76	0.79	12.23	1.259	8.348	56.27	1.28	12.50	2.572	7.060	57.16	-0.76	8.73	4.378	3.700
56.03	0.17	10.91	0.389	5.390	54.90	1.27	11.73	1.777	6.710	57.33	-1.07	7.74	5.225	3.057
52.52	1.05	14.50	4.232	9.961	52.08	0.45	14.55	0.746	10.306	57.32	-0.85	10.06	7.022	3.707
56.03	1.00	12.70	3.390	6.877	55.81	1.03	12.62	0.236	6.965	58.36	-0.53	8.31	5.245	2.733
52.98	0.78	13.19	2.012	9.709	54.16	0.70	12.59	1.326	8.458	58.32	-0.81	9.69	5.291	3.826
53.37	0.78	12.71	1.136	9.002	53.84	1.07	12.38	0.643	8.450	58.24	-0.51	8.44	6.114	2.694
58.10	0.77	9.90	2.338	4.265	57.35	0.29	10.77	1.245	5.365	58.62	0.07	8.65	2.481	2.900
57.30	0.39	11.22	1.449	5.155	56.90	0.57	11.84	0.759	5.890	57.85	-0.91	8.46	3.810	3.069
56.42	0.99	13.81	2.027	7.540	54.91	0.84	12.37	2.092	7.765	58.53	-0.53	9.48	4.830	3.652
57.07	0.58	10.98	1.465	5.221	55.65	0.87	12.37	2.008	7.176	59.11	-0.05	8.46	5.302	2.127
56.88	0.55	9.78	1.563	4.557	57.22	0.10	8.80	1.131	3.867	57.94	-0.81	6.46	2.612	3.345
57.04	0.22	9.40	0.834	3.636	56.05	0.36	11.17	2.033	5.641	57.85	-0.38	7.96	3.754	2.219
54.77	0.18	11.46	1.996	7.416	54.67	0.39	11.85	0.698	7.726	58.75	-0.64	7.94	5.744	2.341
54.10	1.68	12.68	1.578	8.167	53.14	1.55	13.08	1.048	9.162	58.56	-0.34	8.21	7.528	2.496
57.17	0.80	11.12	0.991	5.420	55.73	0.69	11.49	1.491	6.722	59.01	-0.62	7.62	5.239	2.391
55.20	0.82	12.91	1.499	7.357	55.50	1.45	13.59	0.974	7.602	58.71	-0.67	8.89	6.074	2.963
53.70	0.49	11.13	1.526	7.913	53.75	0.66	11.93	0.819	8.354	57.90	-0.64	8.68	5.429	3.281

GROUP T

2nd bleaching –9					3rd bleaching - 10					Rehydration –11				
L^*_9	a^*_9	b^*_9	**Seq ΔE**	**ΔE_9**	L^*_{10}	a^*_{10}	b^*_{10}	**Seq ΔE**	**ΔE_{10}**	L^*_{11}	a^*_{11}	b^*_{11}	**Seq ΔE**	**ΔE_{11}**
58.89	-0.45	9.83	0.315	3.443	58.92	-0.07	9.50	0.504	3.070	57.71	-0.19	10.12	1.365	4.251
58.90	-0.32	7.08	0.935	1.747	58.71	-0.34	6.55	0.563	1.614	58.38	-0.25	6.74	0.391	1.946
58.20	-0.40	7.78	0.799	2.219	57.09	-0.44	7.62	1.122	3.111	56.32	-0.43	7.43	0.793	3.782
57.11	-0.88	8.38	0.373	3.570	57.33	-0.55	8.09	0.491	3.123	57.11	-0.32	8.46	0.488	3.436
57.46	-0.85	7.23	0.570	2.702	57.29	-0.76	6.73	0.536	2.746	56.71	-0.53	6.96	0.665	3.112
57.81	-0.90	9.25	0.948	2.911	57.51	-0.72	9.46	0.408	3.150	56.71	-0.48	9.77	0.891	3.836
58.68	-0.51	7.82	0.586	2.360	58.95	-0.16	7.88	0.446	1.951	58.29	-0.14	7.64	0.703	2.482
58.21	-0.51	9.08	0.689	3.275	58.39	-0.15	9.56	0.626	3.553	57.20	-0.73	8.68	1.590	3.659
58.04	-0.54	8.74	0.362	3.039	57.60	-0.50	8.74	0.442	3.327	57.38	-0.50	8.68	0.228	3.453
58.83	-0.39	7.64	1.130	2.065	58.85	-0.14	8.00	0.439	2.262	58.42	0.09	7.61	0.624	2.246
58.96	0.15	8.86	1.586	2.319	57.76	-0.52	8.39	1.453	2.929	58.40	-0.25	7.47	1.153	1.895
58.45	-0.43	11.28	1.805	4.620	58.76	-0.30	10.55	0.804	3.897	58.79	0.11	9.50	1.128	3.172
59.02	-0.28	7.71	0.790	1.660	58.66	-0.26	7.31	0.539	1.651	57.75	-0.45	7.16	0.942	2.436
58.00	-0.29	6.52	0.527	2.993	58.40	0.17	6.85	0.693	2.376	58.80	0.46	6.90	0.497	1.887
57.41	-0.97	7.84	0.746	2.720	57.40	-0.24	7.61	0.765	2.239	56.38	0.12	8.05	1.168	3.196
58.75	-0.60	7.38	0.561	2.046	58.82	-0.57	6.98	0.407	1.852	58.98	-0.10	7.40	0.650	1.661
58.52	-0.41	8.05	0.179	2.534	58.22	-0.31	7.82	0.391	2.683	57.66	-0.34	7.93	0.571	3.188
58.35	-0.75	7.28	0.754	2.966	58.56	-0.63	7.06	0.327	2.724	58.61	-0.25	7.14	0.392	2.496
58.48	-0.67	9.50	0.652	3.345	58.60	-0.33	8.28	1.272	2.684	58.15	-0.23	8.02	0.529	2.971
58.39	-0.73	8.42	0.562	2.819	57.71	-0.59	7.96	0.833	3.031	57.19	-0.50	8.39	0.681	3.632

GROUP W

	Baseline			Day 1 of staining				Day 2 of staining				
Specimen	L^*_0	a^*_0	b^*_0	L^*_1	a^*_1	b^*_1	**ΔE_1**	L^*_2	a^*_2	b^*_2	**Seq ΔE**	**ΔE_2**
1	60.59	1.24	7.14	56.70	0.28	9.81	4.815	54.39	0.00	16.68	7.253	11.445
2	59.04	0.16	5.38	56.71	-1.20	12.12	7.260	55.22	-0.89	13.58	2.109	9.107
3	60.06	0.78	6.07	54.62	-0.67	13.77	9.539	52.78	-0.08	18.82	5.407	14.707
4	59.89	1.36	7.07	56.08	-0.06	10.26	5.168	52.58	0.27	16.33	7.015	11.848
5	59.74	0.85	6.68	54.36	0.51	11.12	6.984	54.06	0.83	13.45	2.371	8.837
6	60.01	0.96	6.38	54.44	0.35	13.73	9.242	53.89	0.74	15.72	2.101	11.169
7	60.24	1.01	6.25	54.52	-0.21	15.16	10.658	53.75	0.68	18.51	3.551	13.876
8	61.08	1.18	8.03	54.31	-0.30	19.33	13.256	52.71	0.79	22.19	3.454	16.453
9	60.18	0.49	6.27	56.38	-0.66	17.23	11.657	54.04	-0.64	20.16	3.750	15.229
10	59.25	0.29	6.25	56.42	-0.42	12.36	6.771	55.68	-0.47	15.27	3.003	9.731
11	59.49	0.54	6.28	55.82	-0.6	9.94	5.307	54.85	-1.30	14.07	4.300	9.252
12	58.72	0.02	5.79	55.13	-0.71	16.86	11.660	53.44	-0.17	20.83	4.348	15.941
13	59.02	-0.13	5.75	55.99	-0.95	16.52	11.218	55.25	-0.79	18.26	1.898	13.082
14	60.47	0.57	7.08	55.44	-1.03	15.37	9.828	54.57	-0.33	17.76	2.638	12.234
15	59.48	0.12	6.50	54.28	0.53	20.08	14.547	52.05	1.42	22.62	3.495	17.797
16	59.49	0.06	5.66	54.92	-1.28	16.78	12.097	54.46	-0.85	18.69	2.011	13.997
17	59.95	0.52	6.97	55.30	-0.26	15.24	9.520	54.43	0.18	18.03	2.955	12.366
18	59.95	0.17	6.95	54.80	0.22	19.41	13.482	53.13	0.48	23.07	4.031	17.506
19	60.41	0.63	6.54	55.54	-0.61	14.72	9.600	55.06	-0.25	15.78	1.218	10.713
20	60.21	0.70	7.19	54.86	-0.84	14.44	9.141	53.65	-0.63	17.11	2.939	11.967

GROUP W

Day 3 of staining					Day 4 of staining					Day 5 of staining				
L^*_3	a^*_3	b^*_3	**Seq ΔE**	**ΔE_3**	L^*_4	a^*_4	b^*_4	**Seq ΔE**	**ΔE_4**	L^*_5	a^*_5	b^*_5	**Seq ΔE**	**ΔE_5**
55.23	-0.33	15.80	1.261	10.305	53.50	0.5	18.44	3.264	13.361	53.64	1.07	20.85	2.480	15.372
54.46	-0.80	17.57	4.063	13.057	54.17	-0.56	18.42	0.930	13.938	53.98	-0.11	18.01	0.638	13.609
51.13	0.51	19.25	1.804	15.923	50.72	1.26	21.05	1.993	17.660	51.24	1.47	21.86	0.985	18.100
52.44	0.70	18.09	1.817	13.318	51.20	1.49	21.44	3.658	16.794	49.58	2.19	21.64	1.776	17.868
52.64	0.87	19.20	5.923	14.393	51.68	1.77	20.81	2.079	16.293	48.93	2.70	22.88	3.565	19.563
52.39	0.91	19.18	3.775	14.897	51.58	1.59	21.05	2.148	16.931	49.68	2.87	23.02	3.021	19.679
52.80	0.93	19.07	1.131	14.823	51.51	1.82	21.40	2.808	17.504	50.04	2.00	22.33	1.749	19.068
51.36	1.53	24.69	2.936	19.291	48.65	3.60	26.70	3.958	22.559	47.36	5.50	25.26	2.711	22.445
54.21	0.25	21.24	1.410	16.118	53.55	0.86	22.89	1.879	17.897	53.48	1.17	23.39	0.592	18.397
54.62	-0.35	19.21	4.082	13.777	53.58	0.06	20.32	1.575	15.171	53.23	0.50	22.27	2.029	17.115
54.64	-0.33	18.72	4.755	13.380	53.76	-0.13	19.66	1.303	14.571	53.38	0.49	21.14	1.649	16.067
53.40	0.40	22.30	1.577	17.350	52.19	1.05	22.08	1.391	17.580	52.01	1.55	23.20	1.240	18.721
54.45	0.05	21.39	3.338	16.295	53.72	0.20	22.35	1.215	17.429	52.26	1.06	23.19	1.891	18.742
55.74	-0.13	20.24	2.749	14.002	54.25	0.20	21.80	2.182	15.984	52.98	0.55	23.31	2.004	17.875
51.99	2.17	23.98	1.554	19.127	50.40	3.42	24.97	2.252	20.844	49.72	3.79	24.63	0.846	20.915
53.01	-0.57	21.73	3.380	17.339	52.87	0.26	22.66	1.254	18.245	52.65	0.42	22.61	0.277	18.282
53.71	0.41	19.24	1.427	13.766	52.96	0.83	20.94	1.905	15.624	52.29	1.37	22.87	2.113	17.669
51.74	2.47	26.24	3.993	21.090	50.95	3.40	26.25	1.220	21.539	50.16	4.70	25.47	1.710	21.433
54.59	0.09	18.48	2.762	13.294	53.90	0.15	19.44	1.184	14.458	54.00	0.30	21.07	1.640	15.885
52.59	-0.53	18.60	1.831	13.776	52.26	0.69	21.64	3.292	16.493	51.38	0.40	22.26	1.115	17.469

