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DEPARTMENT OF PROSTHODONTICS

EVALUATION OF THE COLOR STABILITY OF HEAT-CURED AND CAD/CAM ACRYLIC RESIN MATERIALS USED IN REMOVABLE DENTURES AGAINST VARIOUS STAINING AGENTS

DOCTOR OF PHILOSOPHY THESIS

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DECLARATION

I declare that this study is my own work, that I complied all academic guidelines when collecting data for my study and behave ethically in planning and writing it. I cited all sources for each data and comments not used in the production of my study, also that my study does not contain any material previously published or written by another researcher.

02.02.2023

Ceren Üstünel

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LIST OF SYMBOLS AND ABBREVIATIONS

3D	Three Dimensional
ANOVA test	Analysis of Variance
Bis-GMA	Bisphenol A-glycidyl Di Methyl Acrylate
°C	Degree Celsius
CAD / CAM	Computer Aided Design / Computer Aided Manufacture
CBCT	Cone Beam Computed Tomography
CC1.D	CAD/CAM 1 Dentsply
CC2.M	CAD/CAM 2 Merz
CC3.O	CAD/CAM 3 Ondent
CCD	Charge Coupled Device
CIE	International Commission on Illumination
CNC	Computerized Numerical Control Machine
COS	Chairside Oral Scanner
CTM	Complete Tooth Measurement
ΔE	Change in Color
g	Gram
HC.H	Heat-cured Heraeus Kulzer
HPP	High Performance Polymers
K	Kelvin
kcal	Kilocalorie
LED	Light Emitting Diode
LL	Laser Lithography
LMS	Long, Medium, Short
ml	Milliliter
mm	Millimeter
NBS	National Bureau of Standards
nm	Nanometer
p	Probability
PMMA	Polymethylmethacrylate
POM	Polyoxymethylene
RGB	Red, Green, Blue

RP	Rapid Prototyping
SM	Spot Measurement
STL	Stereolithography
Tg	Glass Transition Temperature
UDMA	Urethane Dimethacrylate
UV	Ultraviolet
Y-TZP	Yttria Stabilized Zirconia

ABSTRACT

Üstünel, C. (2023). EVALUATION OF THE COLOR STABILITY OF HEAT-CURED AND CAD/CAM ACRYLIC RESIN MATERIALS USED IN REMOVABLE DENTURES AGAINST VARIOUS STAINING AGENTS

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Aim: The aim of this study is to evaluate and compare the changes in the color of heat-cured and CAD/CAM acrylic resin materials used in removable dentures against various staining agents.

Materials and Method: 3 brands of CAD/CAM acrylic resin blocks (Dentsply/Lucitone 199, Merz/M-PM, and Ondent/Tempo-Cad) and 1 brand of heat-cured acrylic resin material (Heraeus Kulzer/Paladent 20) were tested. The samples were immersed in 4 different solutions: Distilled water, tea, coffee and red wine. For each acrylic groups, 40 disc-shaped samples with 15 mm in diameter and 2 mm in height were prepared (n=10). Before the samples were immersed in the solutions, a spectrophotometer was used to measure the baseline color measurement (T0). 6 color measurements were made in total. The CIE L*a*b*color space was used to determine color differences (ΔE). ANOVA tests and sequential Bonferroni adjustment were used in statistical analysis.

Results: At the end of 5 weeks of immersion, the highest color change (ΔE) was observed for the CAD/CAM 1 Dentsply group immersed in red wine (ΔE=6.28±0.24). The highest ΔE values for tea and coffee solutions were observed in CAD/CAM 3 Ondent group, respectively (ΔE=5.54±0.21), (ΔE=5.25±0.25) at the end of the 5 weeks of immersion. The lowest color change (ΔE) was observed for the CAD/CAM 2 Merz group was immersed in tea, coffee and red wine (except distilled water), respectively (ΔE=4.23±0.14), (ΔE=3.48±0.18) (ΔE=5.24±0.18). In all acrylic resin groups, all solutions caused clinically marked color change except distilled water, at the end of the study (3<NBS<6). The color stability of 4 different acrylic resin groups in 4 different solutions showed a significant difference ($p<0.05$).

Conclusion: ΔE values of all acrylic resin groups increased proportionally with increasing immersion time. The color stability of heat-cured acrylic resin was comparable to that of the CAD/CAM acrylic resins.

Keywords: Acrylic resin, color stability, spectrophotometer, CAD/CAM

ÖZET

Üstünel, C. (2023). HAREKETLİ PROTEZLERDE KULLANILAN ISI İLE SERTLEŞEN VE CAD/CAM AKRİLİK REZİN MATERYALLERİNİN ÇEŞİTLİ BOYAYICI AJANLARA KARŞI RENK STABİLİTELERİNİN DEĞERLENDİRİLMESİ

Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Protetik Diş Tedavisi Anabilim Dalı, PhD Tezi, İstanbul.

Amaç: Bu çalışmanın amacı, hareketli protezlerde kullanılan ısı ile sertleşen ve CAD/CAM akrilik rezin materyallerinin çeşitli boyama ajanlarına karşı oluşabilecek renk değişimlerini değerlendirmek ve karşılaştırmaktır.