Day 6 of staining					**Day 7 of staining**									
L^*_6	a^*_6	b^*_6	**Seq ΔE**	**ΔE_6**	L^*_7	a^*_7	b^*_7	**Seq ΔE**	**ΔE_7**	L^*_8	a^*_8	b^*_8	**Seq ΔE**	**ΔE_8**
52.68	1.02	21.02	0.976	15.977	52.25	2.38	23.79	3.116	18.657	55.16	0.11	21.89	4.151	15.758
53.65	0.66	21.19	3.288	16.711	52.96	0.57	20.63	0.893	16.422	54.55	-0.71	21.44	2.196	16.699
49.96	2.20	22.28	1.532	19.152	50.59	2.22	22.31	0.631	18.854	51.70	1.59	24.81	2.807	20.536
48.67	3.71	23.95	2.911	20.405	49.12	2.89	20.72	3.363	17.454	50.10	3.04	26.89	6.249	22.170
49.52	2.97	23.08	0.679	19.440	49.14	3.22	23.01	0.460	19.612	50.21	2.79	26.17	3.364	21.782
49.01	2.88	22.91	0.679	19.948	48.97	3.25	22.84	0.379	19.951	50.38	2.58	25.79	3.338	21.728
49.83	2.73	22.58	0.800	19.442	50.34	3.30	22.84	0.808	19.455	50.70	2.26	25.28	2.677	21.324
48.03	4.80	26.36	1.466	22.790	47.63	5.28	27.04	0.923	23.645	51.20	3.59	28.63	4.258	22.974
53.35	1.11	23.58	0.238	18.619	51.71	2.17	24.31	2.085	20.000	54.60	0.74	25.69	3.507	20.207
52.66	0.86	22.46	0.700	17.508	52.24	1.28	22.86	0.716	18.056	54.03	-0.17	24.28	2.706	18.776
53.09	1.06	22.25	1.281	17.213	52.83	1.18	22.47	0.361	17.518	54.17	-0.47	22.80	2.151	17.385
52.14	2.27	24.61	1.589	20.064	51.49	2.67	24.52	0.769	20.251	53.55	0.99	26.07	3.077	20.951
51.83	1.59	23.79	0.909	19.496	50.99	1.87	23.98	0.906	20.020	53.35	0.60	25.59	3.126	20.647
52.63	1.25	23.98	1.030	18.642	50.62	1.59	23.46	2.104	19.141	52.60	0.29	25.58	3.179	20.106
49.15	4.97	24.79	1.320	21.558	48.81	4.95	24.78	0.341	21.710	50.77	3.78	28.26	4.162	23.723
52.12	0.92	23.48	1.135	19.303	51.59	1.54	23.71	0.847	19.759	53.33	0.03	24.84	2.566	20.145
52.45	0.89	22.65	0.552	17.385	52.01	1.44	23.4	1.029	18.271	53.32	0.44	24.61	2.045	18.845
49.02	5.99	25.52	1.722	22.320	48.97	5.93	24.96	0.565	21.865	51.79	3.51	28.83	5.365	23.590
53.13	0.79	22.46	1.711	17.506	53.17	0.98	22.95	0.527	17.940	54.97	-0.37	23.60	2.342	17.934
50.66	0.97	22.31	0.920	17.885	51.12	1.11	23.19	1.003	18.406	52.40	0.79	24.81	2.089	19.274

2nd bleaching - 9					3rd bleaching - 10					Rehydration - 11				
L^*_9	a^*_9	b^*_9	**Seq ΔE**	**ΔE_9**	L^*_{10}	a^*_{10}	b^*_{10}	**Seq ΔE**	**ΔE_{10}**	L^*_{11}	a^*_{11}	b^*_{11}	**Seq ΔE**	**ΔE_{11}**
5.26	0.35	21.79	0.279	15.615	54.68	1.27	22.78	1.471	16.719	54.74	1.70	21.58	1.276	15.587
54.11	0.41	22.71	1.750	18.019	54.61	0.40	22.34	0.622	17.531	54.41	0.94	21.59	0.946	16.876
52.34	1.58	24.69	0.651	20.173	49.91	2.74	25.10	2.724	21.657	50.78	2.68	24.34	1.157	20.580
52.04	2.66	25.65	2.334	20.212	52.69	2.10	24.57	1.379	18.938	52.42	2.94	23.29	1.555	17.927
50.99	3.25	26.35	0.923	21.662	53.26	2.36	24.35	3.154	18.881	50.42	3.40	25.32	3.176	20.996
51.59	2.95	25.57	1.284	21.050	52.81	2.78	25.20	1.286	20.232	51.47	2.78	25.03	1.351	20.593
52.87	2.31	25.07	2.181	20.253	51.94	3.24	25.46	1.372	21.045	52.00	3.79	24.65	0.981	20.352
52.14	3.69	28.11	1.079	22.123	52.52	3.29	27.50	0.822	21.373	51.26	4.75	27.70	1.939	22.273
54.80	0.99	24.43	1.300	18.947	54.20	0.73	23.60	1.057	18.334	54.58	1.99	22.71	1.589	17.432
53.48	0.91	24.5	1.232	19.150	54.22	1.36	23.59	1.256	18.086	53.65	1.64	22.77	1.037	17.496
54.21	0.44	22.63	0.927	17.182	54.57	0.67	21.14	1.550	15.654	53.40	1.48	21.22	1.425	16.161
53.32	1.25	25.74	0.479	20.704	53.19	1.04	24.01	1.748	19.068	52.98	2.13	23.74	1.142	18.963
53.34	0.85	24.92	0.715	20.018	53.86	0.88	23.85	1.190	18.848	53.37	2.05	23.26	1.399	18.528
53.98	1.03	24.95	1.688	19.018	54.19	0.89	23.56	1.413	17.639	53.11	1.85	22.78	1.642	17.387
51.37	3.86	28.17	0.612	23.438	51.92	3.50	27.48	0.953	22.555	51.36	4.68	27.07	1.369	22.580
52.95	1.06	24.78	1.099	20.232	53.64	0.54	22.76	2.197	18.079	53.12	1.74	23.02	1.333	18.568
54.33	0.8	23.67	1.426	17.623	54.50	0.93	22.41	1.278	16.379	53.98	1.85	22.28	1.065	16.487
51.48	4.45	29.1	1.026	24.097	52.43	3.99	28.50	1.214	23.142	51.55	4.94	27.94	1.411	23.106
55.03	0.41	22.69	1.200	17.024	54.42	0.76	22.59	0.710	17.132	53.68	1.77	22.85	1.279	17.681
53.07	2.18	26.35	2.180	20.501	52.71	1.73	24.50	1.938	18.893	53.59	2.46	23.25	1.694	17.460

APPENDIX 2 – CHANGES IN THE L* a* b* AND E* FOR ALL THREE GROUPS

GROUP C – STAINING PERIOD (DAY 1 TO 3)

	ΔL_1^*	Δa_1^*	Δb_1^*	ΔE_1^*	ΔL_2^*	Δa_2^*	Δb_2^*	ΔE_2^*	ΔL_3^*	Δa_3^*	Δb_3^*	ΔE_3^*
Spec 1	0.120	0.550	0.370	0.674	0.320	0.430	0.290	0.609	0.440	0.360	-0.020	0.569
Spec 2	0.450	-0.020	-0.390	0.596	0.130	-0.390	-0.800	0.899	1.180	0.500	0.430	1.352
Spec 3	0.090	0.170	-0.240	0.308	0.070	0.340	0.110	0.364	-0.930	-0.010	-0.430	1.025
Spec 4	-0.550	-0.040	0.070	0.556	-0.440	-0.010	-0.120	0.456	-0.630	0.030	-0.730	0.965
Spec 5	0.680	0.380	0.200	0.804	1.010	0.780	0.880	1.550	0.790	0.820	1.000	1.515
Spec 6	-0.040	0.050	-0.220	0.229	0.550	0.510	0.630	0.980	-0.480	0.010	-0.420	0.638
Spec 7	0.080	-0.240	0.370	0.448	0.020	-0.020	0.680	0.681	0.630	0.060	0.470	0.788
Spec 8	0.250	0.000	-0.270	0.368	0.100	0.010	-0.390	0.403	0.180	0.070	-0.290	0.348
Spec 9	-0.820	0.320	0.450	0.989	-0.800	0.040	-0.050	0.803	0.010	0.120	-0.130	0.177
Spec 10	-0.060	-0.230	-0.710	0.749	0.360	-0.250	-0.590	0.735	0.170	-0.200	-0.750	0.795
Spec 11	-0.980	0.090	-0.480	1.095	0.240	0.390	0.310	0.553	0.830	0.390	-0.170	0.933
Spec 12	-0.230	0.070	-0.140	0.278	-0.230	-0.280	-0.830	0.906	0.370	0.490	0.700	0.931
Spec 13	-0.550	-0.570	-0.910	1.206	-1.010	-0.670	-1.190	1.699	-0.800	-0.600	-1.370	1.696
Spec 14	-0.390	0.310	0.340	0.603	-0.190	0.010	0.010	0.191	-0.360	0.070	-0.060	0.372
Spec 15	-0.330	-0.440	-1.080	1.212	-0.620	-0.560	-1.240	1.495	-0.410	-0.260	-0.570	0.749
Spec 16	-0.330	-0.140	-0.440	0.568	0.490	0.110	-0.390	0.636	-0.230	0.180	-0.350	0.456
Spec 17	0.180	0.630	0.700	0.959	0.200	0.390	-0.010	0.438	-0.070	0.430	0.200	0.479
Spec 18	-0.600	-0.040	-0.350	0.696	-0.140	0.080	-0.250	0.297	0.230	0.070	-0.130	0.273
Spec 19	-0.670	-0.260	-0.900	1.152	-0.590	-0.250	-0.840	1.057	0.450	-0.050	-0.440	0.631
Spec 20	0.040	-0.360	-0.010	0.362	-0.050	-0.020	0.620	0.622	0.790	-0.190	0.190	0.834
Mean	-0.183	0.012	-0.182	0.693	-0.029	0.032	-0.159	0.769	0.108	0.115	-0.144	0.776
SD	0.435	0.319	0.496	0.320	0.492	0.369	0.625	0.420	0.591	0.319	0.547	0.404

Spec - Specimen

GROUP C – STAINING PERIOD (DAY 4 TO 7)