Materyal ve Metod: 3 marka CAD / CAM akrilik rezin bloğu (Dentsply/Lucitone 199, Merz/M-PM ve Ondent/Tempo-Cad) ve 1 marka ısı ile sertleşen akrilik rezin materyali (Heraeus Kulzer/Paladent 20) test edildi. Numuneler 4 farklı solüsyona daldırıldı: distile su, çay, kahve ve kırmızı şarap. Her akrilik grup için 15 mm çapında ve 2 mm kalınlığında 40 adet disk şeklinde örnek hazırlandı (n=10). Örnekler çözeltilere daldırılmadan önce, bir spektrofotometre kullanılarak baseline (T0) renk ölçümü yapıldı. Toplamda 6 renk ölçümü gerçekleştirildi. Renk farklılıklarını (ΔE) belirlemek için CIE L*a*b*renk alanı kullanıldı. İstatistiksel analiz için ANOVA testleri ve Sequential Bonferroni testi kullanıldı.

Bulgular: 5 haftalık daldırma sonunda, en yüksek renk değişimi (ΔE), kırmızı şarap için CAD/CAM 1 Dentsply grubunda (ΔE=6.28±0.24). Çay ve kahve solüsyonlarında ise, 5 haftalık daldırma sonunda en yüksek ΔE deeğrleri CAD/CAM 3 Ondent grubunda bulunmuştur ve ΔE değerleri sırasıyla belirtilmiştir (ΔE=5.54±0.21), (ΔE=5.25±0.25). Distile su haricinde çay, kahve ve kırmızı şarap için en düşük renk değişimi (ΔE) CAD/CAM 2 Merz grubunda gözlenmiştir ve ΔE değerleri sırasıyla belirtilmiştir (ΔE=4.23±0.14), (ΔE=3.48±0.18), (ΔE=5.24±0.18). Tüm akrilik rezin gruplarında çalışma sonunda distile su dışındaki tüm solüsyonlar klinik olarak belirgin renk değişikliğine neden olmuştur (3<NBS<6). 4 farklı akrilik resin grubunun 4 farklı solüsyondaki renk stabilitesi arasında önemli bir fark bulunmuştur ($p<0.05$).

Sonuç: Tüm akrilik rezin gruplarına ait ΔE değerleri artan daldırma zamanıyla doğru orantılı olarak artış göstermiştir. Isı ile sertleşen akrilik rezin, renk stabilitesi açısından CAD/CAM akrilik rezinlerle karşılaştırılabilir değerlerde bulunmuştur.

Anahtar kelimeler: Akrilik rezin, renk stabilitesi, spektrofotometre, CAD/CAM

1. INTRODUCTION AND PURPOSE

People experience tooth loss for various reasons over time, and these losses cause biological, phonetic, esthetic, functional and psychological problems (1). Dental prostheses play a major role in eliminating tooth deficiencies and subsequent problems in patients. Removable dental prostheses have been one of the treatment options for patients who have lost most or all of their teeth. In the past, removable dental prostheses were produced only by the conventional method. With the development of technology, CAD/CAM systems (Computer Aided Design and Computer Aided Manufacturing) have also been used in dentistry since the 1980s, its popularity has increased gradually and it has become an alternative to the conventional method in the production of removable dental prostheses (2,3,4,5,6).

After a removable dental prosthesis base is designed in three dimensions using computer software in CAD/CAM systems, it is produced by scraping from ready-made discs with a fully automatic milling process. These discs are made of ready-made acrylic resin based on polymethylmethacrylate, which has been polymerized (prepolymerized) under high heat and pressure. Thus, prosthesis production is carried out using a high-density acrylic resin material, which is thought to have less monomer released and less porosity. This also provides a better dimensional stability and fracture strength to the denture base material (3,5,7).

The appearance of the denture base in harmony with the tissues and color stability are important for the esthetics of the prosthesis. Color stability is the ability of a material to retain its color in a given environment over time. The denture base material should match the color and appearance of the underlying tissues. Color changes in the prosthesis may result in dissatisfaction of the patient and it may be necessary to replace the prosthesis (8,9,10).

Color changes in acrylic resin materials can be caused by both internal and external factors (11). Internal factors are related to the matrix structure of the material (photoinitator types and inorganic materials such as amorphous silica, glass fillers) (12,13,14).

Most often, this internal discoloration; it is caused by physical and chemical conditions such as temperature and humidity differences, ultraviolet exposure. In addition, smoking, chewing tobacco, some drugs taken and the diet of the person can also

affect the chemical structure of the material. Oxidation of the material, changes in the matrix/matrix interface and fillers, and the destruction of chemical bonds are some chemical changes that occur in the internal structure of the material (15). External factors are related to the absorption and adsorption of coloring agents (16,17). Substances such as coffee, tea, nicotine and red wine may cause discoloration of the prosthesis and esthetic problems (18,19).

A change in color is also an indicator of aging or material damage. Factors triggering color change; stain accumulation, water absorption, dissolution of components, degradation of intrinsic pigments, and surface roughness can be given as examples (11,12,13).