ΔL_4^*	Δa_4^*	Δb_4^*	ΔE_4^*	ΔL_5^*	Δa_5^*	Δb_5^*	ΔE_5^*	ΔL_6^*	Δa_6^*	Δb_6^*	ΔE_6^*	ΔL_7^*	Δa_7^*	Δb_7^*	ΔE_7^*
1.010	0.910	0.310	1.394	0.110	0.390	0.040	0.407	0.630	0.340	-0.180	0.738	1.120	0.370	0.090	1.183
0.300	0.050	-0.760	0.819	0.100	-0.180	-0.840	0.865	0.740	-0.030	-0.800	1.090	-0.500	-0.450	-1.510	1.653
-0.160	0.160	-0.490	0.540	-0.070	0.070	-0.670	0.677	-0.140	-0.180	-0.840	0.870	-0.250	-0.600	-0.930	1.135
-0.210	-0.130	-0.620	0.667	-0.500	0.030	-0.020	0.501	-0.190	-0.190	-0.500	0.568	-0.050	-0.050	0.020	0.073
0.890	0.660	0.150	1.118	0.410	0.110	-0.640	0.768	0.470	0.060	-0.700	0.845	1.100	0.510	0.270	1.242
-0.120	0.330	-0.330	0.482	-0.100	0.210	-0.530	0.579	0.440	0.440	0.050	0.624	0.520	0.430	-0.140	0.689
0.920	0.010	0.360	0.988	1.570	0.120	0.330	1.609	1.640	0.090	0.520	1.723	1.290	-0.120	-0.200	1.311
-0.020	0.090	-0.440	0.450	0.130	0.130	-0.360	0.404	-0.320	-0.180	-0.600	0.703	-0.590	-0.360	-0.900	1.135
-0.360	0.070	-0.350	0.507	-0.200	0.120	-0.200	0.307	-0.090	-0.130	-0.670	0.688	0.000	0.200	-0.140	0.244
0.100	-0.160	-0.850	0.871	0.070	-0.050	-0.780	0.785	0.730	-0.060	-0.670	0.993	0.880	-0.030	-0.740	1.150
-0.080	0.180	-0.350	0.402	0.530	0.470	-0.100	0.715	0.270	0.040	-1.180	1.211	0.020	-0.170	-1.570	1.579
-0.390	0.020	-0.360	0.531	-0.140	-0.210	-1.020	1.051	-0.140	-0.150	-0.810	0.836	0.070	0.040	-0.570	0.576
-0.550	-0.440	-1.310	1.487	-0.320	-0.430	-1.140	1.260	-0.380	-0.500	-1.300	1.444	-0.720	-0.870	-1.300	1.722
0.170	0.090	-0.360	0.408	0.710	0.330	0.240	0.819	-0.320	-0.190	-0.680	0.775	-0.780	-0.490	-1.060	1.404
0.320	-0.120	-0.800	0.870	-0.690	-0.470	-1.350	1.587	-0.040	-0.440	-0.990	1.084	-0.390	-0.440	-1.510	1.620
0.580	0.060	-0.880	1.056	0.020	-0.070	-1.180	1.182	-0.680	-0.230	-1.040	1.264	0.140	0.180	-0.520	0.568
0.110	0.510	0.090	0.529	-0.080	0.390	-0.070	0.404	0.140	0.590	0.260	0.660	0.190	0.230	-0.400	0.499
-0.400	-0.160	-0.720	0.839	-0.780	-0.220	-0.400	0.904	-0.200	0.040	-0.370	0.422	-0.270	-0.240	-0.630	0.726
-0.500	-0.220	-0.900	1.053	-0.410	-0.520	-1.650	1.778	-0.440	-0.350	-0.990	1.139	0.520	-0.250	-0.820	1.003
0.730	-0.270	-0.010	0.778	1.300	-0.150	-0.070	1.310	1.380	-0.190	0.110	1.397	1.000	-0.320	-0.740	1.285
0.117	0.082	-0.431	0.789	0.083	0.003	-0.521	0.896	0.175	-0.061	-0.569	0.954	0.165	-0.122	-0.665	1.040
0.491	0.323	0.443	0.321	0.594	0.288	0.554	0.436	0.611	0.275	0.494	0.335	0.648	0.367	0.550	0.478

GROUP C – BLEACHING AND REHYDRATION PERIOD (DAY 8 TO 11)

ΔL_8^*	Δa_8^*	Δb_8^*	ΔE_8^*	ΔL_9^*	Δa_9^*	Δb_9^*	ΔE_9^*	ΔL_{10}^*	Δa_{10}^*	Δb_{10}^*	ΔE_{10}^*	ΔL_{11}^*	Δa_{11}^*	Δb_{11}^*	ΔE_{11}^*
-0.790	-0.070	-0.400	0.888	-1.150	-0.330	-1.190	1.687	0.070	0.150	-0.260	0.308	-1.510	-0.350	-1.320	2.036
0.470	0.100	0.250	0.542	-0.520	-0.380	-0.820	1.043	-0.350	-0.020	-0.440	0.563	0.070	-0.230	-0.650	0.693
0.320	0.420	-0.100	0.537	-0.570	-0.010	-0.940	1.099	-0.600	0.080	-0.900	1.085	-0.810	-0.040	-0.910	1.219
0.410	0.100	-0.600	0.734	-0.500	-0.360	-1.560	1.677	-0.410	-0.380	-1.500	1.601	-0.080	0.100	-0.710	0.721
-0.500	-0.540	-1.230	1.433	-1.040	-0.910	-2.180	2.581	-1.100	-0.890	-2.120	2.549	-1.450	-0.920	-2.090	2.705
-0.500	-0.650	-0.950	1.255	-0.930	-0.800	-1.600	2.016	-0.920	-0.970	-1.790	2.234	-1.500	-0.990	-1.850	2.579
0.020	-0.060	0.000	0.063	-0.770	-0.370	-1.030	1.338	-0.610	-0.270	-0.850	1.081	-1.510	-0.310	-0.830	1.751
0.780	-0.120	-0.470	0.919	-0.130	-0.140	-0.770	0.793	0.060	-0.450	-1.300	1.377	-0.110	-0.360	-1.330	1.382
-0.060	-0.240	-0.510	0.567	-0.720	-0.760	-1.520	1.846	-0.860	-0.650	-1.410	1.775	-1.190	-0.970	-1.950	2.482
-1.130	-0.170	-0.500	1.247	-1.720	-0.700	-1.610	2.458	-1.790	-0.790	-1.690	2.585	-2.290	-0.890	-2.010	3.174
0.260	0.060	0.050	0.271	0.020	-0.120	-0.430	0.447	0.020	-0.040	0.390	0.393	-1.130	-0.520	-0.480	1.333
-0.450	-0.300	-0.560	0.779	-1.560	-0.850	-1.610	2.398	-1.400	-0.920	-1.920	2.548	-0.850	-0.480	-1.100	1.471
0.230	0.290	-0.360	0.516	-0.840	-0.400	-1.650	1.894	-1.310	-0.530	-1.980	2.433	-1.120	-0.340	-1.760	2.114
0.360	0.250	0.080	0.446	0.000	-0.020	-0.600	0.600	0.000	-0.080	-0.490	0.496	-0.090	-0.290	-1.020	1.064
-0.180	-0.090	-0.280	0.345	-0.330	-0.510	-1.040	1.204	-0.730	-0.500	-1.290	1.564	-1.740	-0.710	-1.600	2.468
0.520	-0.350	-0.770	0.993	-1.040	-0.820	-1.590	2.069	-0.520	-0.890	-1.770	2.048	-1.370	-1.110	-2.430	3.002
0.500	0.530	0.920	1.174	-0.460	-0.190	-0.440	0.664	-0.240	-0.360	-0.800	0.910	-0.450	0.030	-0.050	0.454
-0.390	-0.270	-0.690	0.837	-0.030	-0.050	-0.580	0.583	-0.770	-0.320	-0.940	1.257	-1.490	-0.660	-1.720	2.369
-1.030	-0.550	-1.010	1.544	-1.320	-0.640	-1.270	1.940	-1.090	-0.250	-0.780	1.363	-1.720	-0.660	-1.720	2.520
-0.390	-0.120	0.060	0.412	-0.270	-0.480	-0.930	1.081	-0.610	-0.410	-0.570	0.930	-0.390	-0.450	-0.680	0.904
-0.077	-0.089	-0.354	0.775	-0.694	-0.442	-1.168	1.471	-0.658	-0.425	-1.121	1.455	-1.037	-0.508	-1.311	1.822
0.550	0.316	0.498	0.406	0.509	0.297	0.485	0.673	0.516	0.345	0.666	0.753	0.667	0.350	0.635	0.829

GROUP T – STAINING PERIOD (DAY 1 TO 3)

	ΔL_1^*	Δa_1^*	Δb_1^*	ΔE_1^*	ΔL_2^*	Δa_2^*	Δb_2^*	ΔE_2^*	ΔL_3^*	Δa_3^*	Δb_3^*	ΔE_3^*
Spec 1	3.560	0.490	2.280	4.256	-3.460	1.100	5.320	6.441	-5.050	0.900	5.740	7.698
Spec 2	-3.080	-0.200	1.360	3.373	-4.210	-0.040	2.760	5.034	-2.190	0.090	1.860	2.875
Spec 3	-2.840	-0.510	0.040	2.886	-4.050	-0.310	1.990	4.523	-4.490	-0.400	2.880	5.349
Spec 4	-2.050	-0.340	0.820	2.234	-2.570	0.340	3.550	4.396	-3.310	0.680	4.780	5.854
Spec 5	-1.800	-0.340	0.090	1.834	-2.070	-1.100	0.110	2.347	-2.520	-0.260	2.340	3.449
Spec 6	-3.870	0.970	2.950	4.962	-4.350	0.470	3.800	5.795	-4.850	0.530	4.120	6.386
Spec 7	-2.460	0.050	0.900	2.620	-2.850	-0.110	2.300	3.664	-3.160	-0.010	2.630	4.111
Spec 8	-2.760	0.150	2.030	3.429	-3.750	1.160	5.070	6.412	-4.850	1.070	5.610	7.493
Spec 9	-2.560	0.420	2.030	3.294	-5.070	0.870	4.380	6.756	-4.380	0.800	4.950	6.658
Spec 10	-2.050	0.530	2.140	3.010	-2.220	0.680	2.510	3.419	-1.810	0.230	2.130	2.805
Spec 11	-1.960	-0.110	0.660	2.071	-2.460	-0.200	2.040	3.202	-2.100	0.100	2.670	3.398
Spec 12	-3.250	0.050	2.320	3.993	-4.570	-0.140	3.140	5.547	-3.560	-0.290	3.590	5.064
Spec 13	-0.690	0.750	1.090	1.492	-1.550	0.130	1.740	2.334	-1.420	0.290	2.810	3.162
Spec 14	-2.230	-0.460	-1.340	2.642	-2.420	-0.410	0.600	2.527	-2.100	-0.630	0.790	2.330
Spec 15	-2.210	-0.220	0.710	2.332	-2.990	-0.090	2.550	3.931	-2.760	0.310	2.960	4.059
Spec 16	-1.910	0.610	1.710	2.635	-4.820	-0.300	2.510	5.443	-2.650	0.150	2.800	3.858
Spec 17	-2.630	0.010	1.020	2.821	-3.070	-0.080	1.610	3.467	-3.620	0.050	2.380	4.333
Spec 18	-3.420	-0.220	1.080	3.593	-3.770	-0.250	1.980	4.266	-4.280	0.080	2.970	5.210
Spec 19	-2.760	-0.830	0.610	2.946	-3.550	-0.310	2.380	4.285	-4.090	-0.440	4.340	5.980
Spec 20	-2.400	-0.030	1.200	2.683	-4.400	0.130	3.300	5.502	-3.350	0.190	3.620	4.936
Mean	-2.169	0.039	1.185	2.955	-3.410	0.077	2.682	4.464	-3.327	0.172	3.299	4.750
SD	1.511	0.465	0.979	0.834	1.004	0.554	1.314	1.371	1.110	0.457	1.285	1.573