The discoloration of acrylic resin denture base material and acrylic teeth with different beverage and food colorants has been the subject of some researches (14,20). Although current scientific data show the clinical superiority of CAD/CAM acrylic resin materials over conventional acrylic resin materials, data on material properties are still limited (21).

Therefore, the aim of this study is to evaluate and compare the color changes of conventional heat cured and CAD/CAM acrylic resin materials used in removable dentures against various staining agents. The null hypothesis that the type of acrylic resin denture base material and immersion solution have no effect on the stainability of acrylic resin denture base materials was rejected.

2. GENERAL INFORMATION

2.1. Polymers

Polymers are widely used in dentistry as in many areas. Impression materials such as alginate, silicone, polyether, composite fillings, fissure sealants, canal filling pastes, tissue regulators, acrylic base materials can be given as examples of polymers (22,23).

2.1.1. Structure of Polymers

Polymers are defined as high molecular weight macromolecules formed by combining more than one low molecular weight monomer. In Latin, poly means many and mer means piece (24,25). The structure of monomers and polymers is shown in Figure 2.1.

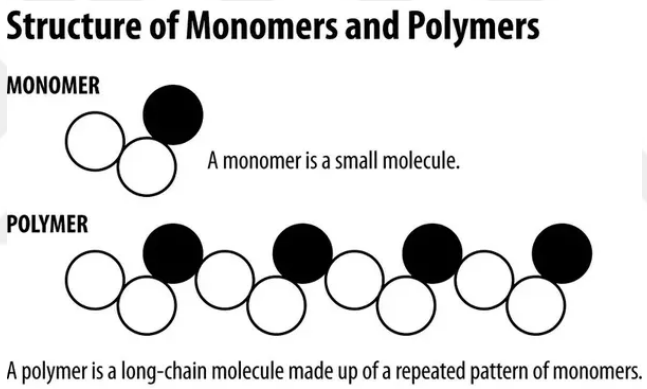

Figure 2.1. Structure of monomers and polymers

While each unit in the polymer itself is connected to each other by covalent bonds, the polymer chains are connected to each other by Van der Waals bonds. Van der Waals bonds are not as heat resistant as covalent bonds and break easily with increasing temperature. The rupture of these bonds causes the polymer to soften and deteriorate (22,23,25).

Polymers have three types of structure. These are linear, branched and cross-linked structures (22,24,26).

The molecules in the structure of linear and branched polymers are connected to each other by weak physical bonds. When exposed to heat, these weak bonds break and the molecular chains slide over each other. This causes the substance to change physically; causes it to become more fluid and softer. When the cooling process is done, the broken bonds are formed again and the substance hardens. Such materials are called 'thermoplastic polymers'. Polystyrene, some PMMAs and polyvinyl acrylics are examples of thermoplastic polymers (22,25,27).

There are network-shaped bonds between the molecules in the structure of cross-linked polymers. Crosslinking is usually created with 'ethylene glycol'. Cross-linked polymers have less liquid absorption, and these polymers generally become fluid at much higher temperatures than others. When cross-linked polymers are exposed to heat, their molecular chains cannot slide past each other, so they do not soften when heated, they burn. Such polymers are called 'thermoset polymers'. Silicones, cis-polysoprene, biphenol A-Diacrylate and cross-linked PMMAs are examples of thermoset polymers (22,25,27,28). The structures of the polymers are shown in Figure 2.2a-c.

Figure 2.2. Spatial structures of polymers

2.1.2. Properties of Polymers

Physical and chemical properties of a polymer; factors such as the diversity of monomers in the structure of the polymer, the number of crosslinks in their structures, the amount of crystallization, chemical structures, molecular weight, degree of polymerization, plasticizers or fillers in the polymer, and the number of side chains formed in the polymer affect it (22,25,27).

The structure formed by the combination of monomers of the same type is called homopolymer, and the structure formed by the combination of monomers of different types is called heteropolymer. The presence of the same or different types of monomers in the structure of the polymer affects the physical properties of the substance. Different monomers can be added to the structure in order for the polymers to have more advanced physical and chemical properties, and in this way, there are two or more different monomers in the structure of the polymer. Such polymers are called copolymers. And the reaction that takes place to form a copolymer is called copolymerization. Copolymers generally have better physical and chemical properties than polymers. For example, it is possible to produce more flexible prostheses as a result of the copolymerization reaction of methyl methacrylate and a small amount of ethyl acrylate (22,25).

Cross-linking occurs when the side branches of linear polymers form links. Cross-linking reduces water absorption and dissolution in liquids, increasing rigidity and durability. In the manufacture of artificial teeth, cross-linked polymers are preferred because they are more resistant to surface tensions and solvents. At the same time, the presence of cross-links allows easy leveling and polishing of acrylics. The length of the chain also affects dissolution. Long-chain polymers dissolve more slowly in water than short-chain polymers (22).

The atoms of polymers with more crystals in their structure are more regular. The regular arrangement of atoms makes the polymer more durable and rigid, while absorbing less water (26).