GROUP T – STAINING PERIOD (DAY 4 TO 7)

ΔL_4^*	Δa_4^*	Δb_4^*	ΔE_4^*	ΔL_5^*	Δa_5^*	Δb_5^*	ΔE_5^*	ΔL_6^*	Δa_6^*	Δb_6^*	ΔE_6^*	ΔL_7^*	Δa_7^*	Δb_7^*	ΔE_7^*
-4.560	0.490	5.770	7.371	-5.590	0.530	6.550	8.627	-5.950	1.100	7.970	10.007	-5.670	0.360	6.890	8.930
-1.540	-0.340	2.000	2.547	-1.740	-0.340	1.990	2.665	-2.180	-0.030	3.520	4.140	-2.030	-0.260	3.600	4.141
-4.430	-0.300	3.620	5.729	-4.370	-0.390	4.050	5.971	-6.610	-0.310	4.840	8.198	-5.100	-0.580	3.830	6.404
-4.140	0.390	5.820	7.153	-4.700	0.230	5.700	7.391	-5.950	0.260	5.850	8.348	-3.440	0.750	6.120	7.060
-3.470	-0.190	4.080	5.359	-3.250	-0.810	3.750	5.028	-3.430	-0.750	4.090	5.390	-4.560	0.350	4.910	6.710
-6.160	0.500	5.180	8.064	-5.590	0.520	2.980	6.356	-7.160	0.460	6.910	9.961	-7.600	-0.140	6.960	10.306
-2.480	-0.360	2.350	3.435	-2.550	-0.630	2.520	3.640	-4.500	0.080	5.200	6.877	-4.720	0.110	5.120	6.965
-4.820	0.810	6.000	7.739	-5.230	0.280	5.700	7.741	-6.860	0.540	6.850	9.709	-5.680	0.460	6.250	8.458
-4.690	0.680	5.570	7.313	-5.610	0.330	5.570	7.912	-6.620	0.350	6.090	9.002	-6.150	0.640	5.760	8.450
-1.900	0.290	3.430	3.932	-2.530	0.500	5.990	6.522	-2.060	0.510	3.700	4.265	-2.810	0.030	4.570	5.365
-1.980	0.180	3.760	4.253	-2.040	-0.410	3.120	3.750	-2.680	-0.180	4.400	5.155	-3.080	0.000	5.020	5.890
-4.790	-0.610	4.190	6.393	-4.010	-0.520	3.880	5.604	-5.020	-0.270	5.620	7.540	-6.530	-0.420	4.180	7.765
-2.710	0.160	4.310	5.094	-1.760	-0.060	3.380	3.811	-2.810	0.000	4.400	5.221	-4.230	0.290	5.790	7.176
-2.410	-0.800	1.640	3.023	-2.390	-0.730	1.720	3.034	-3.630	-0.680	2.670	4.557	-3.290	-1.130	1.690	3.867
-2.100	-0.170	2.800	3.504	-1.720	-0.390	2.210	2.827	-2.040	-0.420	2.980	3.636	-3.030	-0.280	4.750	5.641
-2.840	0.010	3.330	4.377	-4.060	-0.770	3.560	5.454	-5.550	-0.620	4.880	7.416	-5.650	-0.050	5.270	7.726
-6.080	-0.170	3.880	7.215	-5.980	-0.170	3.740	7.055	-6.350	0.520	5.110	8.167	-7.310	0.390	5.510	9.162
-4.560	-0.440	3.610	5.833	-3.660	-0.580	3.150	4.864	-3.700	-0.010	3.960	5.420	-5.140	-0.120	4.330	6.722
-3.920	-0.540	3.510	5.289	-4.540	-0.780	3.740	5.934	-5.440	-0.570	4.920	7.357	-5.140	0.060	5.600	7.602
-4.170	0.010	4.560	6.179	-5.030	-0.160	4.690	6.879	-6.530	0.010	4.470	7.913	-6.480	0.180	5.270	8.354
-3.688	-0.020	3.971	5.490	-3.818	-0.218	3.900	5.553	-4.754	0.000	4.922	6.914	-4.882	0.032	5.071	7.135
1.376	0.448	1.253	1.687	1.471	0.467	1.394	1.805	1.783	0.490	1.347	2.048	1.564	0.440	1.209	1.635

GROUP T – BLEACHING AND REHYDRATION PERIOD (DAY 8 TO 11)

ΔL_8^*	Δa_8^*	Δb_8^*	ΔE_8^*	ΔL_9^*	Δa_9^*	Δb_9^*	ΔE_9^*	ΔL_{10}^*	Δa_{10}^*	Δb_{10}^*	ΔE_{10}^*	ΔL_{11}^*	Δa_{11}^*	Δb_{11}^*	ΔE_{11}^*
4.060	-1.180	-3.610	5.560	4.270	-1.130	-3.840	5.853	4.300	-0.750	-4.170	6.037	3.090	-0.870	-3.550	4.786
-0.010	-0.640	-2.320	2.407	0.860	-0.320	-2.440	2.607	0.670	-0.340	-2.970	3.064	0.340	-0.250	-2.780	2.812
2.860	-1.280	-3.300	4.551	3.480	-0.790	-3.180	4.780	2.370	-0.830	-3.340	4.179	1.600	-0.820	-3.530	3.961
0.890	-2.040	-3.770	4.378	0.840	-2.160	-4.120	4.727	1.060	-1.830	-4.410	4.891	0.840	-1.600	-4.040	4.426
2.430	-2.340	-3.990	5.225	2.560	-2.120	-4.500	5.594	2.390	-2.030	-5.000	5.902	1.810	-1.800	-4.770	5.410
5.240	-1.300	-4.490	7.022	5.730	-1.350	-5.300	7.921	5.430	-1.170	-5.090	7.534	4.630	-0.930	-4.780	6.719
2.550	-1.560	-4.310	5.245	2.870	-1.540	-4.800	5.801	3.140	-1.190	-4.740	5.809	2.480	-1.170	-4.980	5.685
4.160	-1.510	-2.900	5.291	4.050	-1.210	-3.510	5.494	4.230	-0.850	-3.030	5.272	3.040	-1.430	-3.910	5.155
4.400	-1.580	-3.940	6.114	4.200	-1.610	-3.640	5.786	3.760	-1.570	-3.640	5.464	3.540	-1.570	-3.700	5.356
1.270	-0.220	-2.120	2.481	1.480	-0.680	-3.130	3.528	1.500	-0.430	-2.770	3.179	1.070	-0.200	-3.160	3.342
0.950	-1.480	-3.380	3.810	2.060	-0.420	-2.980	3.647	0.860	-1.090	-3.450	3.719	1.500	-0.820	-4.370	4.692
3.620	-1.370	-2.890	4.830	3.540	-1.270	-1.090	3.916	3.850	-1.140	-1.820	4.408	3.880	-0.730	-2.870	4.881
3.460	-0.920	-3.910	5.302	3.370	-1.150	-4.660	5.865	3.010	-1.130	-5.060	5.995	2.100	-1.320	-5.210	5.770
0.720	-0.910	-2.340	2.612	0.780	-0.390	-2.280	2.441	1.180	0.070	-1.950	2.280	1.580	0.360	-1.900	2.497
1.800	-0.740	-3.210	3.754	1.360	-1.330	-3.330	3.835	1.350	-0.600	-3.560	3.854	0.330	-0.240	-3.120	3.147
4.080	-1.030	-3.910	5.744	4.080	-0.990	-4.470	6.132	4.150	-0.960	-4.870	6.470	4.310	-0.490	-4.450	6.214
5.420	-1.890	-4.870	7.528	5.380	-1.960	-5.030	7.621	5.080	-1.860	-5.260	7.545	4.520	-1.890	-5.150	7.108
3.280	-1.310	-3.870	5.239	2.620	-1.440	-4.210	5.164	2.830	-1.320	-4.430	5.420	2.880	-0.940	-4.350	5.301
3.210	-2.120	-4.700	6.074	2.980	-2.120	-4.090	5.487	3.100	-1.780	-5.310	6.401	2.650	-1.680	-5.570	6.393
4.150	-1.300	-3.250	5.429	4.640	-1.390	-3.510	5.982	3.960	-1.250	-3.970	5.745	3.440	-1.160	-3.540	5.071
2.927	-1.336	-3.554	4.930	3.058	-1.269	-3.706	5.109	2.911	-1.103	-3.942	5.158	2.482	-0.978	-3.987	4.936
1.561	0.521	0.777	1.387	1.489	0.564	1.027	1.450	1.446	0.546	1.070	1.442	1.338	0.605	0.953	1.278

GROUP W – STAINING PERIOD (DAY 1 TO 3)

	ΔL_1^*	Δa_1^*	Δb_1^*	ΔE_1^*	ΔL_2^*	Δa_2^*	Δb_2^*	ΔE_2^*	ΔL_3^*	Δa_3^*	Δb_3^*	ΔE_3^*
Spec 1	-3.890	-0.960	2.670	4.815	-6.200	-1.240	9.540	11.445	-5.360	-1.570	8.660	10.305
Spec 2	-2.330	-1.360	6.740	7.260	-3.820	-1.050	8.200	9.107	-4.580	-0.960	12.190	13.057
Spec 3	-5.440	-1.450	7.700	9.539	-7.280	-0.860	12.750	14.707	-8.930	-0.270	13.180	15.923
Spec 4	-3.810	-1.420	3.190	5.168	-7.310	-1.090	9.260	11.848	-7.450	-0.660	11.020	13.318
Spec 5	-5.380	-0.340	4.440	6.984	-5.680	-0.020	6.770	8.837	-7.100	0.020	12.520	14.393
Spec 6	-5.570	-0.610	7.350	9.242	-6.120	-0.220	9.340	11.169	-7.620	-0.050	12.800	14.897
Spec 7	-5.720	-1.220	8.910	10.658	-6.490	-0.330	12.260	13.876	-7.440	-0.080	12.820	14.823
Spec 8	-6.770	-1.480	11.300	13.256	-8.370	-0.390	14.160	16.453	-9.720	0.350	16.660	19.291
Spec 9	-3.800	-1.150	10.960	11.657	-6.140	-1.130	13.890	15.229	-5.970	-0.240	14.970	16.118
Spec 10	-2.830	-0.710	6.110	6.771	-3.570	-0.760	9.020	9.731	-4.630	-0.640	12.960	13.777
Spec 11	-3.670	-1.140	3.660	5.307	-4.640	-1.840	7.790	9.252	-4.850	-0.870	12.440	13.380
Spec 12	-3.590	-0.730	11.070	11.660	-5.280	-0.190	15.040	15.941	-5.320	0.380	16.510	17.350
Spec 13	-3.030	-0.820	10.770	11.218	-3.770	-0.660	12.510	13.082	-4.570	0.180	15.640	16.295
Spec 14	-5.030	-1.600	8.290	9.828	-5.900	-0.900	10.680	12.234	-4.730	-0.700	13.160	14.002
Spec 15	-5.200	0.410	13.580	14.547	-7.430	1.300	16.120	17.797	-7.490	2.050	17.480	19.127
Spec 16	-4.570	-1.340	11.120	12.097	-5.030	-0.910	13.030	13.997	-6.480	-0.630	16.070	17.339
Spec 17	-4.650	-0.780	8.270	9.520	-5.520	-0.340	11.060	12.366	-6.240	-0.110	12.270	13.766
Spec 18	-5.150	0.050	12.460	13.482	-6.820	0.310	16.120	17.506	-8.210	2.300	19.290	21.090
Spec 19	-4.870	-1.240	8.180	9.600	-5.350	-0.880	9.240	10.713	-5.820	-0.540	11.940	13.294
Spec 20	-5.350	-1.540	7.250	9.141	-6.560	-1.330	9.920	11.967	-7.620	-1.230	11.410	13.776
Mean	-4.533	-0.972	8.201	9.588	-5.864	-0.627	11.335	12.863	-6.507	-0.164	13.700	15.266
SD	1.124	0.541	3.137	2.838	1.291	0.681	2.792	2.760	1.541	0.948	2.556	2.575