Another factor affecting the physical properties of polymers is the molecular weight of the polymers. The weight of the polymer molecule is equal to the sum of the molecular weights of the monomers that make it up. Since the polymerization process is never fully completed, a residual monomer remains. The remaining residual monomers also affect the molecular weight. The higher the molecular weight of the polymer, the higher the degree of polymerization and the physical properties of the material change positively (22,26,27).

The glass transition temperature (Tg) is the temperature at which polymers liquefy at or above this temperature, transforming from a brittle and hard structure to a rubbery state, and the glass transition temperature affects the physical properties of polymers (28). The molecular weight of the polymer affects Tg. As the molecular weight of the polymer increases, Tg increases, and if the weight decreases, the Tg value

decreases. In general, polymers with long chains and higher molecular weights are more durable and rigid. In addition, the change in molecular weight causes a change in the elastic modulus of the polymer (22,26).

Composite resins used in dental treatments contain cross-linked molecular chains that have made many covalent bonds with each other. The rigid molecular chains formed by these bonds make the polymer harder and more resistant to occlusal forces. By adding plasticizers and fillers to polymers that do not crosslink, the Tg of the polymer decreases. A decrease in Tg makes a material that is rigid at room temperature more flexible at the same temperature. Therefore, the temperature of the polymer affects the flexibility of the polymer and its resistance to forces (22,26).

Reversible elastic deformation or irreversible plastic deformation may occur as a result of the tension forces occurring inside the polymers. In addition, if elastic and plastic deformation occur together, it is called viscoelastic deformation, in this type of deformation, it is not easy for the material to return to its original state after the force is removed. The attempt of polymers to return to their original state after viscoelastic deformation is also called viscoelastic recovery (22).

2.1.3. Polymerization

Polymerization is when more than one low molecular weight monomer combines with a chemical reaction to form a high molecular weight macromolecule, that is, a polymer (22).

2.1.3.1. Polymerization Types

The conversion of monomers to polymers occurs by an addition or condensation type reaction (24,27).

Reactions in which two molecules react and combine to form a larger molecule, while a small molecule is released as a by-product, is called condensation polymerization. These by-products are substances such as alcohol, water, ammonia and halogen acids (23).

With the condensation method, the formation of polymers is slower and the molecules tend to stop before they reach a very large size, as the chains become fewer and less mobile as the chains grow (29,30).

It is very difficult to form molecules with very high molecular weights by condensation polymerization. For this reason, it is not generally preferred in dental restorations and prosthetic applications. The polymerization of silicone and polysulfide elastomeric impression materials used in dentistry occurs with a condensation reaction (29,30).

Addition type polymerization is when two molecules react and combine to form a larger molecule. No by-products are formed in addition-type polymerizations (23). Heat, light or chemicals can be used to accelerate the reaction (1). Polymethyl methacrylate (PMMA) and bisphenol A glycidyl methacrylate (Bis-GMA), which are frequently used in dentistry, are formed by addition type polymerization (30).

Polymerization takes place in four stages: initiation, propagation, chain transfer, termination.

In the initiation phase, first of all, free radicals must be produced, which will initiate the growth of the polymer chain. Activating agents that produce free radicals are called initiators. In chemically activated systems, free radicals are usually generated by the reaction of an organic peroxide initiator and an amine accelerator. "N, N-dihydroxyethyl-paratoluidine" is generally used as an accelerator in dentistry. In light-activated systems, the initiating agent is camphoroquinone (23,30,31). Free radicals occur when the bonds of initiating agents are broken. The free radicals formed then react with the monomers. They allow the release of electrons located at the end of the monomers. This causes the activated monomer to bind with the double bonds of the other monomer and free radicals are formed again (22,26,30).

In this second stage, propagation, longitudinal growth of the chain is observed. Monomers start to make chains. While these chain-forming reactions continue until all monomers are converted into polymers, it is reported that the polymerization is not completely completed. The chain-formed reaction ends with direct double bonds or by the passage of hydrogen atoms from one enlarged chain to another (30).

Although chain formation is terminated by chain transfer, it differs from the termination reactions described. The active state is transferred from an active radical to an inactive monomer and a new nucleus is formed where further growth will take place. Growth stops in the chain to which the active site is transferred (30,32).

Termination stage is defined as the termination of the proliferating free radicals occurs by various mechanisms, resulting in the formation of branching and crosslinks. In other words, both molecules become inactive with an energy change (30).

2.1.4. Denture Base Materials (Polymers)

Denture base is defined as the part of removable dentures that carries the artificial teeth and is located on the supporting tissues (33).

2.1.4.1. Development of Denture Base Materials

It has been observed that materials suitable for the technology of the current period were used in the dental treatments carried out in history. Since the past, denture base materials, like other materials, have made progress as technology develops (34).

The first prostheses were made using the carving method from materials such as bone, wood and ivory, and retention was tried to be provided by using tools such as springs. Following the development of molding and casting systems, metal and metal alloys such as gold, silver and aluminum have also been used in the construction of denture bases (34).

In the 18th century, porcelain began to be used as a base material. Although porcelain has many advantages, it is not preferred because it is not easy to manipulate and is fragile. In 1839, Charles Goodyear found that natural rubber could be vulcanized with dry air, and his brother Nelson Goodyear used this knowledge to discover "vulcanite", a hard rubber, in 1851 (34). Although it is an advantage that vulcanite is resistant to chewing forces, it is a disadvantage that it is dark in color and less transparent. In later times, materials such as polystyrene, epoxy, polyvinyl acrylic, polycarbonate, polyamide and light-activated urethane dimethacrylate were also used in the construction of the denture base (34).

Polymethyl methacrylate polymers, namely acrylic resins, introduced by Wright in 1937, were frequently preferred as base material. Until 1946, 98% of methyl methacrylate polymers and copolymers were used in the production of denture bases (35). Acrylic resins were not much superior to vulcanite in terms of physical properties at the beginning of their use, but the easy production and repair of prostheses made of acrylic resins, the good aesthetics of acrylic resins, and their colors being compatible with the tissues in the mouth have made them more preferred (34).

At the beginning of 1959, the structure of acrylic resins was developed and cross-links were included in the structure. Acrylic resins have started to be preferred very often as denture base material thanks to these advanced properties (34).

2.1.4.2. Characteristics expected from an ideal denture base material

- It should have mechanical properties such as high elastic modulus, high fatigue, high wear and impact resistance. In other words, it should be resistant to chewing and swallowing forces and should not be easily worn.
- It should be resistant to breakage and impact. The prosthesis should not break easily when it falls.
- The expansion coefficients should be similar to those of artificial teeth and there should be a strong chemical bond with artificial teeth.
- It should be dimensionally stable.
- It should provide good surface details.
- It should have a low water absorption rate and low solubility in oral fluids.
- The amount of residual monomer it contains should be low.
- While repairing, its shape and size should not change, and procedures such as relining, rebasing and polishing should be done easily.
- Its color and appearance should be in harmony with the tissues of the mouth. For a good aesthetic, it should pass the light in a sufficient amount. Its color should not change easily with use.
- It should have a low specific gravity and be light.
- It should be radiopaque to detect on the radiograph against the risk of ingestion.
- It should not be toxic and irritant, that is, it should be biocompatible.
- It should not allow microorganisms such as bacteria and fungi to settle and reproduce.
- It should have sufficient thermal conductivity so that patients can easily perceive hot and cold stimuli during food intake and not be harmed.
- It should not cause local tissue reaction or allergy.
- It should have no taste or smell.
- It should be easy to clean.
- Its shelf life should be long and economical (1,22,25,27)

2.1.5. Polymethylmethacrylate (PMMA)

Acrylic resins, namely PMMA, are used most often as denture base material. PMMA is a tough polymer derived from methylmethacrylate, with a Knoop hardness number of 18-20. It has a tensile strength of 59 MPa and a specific gravity of 1.19 g/ml (22,24,25,27). A pure PMMA is transparent. Pure PMMA is colored with the help of many pigments to ensure harmony with the tissues in the mouth. PMMA used in dentistry exists in many forms. Generally, powder and liquid form are frequently used (22,24,25).

2.1.5.1. Contents of polymethylmethacrylate powder

Polymers and copolymers are the structures that make up the largest part of the powder. PMMA is a clear glassy polymer. It can even transmit UV rays with a wavelength of 0.25 (1,25).

Reaction initiating agents are substances that initiate the polymerization of acrylic resins. One of these substances is benzoyl peroxide (22). One of the free radicals formed by the breakdown of the bonds in the structure of benzoyl peroxide reacts with the monomer molecule and a new free radical is formed. The newly formed free radical binds to another monomer molecule. The polymerization reaction continues in this way (1,22,24,25,27).

Coloring agents are the substances in PMMA powder so that the prosthesis has a compatible appearance with the oral tissues. These items are; mercury sulfide, mercury, sulfite, cadmium selenide, cadmium sulfide, ferric acid or carbon. Denture base materials often contain cadmium salts as colorants (22). It was determined that the color stability of the denture bases containing cadmium salts was good and the residual cadmium was found in very low amount. However, since cadmium salts cause allergic reactions, the use of iron oxide and organic pigments has been suggested instead (25,27).

These pigments are added to the content during the polymerization stage during the production of the material or mixed into the polymers after polymerization. Usually, pigment addition and mixing done after polymerization. The more randomly the pigment is dispersed in the denture acrylic, the more natural the appearance of the denture base becomes (36).

Dyes are also other coloring agents, but they are not as successful as pigments. This is because the dyes dissolve in oral fluids and are released into the oral environment,

resulting in discoloration of the denture. Also, dyed synthetic fibers such as nylon or acrylic fiber are used to imitate small vessels under the oral mucosa (24,25,27).

Opacifying agents are substances included in PMMA powder to allow the prosthesis to be visible on the radiograph. Zinc oxide and titanium oxide powders are examples of opacifying agents. Heavy metal components such as barium are also used (22,24,25,27).