GROUP W – STAINING PERIOD (DAY 4 TO 7)

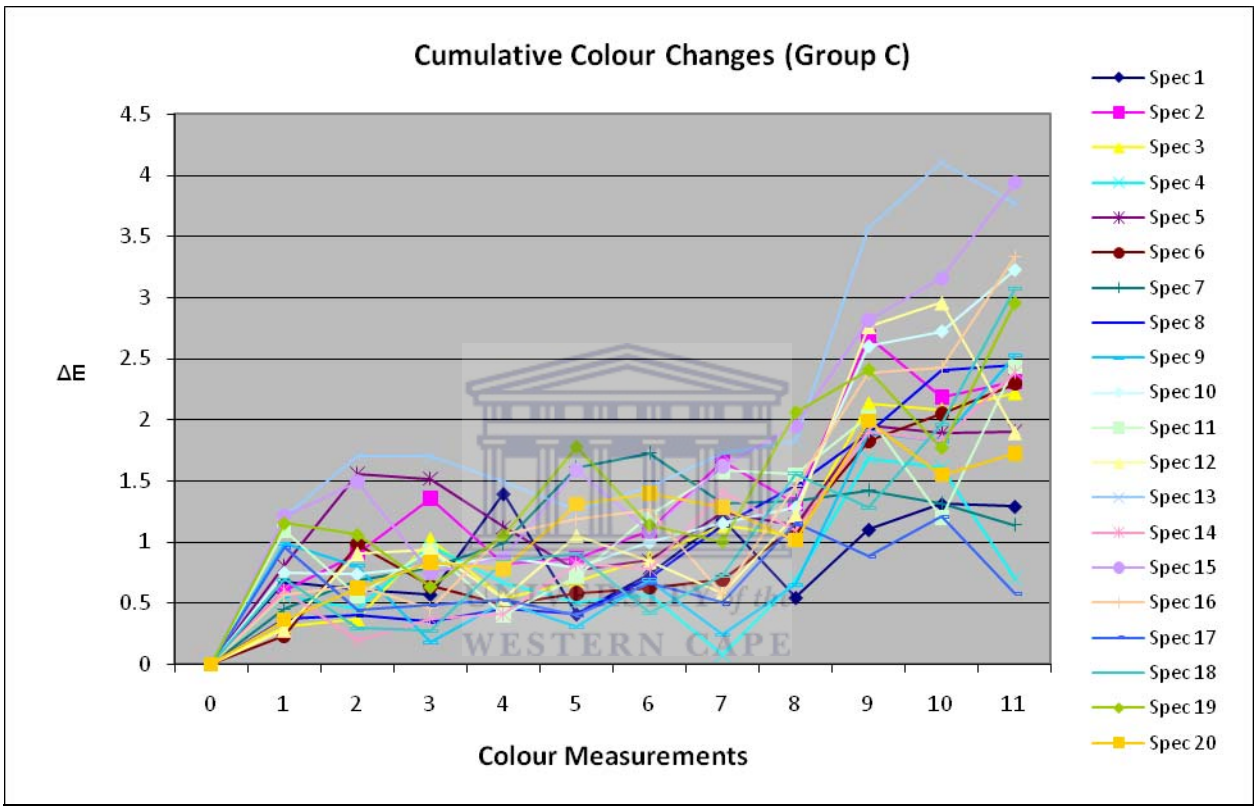
ΔL_4^*	Δa_4^*	Δb_4^*	ΔE_4^*	ΔL_5^*	Δa_5^*	Δb_5^*	ΔE_5^*	ΔL_6^*	Δa_6^*	Δb_6^*	ΔE_6^*	ΔL_7^*	Δa_7^*	Δb_7^*	ΔE_7^*
-7.090	-0.740	11.300	13.361	-6.950	-0.170	13.710	15.372	-6.950	-0.170	13.710	15.977	-7.910	-0.220	13.880	18.657
-4.870	-0.720	13.040	13.938	-5.060	-0.270	12.630	13.609	-5.060	-0.270	12.630	16.711	-5.390	0.500	15.810	16.422
-9.340	0.480	14.980	17.660	-8.820	0.690	15.790	18.100	-8.820	0.690	15.790	19.152	-10.100	1.420	16.210	18.854
-8.690	0.130	14.370	16.794	-10.310	0.830	14.570	17.868	-10.310	0.830	14.570	20.405	-11.220	2.350	16.880	17.454
-8.060	0.920	14.130	16.293	-10.810	1.850	16.200	19.563	-10.810	1.850	16.200	19.440	-10.220	2.120	16.400	19.612
-8.430	0.630	14.670	16.931	-10.330	1.910	16.640	19.679	-10.330	1.910	16.640	19.948	-11.000	1.920	16.530	19.951
-8.730	0.810	15.150	17.504	-10.200	0.990	16.080	19.068	-10.200	0.990	16.080	19.442	-10.410	1.720	16.330	19.455
-12.430	2.420	18.670	22.559	-13.720	4.320	17.230	22.445	-13.720	4.320	17.230	22.790	-13.050	3.620	18.330	23.645
-6.630	0.370	16.620	17.897	-6.700	0.680	17.120	18.397	-6.700	0.680	17.120	18.619	-6.830	0.620	17.310	20.000
-5.670	-0.230	14.070	15.171	-6.020	0.210	16.020	17.115	-6.020	0.210	16.020	17.508	-6.590	0.570	16.210	18.056
-5.730	-0.670	13.380	14.571	-6.110	-0.050	14.860	16.067	-6.110	-0.050	14.860	17.213	-6.400	0.520	15.970	17.518
-6.530	1.030	16.290	17.580	-6.710	1.530	17.410	18.721	-6.710	1.530	17.410	20.064	-6.580	2.250	18.820	20.251
-5.300	0.330	16.600	17.429	-6.760	1.190	17.440	18.742	-6.760	1.190	17.440	19.496	-7.190	1.720	18.040	20.020
-6.220	-0.370	14.720	15.984	-7.490	-0.020	16.230	17.875	-7.490	-0.020	16.230	18.642	-7.840	0.680	16.900	19.141
-9.080	3.300	18.470	20.844	-9.760	3.670	18.130	20.915	-9.760	3.670	18.130	21.558	-10.330	4.850	18.290	21.710
-6.620	0.200	17.000	18.245	-6.840	0.360	16.950	18.282	-6.840	0.360	16.950	19.303	-7.370	0.860	17.820	19.759
-6.990	0.310	13.970	15.624	-7.660	0.850	15.900	17.669	-7.660	0.850	15.900	17.385	-7.500	0.370	15.680	18.271
-9.000	3.230	19.300	21.539	-9.790	4.530	18.520	21.433	-9.790	4.530	18.520	22.320	-10.930	5.820	18.570	21.865
-6.510	-0.480	12.900	14.458	-6.410	-0.330	14.530	15.885	-6.410	-0.330	14.530	17.506	-7.280	0.160	15.920	17.940
-7.950	-0.010	14.450	16.493	-8.830	-0.300	15.070	17.469	-8.830	-0.300	15.070	17.885	-9.550	0.270	15.120	18.406
-7.494	0.547	15.204	17.044	-8.264	1.124	16.052	18.214	-8.264	1.124	16.052	19.068	-8.685	1.606	16.751	19.349
1.784	1.186	2.080	2.426	2.163	1.488	1.489	2.083	2.163	1.488	1.489	1.817	2.098	1.595	1.273	1.696

GROUP W – BLEACHING AND REHYDRATION PERIOD (DAY 8 TO 11)