Plasticizers are used to increase the solubility of PMMA particles, which dissolve very slowly in the monomer. Dibutyl phthalate is the most commonly used plasticizer. The amount of plasticizer should be 10% to prevent too much dissolution and deterioration of acrylic in oral fluids (22,24,25).

Inorganic substances are used to increase the wettability and hardness of acrylic resins. Glass fibers or inorganic particles such as zirconium silicate are examples of these materials (22,24,25).

2.1.5.2. Contents of polymethylmethacrylate liquid

Monomer is the methylmethacrylate content that constitutes the most important part of the liquid. It is a very good liquid solvent with clear, colorless and low viscosity. Its boiling point is 100.3°C. It is volatile at room temperature. Its density is 0.945 g/ml at 20°C, and its heat of polymerization is 12.9 kcal/mol (22,23,24).

Inhibitors are substances added to the PMMA content in order to extend the shelf life of the liquid. In the absence of an inhibitor, polymerization can occur at room temperature or even colder. Generally, inhibitors obtained from hydroquinone react with free radicals in the liquid and stop them, so that free radicals cannot initiate polymerization (22,23,25).

Another way to stop the formation of free radicals is to store the material in a dark place. Visible light or UV radiation can trigger the formation of free radicals. Eliminating the light source is therefore beneficial to prevent polymerization from starting. In addition, the amount of polymerization decreases in the presence of oxygen (22,23,26,27,36,37).

Crosslinking agents are added to the liquid to improve the physical properties of PMMA. The most widely used crosslinking agent is ethyleneglycodimethylacrylate. It increases the resistance of acrylic against cracks and scratches (22,23,26,27,36,37).

2.1.6. Classification of Denture Base Materials (Polymers)

Denture base materials used in dentistry have been classified in different ways.

Denture base materials are classified according to their thermal behavior as follows (22,30):

1.Thermoplastic

2.Tremoset

Denture base materials are classified according to their polymer structure as follows (30):

1. Polyacrylic acid ester
2. Polyvinyl ester
3. Polystyrene
4. Polycarbonate
5. Polysulfone
6. Rubber modified polymethacrylic acid ester
7. Polydimethacrylic acid ester
8. Polyacetal resin/Polyoxymethylene (POM)
9. Epoxy resin
10. Polyurethane
11. Cyanoacrylate
12. Acrylic resin

2.1.6.1. Acrylic Resin Denture Base Materials According to Polymerization Types

Many polymerization methods have been developed since the use of PMMA as a denture base material (22). Different studies have been carried out to reduce the polymerization time of PMMA and to improve its physical and mechanical properties (22,24,25).

Denture base materials are classified as follows in international standards according to their polymerization methods (38):

Type 1: Heat-cured polymers

- Class 1: Powder and liquid
- Class 2: Pat

Type 2: Autopolymerized polymers

- Class 1: Powder and liquid

- Class 2: Powder and liquid flowable type

Type 3: Microwave-polymerized polymers

Type 4: Polymers that polymerize with visible light.

According to another classification, denture base materials are classified as follows according to their polymerization methods (1,23,39):

1. Heat polymerized acrylic resins

a. Acrylic resins polymerized by conventional compression molding technique

i. Unfilled acrylic resins

ii. Reinforced acrylic resins

- Reinforced with carbon
- Reinforced with rubber
- Reinforced with fiber

b. Acrylic resins polymerized by injection molding technique

2. Chemically polymerized acrylic resins (Autopolymerizing acrylics, cold acrylics, repair acrylics)

a. Conventional autopolymerizing resins

b. Pourable (fluid) acrylic resins

c. Resins polymerized at room temperature by injection molding technique

3. Light-cured acrylic resins

4. Microwave-polymerized acrylic resins

2.1.6.1.1. Heat Polymerized Acrylic Resin Denture Based Materials

Most denture base materials are polymerized by heat. Acrylics that polymerize with heat are available in powder and liquid form prior to polymerization. The liquid (monomer) consists of pure methyl methacrylate. The powder (polymer) consists of PMMA powder (1).

a. Acrylic resins polymerized by conventional compression molding technique

Conventional compression molding technique is the most commonly used method in the production of denture base materials today. Advantages of the method; providing adequate aesthetics, low cost, easy production, finishing and polishing. The disadvantages are; its resistance to impact and bending is low, local tissue reaction and allergic reactions can be seen (23).

Acrylic resins polymerized by this method are prepared in a dough by mixing PMMA powder and liquid at the rates determined by the manufacturer. By volume, the powder/liquid, ie polymer/monomer ratio is 3:1. As the amount of polymer in the mixture increases, the polymerization time is shorter and the polymerization shrinkage is less. In addition, if the room temperature increases while the mixture is being prepared, the working time will be shortened (34).

The mixed polymer dissolves in the monomer at a certain rate. The powder and liquid mixture formed during this physical reaction of polymer and monomer goes through four stages: sandy, stringy, dough-like (working) and rubbery (elastic) stages. This prepared dough is placed in the muffle under pressure. The process of placing the acrylic in the muffle should be done in the third stage, the dough stage (34).

It has been determined that the majority of acrylic resins in clinical use reach the dough-like stage in less than 10 minutes. The residence time of an acrylic resin material in the dough-like stage is defined as the working time of that resin. According to ANSI/ADA Specification No: 12, the dough must be formable for at least 5 minutes (22,31,39).

After being placed in the acrylic muffle, the muffle kept under pressure is placed in a hot water bath and polymerization is achieved. The reaction that takes place in this technique is exothermic. With the increase in temperature, the double bonds of the carbon atoms are broken and a single bond is formed. At this stage, energy is output and the energy is converted into heat, increasing the temperature of the mass. An increase in temperature causes the monomer to move faster. At this point, the material becomes quite fluid. The benzoyl peroxide, which is a reaction initiator, is decomposed and the polymerization reaction begins in this way (22,23,25).

The physical and chemical properties of denture base materials that polymerize with heat can change according to the duration of the polymerization process (22). The process of heating the acrylic placed in the muffle under pressure can take place in different ways. The conditions, structure and duration of the heating process are called the polymerization cycle (22).

Polymerization Cycles:

There are two different cycles: long polymerization and short polymerization (22).

Long polymerization takes place by polymerizing the acrylic resin in a water bath at 74^{o}C for 8 hours or longer. In the heat polymerization method, the polymerization reaction is

exothermic and the polymerization temperature should be kept around 74°C. After the temperature of the dough in plastic consistency approaches 70ºC, the temperature in different parts of the muffle continues to increase at the same rate. This process should be carried out in a controlled manner in order to prevent undesirable effects such as monomer loss and porosity formation as a result of uncontrolled temperature increase. No additional boiling is done at the end of this process (22,31,37).

There are also methods with additional boiling. In one of these methods, after the acrylic resin is polymerized at 74ºC for 8 hours, the temperature of the water is increased to 100 ºC and an additional boiling is done for 1 hour (22,37).

In another method, acrylic resin is polymerized at 74ºC for 2 hours, then the temperature of the water is increased to 100ºC and an additional boiling is done for 1 hour. Performing the polymerization process at low temperature allows most of the monomers with a boiling point of 100.3ºC -100.8ºC to be converted into low molecular weight polymers without boiling. Then, increasing the temperature to higher degrees allows the polymerization to take place at the maximum level (22,23,25).

In the short polymerization method, the muffles are pressed with brit after being pressed and placed in a container filled with cold water so that it is completely submerged in water. The water in which the muffle is immersed is increased from room temperature to 100ºC and boiling is done for half an hour. Then the muffles are left to cool in their own water or by removing them from the water. As the acrylic resin polymerizes, the amount of monomer decreases, as the temperature of the mass increases, the mobility in the molecules increases and polymer formation begins (22,25,40).

b. Acrylic resins polymerized by injection molding technique

Injection molding technique is the method in which the acrylic dough is injected into the cavity in a special muffle under 6 atmospheres air pressure and polymerization takes place with heat. In this method, the mechanism works on the principle of constant availability of additional resin under a constant pressure (1,23,24).

Acrylic resin is contained in a special box with dose adjustment. In this way, mistakes that can be made in dose adjustment are prevented. Mixing is done with the help of a vibrator. In this way, a more uniform mixing process takes place and a more homogeneous structure is obtained. In this way, contamination of the material and any irritation that may occur on the skin are prevented (1).

Less polymerization shrinkage, less free monomer content, good dimensional stability and tissue compatibility of the prostheses produced by this method, acrylic reaching the thinnest areas, obtaining more homogeneous base plates, and almost no porosity can be considered as the advantages of acrylic resins that are heat-polymerized using injection molding technique. The disadvantages are that it requires special muffles, tools such as auxiliary injection equipment, high cost, crack formation can be observed and low yield strength (23,24).

2.1.6.1.2. Chemically Activated Acrylic Resin Denture Based Materials

Chemically polymerized acrylic resins are base materials that can be polymerized at room temperature through a chemical activator without the need for external heat application. They are generally called cold acrylic resin, self-curing acrylic resin, autopolymerizing acrylic resin. Unlike other polymerized resins, a chemical activator is used to break down benzoylperoxide, the initiating agent of polymerization, to form free radicals (22,26).

When polymerization is desired to take place at room temperature, chemicals such as a tertiary amine such as dimethyl-para-toluidine, sulfuric acid or more stable salts of this acid are added to the liquid so that the peroxide structure can be broken down by a method other than heat. In this way, polymerization can take place at room temperature. During chemical polymerization, it is recommended to perform the polymerization in a pressure due to the inhibitory effect of oxygen in the air on free radicals (1,22,24).

Chemically polymerized resins have some distinct advantages and disadvantages. Chemically polymerized resins have less color stability compared to heat-cured resins. The reason for this is the presence of tertiary amines sensitive to oxidation and color changes in their structures (24,30).

Since the working time of chemically polymerized acrylics is shorter, flaking should be done more quickly. This type of acrylic does not require heating tools and equipment (22,30).

The initial durability of chemically polymerized acrylics is not as high as heat-polymerized resins, but it approaches them after a while (22).