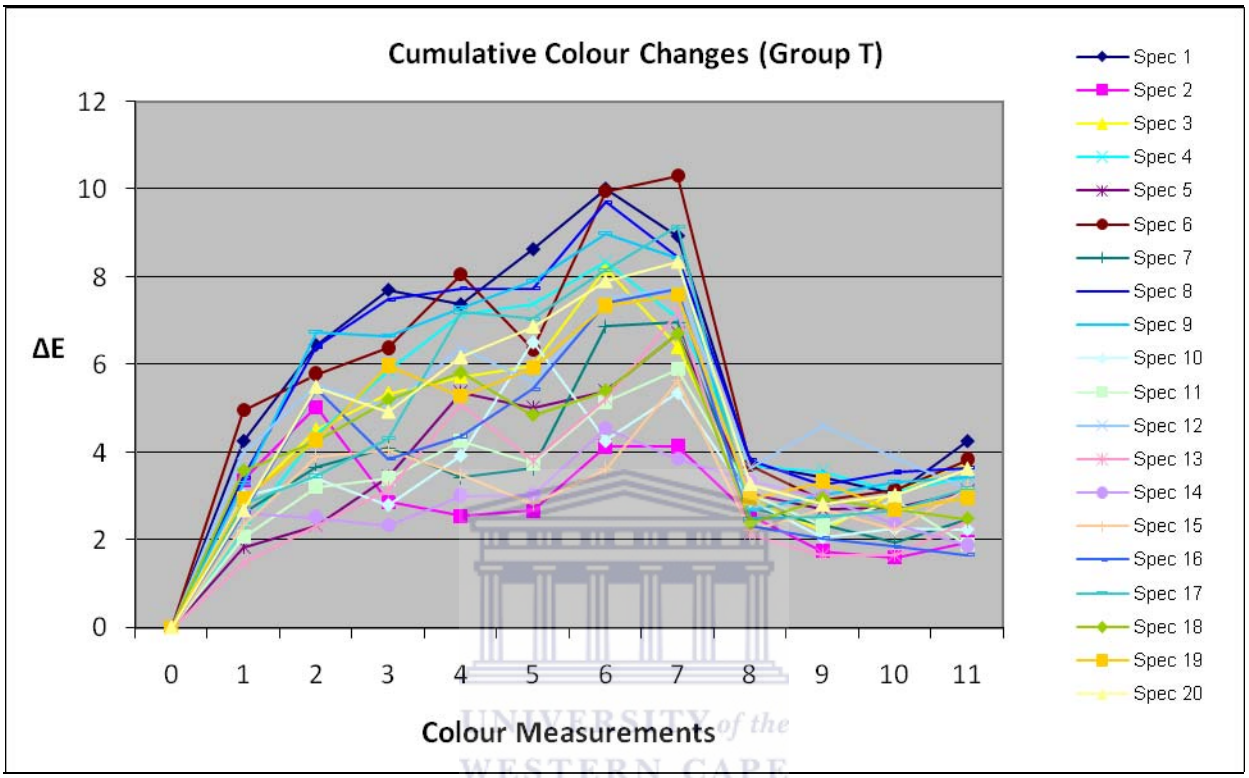
ΔL_8^*	Δa_8^*	Δb_8^*	ΔE_8^*	ΔL_9^*	Δa_9^*	Δb_9^*	ΔE_9^*	ΔL_{10}^*	Δa_{10}^*	Δb_{10}^*	ΔE_{10}^*	ΔL_{11}^*	Δa_{11}^*	Δb_{11}^*	ΔE_{11}^*
2.910	-2.270	-1.900	4.151	3.010	-2.030	-2.000	4.145	2.430	-1.110	-1.010	2.856	2.490	-0.680	-2.210	3.398
1.590	-1.280	0.810	2.196	1.150	-0.160	2.080	2.382	1.650	-0.170	1.710	2.382	1.450	0.370	0.960	1.778
1.110	-0.630	2.500	2.807	1.750	-0.640	2.380	3.023	-0.680	0.520	2.790	2.918	0.190	0.460	2.030	2.090
0.980	0.150	6.170	6.249	2.920	-0.230	4.930	5.734	3.570	-0.790	3.850	5.310	3.300	0.050	2.570	4.183
1.070	-0.430	3.160	3.364	1.850	0.030	3.340	3.818	4.120	-0.860	1.340	4.417	1.280	0.180	2.310	2.647
1.410	-0.670	2.950	3.338	2.620	-0.300	2.730	3.796	3.840	-0.470	2.360	4.532	2.500	-0.470	2.190	3.357
0.360	-1.040	2.440	2.677	2.530	-0.990	2.230	3.515	1.600	-0.060	2.620	3.071	1.660	0.490	1.810	2.504
3.570	-1.690	1.590	4.258	4.510	-1.590	1.070	4.900	4.890	-1.990	0.460	5.299	3.630	-0.530	0.660	3.727
2.890	-1.430	1.380	3.507	3.090	-1.180	0.120	3.310	2.490	-1.440	-0.710	2.963	2.870	-0.180	-1.600	3.291
1.790	-1.450	1.420	2.706	1.240	-0.370	1.640	2.089	1.980	0.080	0.730	2.112	1.410	0.360	-0.090	1.458
1.340	-1.650	0.330	2.151	1.380	-0.740	0.160	1.574	1.740	-0.510	-1.330	2.249	0.570	0.300	-1.250	1.406
2.060	-1.680	1.550	3.077	1.830	-1.420	1.220	2.618	1.700	-1.630	-0.510	2.410	1.490	-0.540	-0.780	1.766
2.360	-1.270	1.610	3.126	2.350	-1.020	0.940	2.729	2.870	-0.990	-0.130	3.039	2.380	0.180	-0.720	2.493
1.980	-1.300	2.120	3.179	3.360	-0.560	1.490	3.718	3.570	-0.700	0.100	3.639	2.490	0.260	-0.680	2.594
1.960	-1.170	3.480	4.162	2.560	-1.090	3.390	4.386	3.110	-1.450	2.700	4.366	2.550	-0.270	2.290	3.438
1.740	-1.510	1.130	2.566	1.360	-0.480	1.070	1.796	2.050	-1.000	-0.950	2.471	1.530	0.200	-0.690	1.690
1.310	-1.000	1.210	2.045	2.320	-0.640	0.270	2.422	2.490	-0.510	-0.990	2.728	1.970	0.410	-1.120	2.303
2.820	-2.420	3.870	5.365	2.510	-1.480	4.140	5.063	3.460	-1.940	3.540	5.317	2.580	-0.990	2.980	4.064
1.800	-1.350	0.650	2.342	1.860	-0.570	-0.260	1.963	1.250	-0.220	-0.360	1.319	0.510	0.790	-0.100	0.946
1.280	-0.320	1.620	2.089	1.950	1.070	3.160	3.864	1.590	0.620	1.310	2.151	2.470	1.350	0.060	2.815
1.817	-1.221	1.905	3.268	2.308	-0.720	1.705	3.342	2.486	-0.731	0.876	3.277	1.966	0.087	0.431	2.597
0.785	0.625	1.616	1.112	0.827	0.680	1.656	1.158	1.248	0.741	1.659	1.194	0.921	0.552	1.594	0.929

APPENDIX 3 - CUMULATIVE COLOUR CHANGES FOR INDIVIDUAL SPECIMENS OVER THE COURSE OF THE STUDY FOR ALL THREE GROUPS.

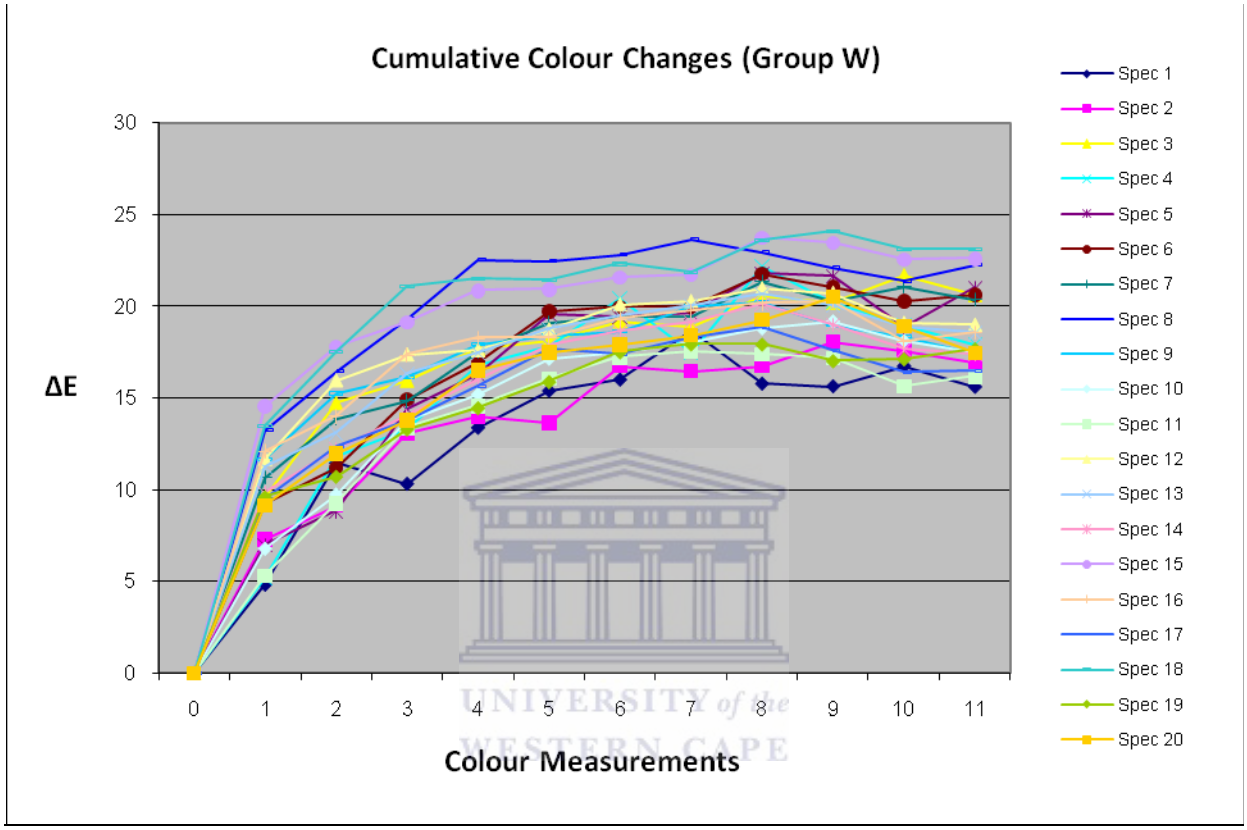
GROUP C



GROUP T



GROUP W



APPENDIX 4

CUMULATIVE ΔE FOR EACH SPECIMEN OVER THE 11 COLOUR MEASUREMENTS TAKEN IN THE STUDY AND THE STATISTICS – GROUP C

	1	2	3	4	5	6	7	8	9	10	11
Spec 1	0.674	0.609	0.569	1.394	0.407	0.738	1.183	0.543	1.101	1.310	1.291
Spec 2	0.596	0.899	1.352	0.819	0.865	1.090	1.653	1.308	2.675	2.179	2.305
Spec 3	0.308	0.364	1.025	0.540	0.677	0.870	1.135	1.048	2.131	2.084	2.218
Spec 4	0.556	0.456	0.965	0.667	0.501	0.568	0.073	0.684	1.686	1.608	0.704
Spec 5	0.804	1.550	1.515	1.118	0.768	0.845	1.242	1.132	1.952	1.889	1.898
Spec 6	0.229	0.980	0.638	0.482	0.579	0.624	0.689	1.112	1.826	2.044	2.288
Spec 7	0.448	0.681	0.788	0.988	1.609	1.723	1.311	1.337	1.422	1.310	1.138
Spec 8	0.368	0.403	0.348	0.450	0.404	0.703	1.135	1.464	1.886	2.404	2.446
Spec 9	0.989	0.803	0.177	0.507	0.307	0.688	0.244	0.654	1.894	1.829	2.525
Spec 10	0.749	0.735	0.795	0.871	0.785	0.993	1.150	1.281	2.600	2.721	3.224
Spec 11	1.095	0.553	0.933	0.402	0.715	1.211	1.579	1.549	2.021	1.199	2.431
Spec 12	0.278	0.906	0.931	0.531	1.051	0.836	0.576	1.220	2.762	2.957	1.895
Spec 13	1.206	1.699	1.696	1.487	1.260	1.444	1.722	1.825	3.571	4.104	3.770
Spec 14	0.603	0.191	0.372	0.408	0.819	0.775	1.404	1.093	1.904	1.826	2.386
Spec 15	1.212	1.495	0.749	0.870	1.587	1.084	1.620	1.952	2.815	3.159	3.941
Spec 16	0.568	0.636	0.456	1.056	1.182	1.264	0.568	1.459	2.382	2.427	3.329
Spec 17	0.959	0.438	0.479	0.529	0.404	0.660	0.499	1.151	0.883	1.208	0.581
Spec 18	0.696	0.297	0.273	0.839	0.904	0.422	0.726	1.561	1.280	1.965	3.071
Spec 19	1.152	1.057	0.631	1.053	1.778	1.139	1.003	2.061	2.408	1.771	2.953
Spec 20	0.362	0.622	0.834	0.778	1.310	1.397	1.285	1.014	1.990	1.550	1.727
Mean	0.693	0.769	0.776	0.789	0.896	0.954	1.040	1.273	2.060	2.077	2.306
SD	0.320	0.420	0.404	0.321	0.436	0.335	0.478	0.404	0.643	0.732	0.930
Minimum	0.229	0.191	0.177	0.402	0.307	0.422	0.073	0.543	0.883	1.199	0.581
Median	0.638	0.658	0.769	0.798	0.802	0.858	1.142	1.250	1.971	1.927	2.345
Maximum	1.212	1.699	1.696	1.487	1.778	1.723	1.722	2.061	3.571	4.104	3.941

Spec – specimen, SD – standard deviation.

CUMULATIVE ΔE FOR EACH SPECIMEN OVER THE 11 COLOUR MEASUREMENTS TAKEN IN THE STUDY AND THE STATISTICS – GROUP T

	1	2	3	4	5	6	7	8	9	10	11
Spec 1	4.256	6.441	7.698	7.371	8.627	10.007	8.930	3.745	3.443	3.070	4.251
Spec 2	3.373	5.034	2.875	2.547	2.665	4.140	4.141	2.571	1.747	1.614	1.946
Spec 3	2.886	4.523	5.349	5.729	5.971	8.198	6.404	2.959	2.219	3.111	3.782
Spec 4	2.234	4.396	5.854	7.153	7.391	8.348	7.060	3.700	3.570	3.123	3.436
Spec 5	1.834	2.347	3.449	5.359	5.028	5.390	6.710	3.057	2.702	2.746	3.112
Spec 6	4.962	5.795	6.386	8.064	6.356	9.961	10.306	3.707	2.911	3.150	3.836
Spec 7	2.620	3.664	4.111	3.435	3.640	6.877	6.965	2.733	2.360	1.951	2.482
Spec 8	3.429	6.412	7.493	7.739	7.741	9.709	8.458	3.826	3.275	3.553	3.659
Spec 9	3.294	6.756	6.658	7.313	7.912	9.002	8.450	2.694	3.039	3.327	3.453
Spec 10	3.010	3.419	2.805	3.932	6.522	4.265	5.365	2.900	2.065	2.262	2.246
Spec 11	2.071	3.202	3.398	4.253	3.750	5.155	5.890	3.069	2.319	2.929	1.895
Spec 12	3.993	5.547	5.064	6.393	5.604	7.540	7.765	3.652	4.620	3.897	3.172
Spec 13	1.492	2.334	3.162	5.094	3.811	5.221	7.176	2.127	1.660	1.651	2.436
Spec 14	2.642	2.527	2.330	3.023	3.034	4.557	3.867	3.345	2.993	2.376	1.887
Spec 15	2.332	3.931	4.059	3.504	2.827	3.636	5.641	2.219	2.720	2.239	3.196
Spec 16	2.635	5.443	3.858	4.377	5.454	7.416	7.726	2.341	2.046	1.852	1.661
Spec 17	2.821	3.467	4.333	7.215	7.055	8.167	9.162	2.496	2.534	2.683	3.188
Spec 18	3.593	4.266	5.210	5.833	4.864	5.420	6.722	2.391	2.966	2.724	2.496
Spec 19	2.946	4.285	5.980	5.289	5.934	7.357	7.602	2.963	3.345	2.684	2.971
Spec 20	2.683	5.502	4.936	6.179	6.879	7.913	8.354	3.281	2.819	3.031	3.632
Mean	2.955	4.464	4.750	5.490	5.553	6.914	7.135	2.989	2.768	2.699	2.937
SD	0.834	1.371	1.573	1.687	1.805	2.048	1.635	0.545	0.700	0.627	0.758
Minimum	1.492	2.334	2.330	2.547	2.665	3.636	3.867	2.127	1.660	1.614	1.661
Median	2.853	4.341	4.634	5.544	5.769	7.387	7.118	2.961	2.769	2.735	3.142
Maximum	4.962	6.756	7.698	8.064	8.627	10.007	10.306	3.826	4.620	3.897	4.251

CUMULATIVE ΔE FOR EACH SPECIMEN OVER THE 11 COLOUR MEASUREMENTS TAKEN IN THE STUDY AND THE STATISTICS – GROUP W

	1	2	3	4	5	6	7	8	9	10	11
Spec 1	4.815	11.445	10.305	13.361	15.372	15.977	18.657	15.758	15.615	16.719	15.587
Spec 2	7.260	9.107	13.057	13.938	13.609	16.711	16.422	16.699	18.019	17.531	16.876
Spec 3	9.539	14.707	15.923	17.660	18.100	19.152	18.854	20.536	20.173	21.657	20.580
Spec 4	5.168	11.848	13.318	16.794	17.868	20.405	17.454	22.170	20.212	18.938	17.927
Spec 5	6.984	8.837	14.393	16.293	19.563	19.440	19.612	21.782	21.662	18.881	20.996
Spec 6	9.242	11.169	14.897	16.931	19.679	19.948	19.951	21.728	21.050	20.232	20.593
Spec 7	10.658	13.876	14.823	17.504	19.068	19.442	19.455	21.324	20.253	21.045	20.352
Spec 8	13.256	16.453	19.291	22.559	22.445	22.790	23.645	22.974	22.123	21.373	22.273
Spec 9	11.657	15.229	16.118	17.897	18.397	18.619	20.000	20.207	18.947	18.334	17.432
Spec 10	6.771	9.731	13.777	15.171	17.115	17.508	18.056	18.776	19.150	18.086	17.496
Spec 11	5.307	9.252	13.380	14.571	16.067	17.213	17.518	17.385	17.182	15.654	16.161
Spec 12	11.660	15.941	17.350	17.580	18.721	20.064	20.251	20.951	20.704	19.068	18.963
Spec 13	11.218	13.082	16.295	17.429	18.742	19.496	20.020	20.647	20.018	18.848	18.528
Spec 14	9.828	12.234	14.002	15.984	17.875	18.642	19.141	20.106	19.018	17.639	17.387
Spec 15	14.547	17.797	19.127	20.844	20.915	21.558	21.710	23.723	23.438	22.555	22.580
Spec 16	12.097	13.997	17.339	18.245	18.282	19.303	19.759	20.145	20.232	18.079	18.568
Spec 17	9.520	12.366	13.766	15.624	17.669	17.385	18.271	18.845	17.623	16.379	16.487
Spec 18	13.482	17.506	21.090	21.539	21.433	22.320	21.865	23.590	24.097	23.142	23.106
Spec 19	9.600	10.713	13.294	14.458	15.885	17.506	17.940	17.934	17.024	17.132	17.681
Spec 20	9.141	11.967	13.776	16.493	17.469	17.885	18.406	19.274	20.501	18.893	17.460
Mean	9.588	12.863	15.266	17.044	18.214	19.068	19.349	20.228	19.852	19.009	18.852
SD	2.838	2.760	2.575	2.426	2.083	1.817	1.696	2.203	2.126	2.066	2.228
Minimum	4.815	8.837	10.305	13.361	13.609	15.977	16.422	15.758	15.615	15.654	15.587
Median	9.570	12.300	14.608	16.863	18.191	19.227	19.298	20.372	20.192	18.865	18.227
Maximum	14.547	17.797	21.090	22.559	22.445	22.790	23.645	23.723	24.097	23.142	23.106

APPENDIX 5

SEQUENTIAL ΔE FOR EACH SPECIMEN OVER THE 11 COLOUR MEASUREMENTS TAKEN IN THE STUDY AND THE STATISTICS - GROUP C

	1	2	3	4	5	6	7	8	9	10	11
Spec 1	0.674	0.247	0.340	0.858	1.074	0.567	0.560	0.888	0.906	1.607	1.967
Spec 2	0.596	0.638	1.846	1.547	0.315	0.659	1.489	0.542	1.535	0.550	0.514
Spec 3	0.308	0.390	1.189	0.791	0.220	0.310	0.443	0.537	1.297	0.103	0.242
Spec 4	0.556	0.222	0.640	0.463	0.685	0.612	0.556	0.734	1.400	0.110	0.982
Spec 5	0.804	0.855	0.254	0.871	1.076	0.098	1.241	1.433	1.154	0.087	0.353
Spec 6	0.229	1.132	1.554	0.490	0.234	0.825	0.206	1.255	0.794	0.255	0.583
Spec 7	0.448	0.385	0.650	0.314	0.660	0.205	0.828	0.063	1.335	0.261	0.901
Spec 8	0.368	0.192	0.141	0.251	0.175	0.597	0.442	0.919	0.958	0.643	0.195
Spec 9	0.989	0.573	0.818	0.433	0.225	0.544	0.631	0.567	1.314	0.209	0.709
Spec 10	0.749	0.437	0.253	0.128	0.134	0.669	0.168	1.247	1.364	0.139	0.602
Spec 11	1.095	1.484	0.761	0.951	0.720	1.191	0.509	0.271	0.566	0.824	1.520
Spec 12	0.278	0.774	1.815	1.386	0.742	0.218	0.371	0.779	1.624	0.356	1.081
Spec 13	1.206	0.548	0.285	0.303	0.286	0.185	0.502	0.516	1.812	0.589	0.347
Spec 14	0.603	0.489	0.193	0.609	0.842	1.476	0.668	0.446	0.815	0.125	0.577
Spec 15	1.212	0.352	0.764	0.778	1.202	0.744	0.627	0.345	0.881	0.472	1.077
Spec 16	0.568	0.859	0.724	0.975	0.648	0.732	1.054	0.993	1.824	0.555	1.098
Spec 17	0.959	0.750	0.344	0.226	0.276	0.444	0.753	1.174	1.814	0.455	0.871
Spec 18	0.696	0.486	0.389	0.893	0.500	0.636	0.388	0.837	0.436	0.866	1.115
Spec 19	1.152	0.100	1.132	1.069	0.813	0.682	0.980	1.544	0.400	0.667	1.204
Spec 20	0.362	0.722	0.959	0.224	0.586	0.201	0.940	0.412	1.060	0.500	0.249
Mean	0.693	0.582	0.753	0.678	0.571	0.580	0.668	0.775	1.164	0.469	0.809
SD	0.320	0.336	0.525	0.399	0.329	0.341	0.338	0.406	0.443	0.362	0.461
Minimum	0.229	0.100	0.141	0.128	0.134	0.098	0.168	0.063	0.400	0.087	0.195
Median	0.638	0.518	0.687	0.694	0.617	0.605	0.594	0.756	1.225	0.463	0.790
Maximum	1.212	1.484	1.846	1.547	1.202	1.476	1.489	1.544	1.824	1.607	1.967

SEQUENTIAL ΔE FOR EACH SPECIMEN OVER THE 11 COLOUR MEASUREMENTS TAKEN IN THE STUDY AND THE STATISTICS - GROUP T

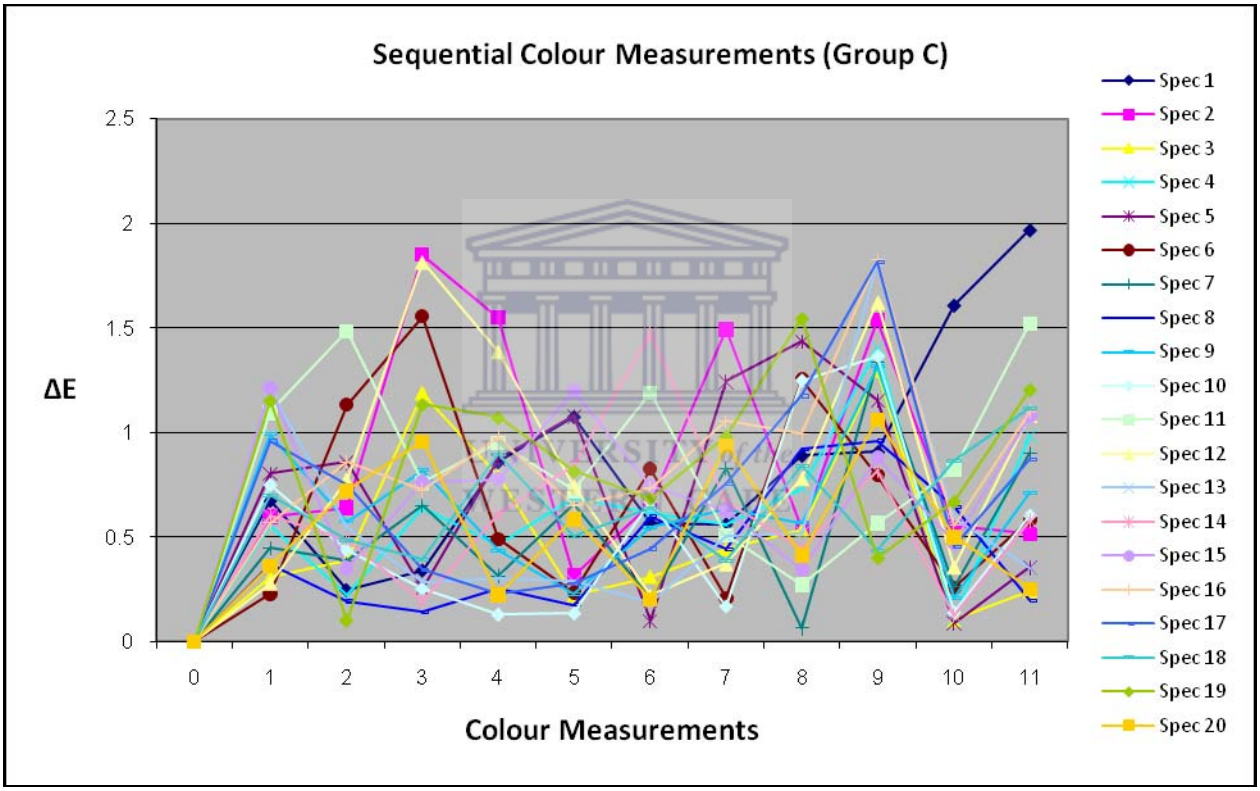
	1	2	3	4	5	6	7	8	9	10	11
Spec 1	4.256	3.102	1.657	0.640	1.293	1.572	1.339	5.560	0.315	0.504	1.365
Spec 2	3.373	1.806	2.215	0.792	0.200	1.622	0.286	2.407	0.935	0.563	0.391
Spec 3	2.886	2.304	0.997	0.749	0.443	2.377	1.837	4.551	0.799	1.122	0.793
Spec 4	2.234	2.861	1.475	1.362	0.595	1.259	2.572	4.378	0.373	0.491	0.488
Spec 5	1.834	0.807	2.425	1.984	0.736	0.389	1.777	5.225	0.570	0.536	0.665
Spec 6	4.962	1.097	0.597	1.685	2.273	4.232	0.746	7.022	0.948	0.408	0.891
Spec 7	2.620	1.462	0.464	0.814	0.327	3.390	0.236	5.245	0.586	0.446	0.703
Spec 8	3.429	3.353	1.229	0.470	0.734	2.012	1.326	5.291	0.689	0.626	1.590
Spec 9	3.294	3.468	0.898	0.703	0.984	1.136	0.643	6.114	0.362	0.442	0.228
Spec 10	3.010	0.434	0.718	1.304	2.645	2.338	1.245	2.481	1.130	0.439	0.624
Spec 11	2.071	1.471	0.785	1.099	0.873	1.449	0.759	3.810	1.586	1.453	1.153
Spec 12	3.993	1.566	1.116	1.405	0.844	2.027	2.092	4.830	1.805	0.804	1.128
Spec 13	1.492	1.244	1.090	1.983	1.348	1.465	2.008	5.302	0.790	0.539	0.942
Spec 14	2.642	1.950	0.432	0.921	0.108	1.563	1.131	2.612	0.527	0.693	0.497
Spec 15	2.332	2.003	0.617	0.832	0.735	0.834	2.033	3.754	0.746	0.765	1.168
Spec 16	2.635	3.152	2.235	0.580	1.466	1.996	0.698	5.744	0.561	0.407	0.650
Spec 17	2.821	0.741	0.955	2.890	0.172	1.578	1.048	7.528	0.179	0.391	0.571
Spec 18	3.593	0.966	1.162	0.871	1.020	0.991	1.491	5.239	0.754	0.327	0.392
Spec 19	2.946	2.007	2.037	0.853	0.703	1.499	0.974	6.074	0.652	1.272	0.529
Spec 20	2.683	2.904	1.099	1.260	0.886	1.526	0.819	5.429	0.562	0.833	0.681
Mean	2.955	1.935	1.210	1.160	0.919	1.763	1.253	4.930	0.743	0.653	0.772
SD	0.834	0.941	0.609	0.599	0.650	0.861	0.640	1.387	0.399	0.310	0.355
Minimum	1.492	0.434	0.432	0.470	0.108	0.389	0.236	2.407	0.179	0.327	0.228
Median	2.853	1.878	1.094	0.896	0.790	1.567	1.188	5.242	0.670	0.537	0.673
Maximum	4.962	3.468	2.425	2.890	2.645	4.232	2.572	7.528	1.805	1.453	1.590

SEQUENTIAL ΔE FOR EACH SPECIMEN OVER THE 11 COLOUR MEASUREMENTS TAKEN IN THE STUDY AND THE STATISTICS - GROUP W

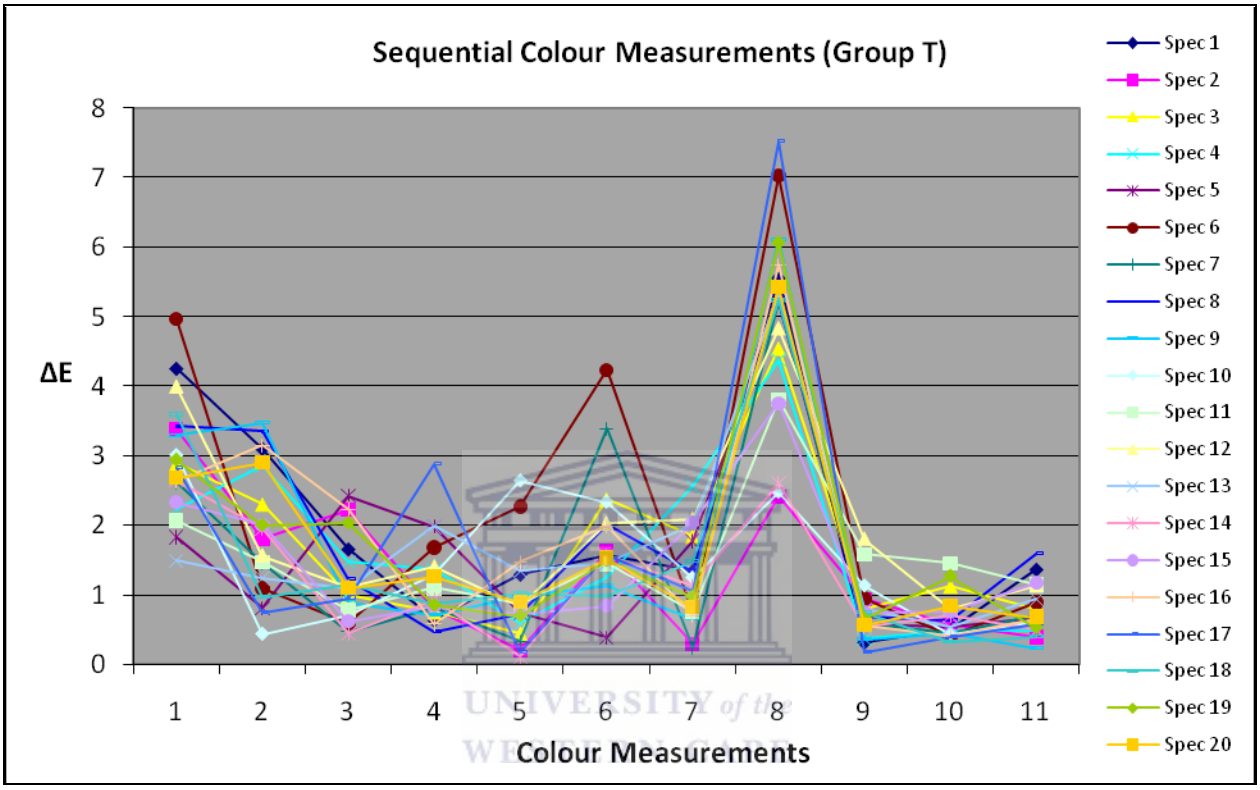
	1	2	3	4	5	6	7	8	9	10	11
Spec 1	4.815	7.253	1.261	3.264	2.480	0.976	3.116	4.151	0.279	1.471	1.276
Spec 2	7.260	2.109	4.063	0.930	0.638	3.288	0.893	2.196	1.750	0.622	0.946
Spec 3	9.539	5.407	1.804	1.993	0.985	1.532	0.631	2.807	0.651	2.724	1.157
Spec 4	5.168	7.015	1.817	3.658	1.776	2.911	3.363	6.249	2.334	1.379	1.555
Spec 5	6.984	2.371	5.923	2.079	3.565	0.679	0.460	3.364	0.923	3.154	3.176
Spec 6	9.242	2.101	3.775	2.148	3.021	0.679	0.379	3.338	1.284	1.286	1.351
Spec 7	10.658	3.551	1.131	2.808	1.749	0.800	0.808	2.677	2.181	1.372	0.981
Spec 8	13.256	3.454	2.936	3.958	2.711	1.466	0.923	4.258	1.079	0.822	1.939
Spec 9	11.657	3.750	1.410	1.879	0.592	0.238	2.085	3.507	1.300	1.057	1.589
Spec 10	6.771	3.003	4.082	1.575	2.029	0.700	0.716	2.706	1.232	1.256	1.037
Spec 11	5.307	4.300	4.755	1.303	1.649	1.281	0.361	2.151	0.927	1.550	1.425
Spec 12	11.660	4.348	1.577	1.391	1.240	1.589	0.769	3.077	0.479	1.748	1.142
Spec 13	11.218	1.898	3.338	1.215	1.891	0.909	0.906	3.126	0.715	1.190	1.399
Spec 14	9.828	2.638	2.749	2.182	2.004	1.030	2.104	3.179	1.688	1.413	1.642
Spec 15	14.547	3.495	1.554	2.252	0.846	1.320	0.341	4.162	0.612	0.953	1.369
Spec 16	12.097	2.011	3.380	1.254	0.277	1.135	0.847	2.566	1.099	2.197	1.333
Spec 17	9.520	2.955	1.427	1.905	2.113	0.552	1.029	2.045	1.426	1.278	1.065
Spec 18	13.482	4.031	3.993	1.220	1.710	1.722	0.565	5.365	1.026	1.214	1.411
Spec 19	9.600	1.218	2.762	1.184	1.640	1.711	0.527	2.342	1.200	0.710	1.279
Spec 20	9.141	2.939	1.831	3.292	1.115	0.920	1.003	2.089	2.180	1.938	1.694
Mean	9.588	3.492	2.778	2.075	1.702	1.272	1.091	3.268	1.218	1.467	1.438
SD	2.838	1.598	1.352	0.894	0.842	0.748	0.876	1.112	0.574	0.635	0.482
Minimum	4.815	1.218	1.131	0.930	0.277	0.238	0.341	2.045	0.279	0.622	0.946
Median	9.570	3.228	2.756	1.949	1.729	1.083	0.828	3.102	1.150	1.329	1.360
Maximum	14.547	7.253	5.923	3.958	3.565	3.288	3.363	6.249	2.334	3.154	3.176

APPENDIX 6 - GRAPHS SHOWING THE SEQUENTIAL COLOUR CHANGES FOR THE THREE GROUPS OVER THE COURSE OF THE STUDY.

GROUP C



GROUP T



GROUP W