More residual monomers are produced during the polymerization of chemically polymerized acrylics. Unreacted residual monomers resulting from the polymerization of chemically polymerized resins have two particularly negative effects. The first is that the

residual monomer causes tissue irritation and impairs the biocompatibility of the denture base (22,24).

The second is that residual monomers lead to a decrease in the flexural strength, hardness, density and fatigue strength of the denture base. In addition, since chemically polymerized acrylic resins are subjected to lower polymerization temperature and less stress, they show lower linear polymerization shrinkage after the prosthesis is removed from the muffle compared to acrylic resins prepared by heat polymerization (22,26,27).

When chemically polymerized acrylics are stored in water, their volumetric expansion and net dimensional changes are slightly higher. There are no dimensional changes in heat cure acrylics (22,24).

2.1.6.1.3. Light Activated Acrylic Resin Denture Based Materials

The chemical structure of acrylic resins polymerized by this method is different from PMMA-based acrylic resins. Their structures are more similar to those of composite-based restoration materials polymerized with blue light (22).

Acrylic resins polymerized by visible light consist of urethanedimethacrylate (UDMA) structure. These materials include acrylic copolymer, silica fillers as well as urethane dimethacrylate matrix. The initiating agent is camphoroquinone in this type of acrylic resin. The first light-cured UDMA-based system is the Triad produced by Dentsply. However, the use of this material is limited due to its low impact strength. Afterwards, another UDMA-containing light-activated Eclipse resin system was developed (22,23,25,36,41).

The raw dough material is produced in the form of rolls and plates. It is stored in opaque plastic packages to prevent unwanted polymerization. Visible blue light with a wavelength of 400-500 nm produced by quartz halogen lamps is required for the polymerization of the material. In the unit that provides this light, the prosthesis is rotated and each part of the prosthesis receives equal light (23,35).

Light-cured acrylic resin materials have no residual monomers, so there is no tissue irritation. In this type of acrylics, there is no heat release during polymerization, the working time is long, the polymerization time is short and the polymerization process is easy. The dimensional stability of the denture base produced by this method is good, so the adaptation to the tissue (22,23,25).

The material can be partially polymerized in the mouth and transferred to the model, and the polymerization can be completed in the model. The baseplate can be polymerized without teeth. The teeth can then be attached to the base with an additional material. The absence of flasking and finishing processes is one of its biggest advantages (22,23).

Light polymerized acrylic resin materials can bond well with other denture base resins. When used as a primer material, it is easy to manipulate but requires special light devices for polymerization. In this method, it is stated that when the strength of the polymerizing light weakens, the amount of polymerization will be low (22,23).

Light-polymerized acrylic resins are comparable to heat-polymerized resins in terms of impact strength and hardness, but have a significantly lower elastic modulus. Therefore, a prosthesis made of light-polymerized acrylic resin shows more elastic deformation than heat-polymerized acrylic resin under masticatory forces (23).

2.1.6.1.4. Microwave Activated Acrylic Resin Denture Based Materials

Another method used for the polymerization of acrylic resins is the use of microwave energy. Microwave heating is a thermal conductivity-dependent method. In resins polymerized with microwave energy, the monomer is a mixture of methyl-ethyl methacrylate (22).

This method uses microwaves generated by a generator called a magnetron. The microwave energy generated by the magnetron is transmitted to the muffle in the heating section. In this method, muffle systems made of Teflon, polyester, quartz or fiber-reinforced plastic insulators that can pass microwave rays are used. Since microwaves are reflected by metallic objects, metal muffles cannot be used in the process (1,22,26).

The substance that absorbs the microwave energy reaching the muffle has a polarized molecule. This means that one molecule in the monomer of the substance is slightly positively charged while another is slightly negatively charged. In an electrostatic field where their orientation changes very rapidly, polarized molecules slide rapidly over each other. Heat is generated by the friction arising from these shifts (22,26).

Molecules are known to change direction on average five million times per second. The sliding and friction that occurs at such high speed raises the temperature in a very short time. The direct absorption of microwave energy by the object and its conversion into heat is called dielectric heating. The difference between dielectric heating

and conventional heating is that the inside and outside of the material are heated by the same amount and the temperature increases much faster. As the temperature increases as a result of dielectric heating, the polymerization reaction continues. As the polymerization reaction continues, the number of monomers in the mass decreases, but the monomers present continue to absorb the same amount of energy. This theoretically allows the amount of polymerization to be higher than other polymerization methods (22,25,26).

While conventional acrylics can be used in this method, there are also special microwave acrylics produced by some companies for this purpose (39).

The advantages of this method include short polymerization time and better dimensional stability of polymerized acrylic resins. However, the disadvantages of this method include the relatively high cost of the plastic muffles used in this method and their increased susceptibility to fracture after several packing processes (1,25).

However, in recent years, it has been reported that the use of silicon bronze coated metal screws instead of polycarbonate screws increases the durability of the muffles (22).

In 2006, Anusavice *et al.* compared the physical properties of acrylic resins polymerized with microwave energy and acrylic resins polymerized with heat. They stated that the dimensional stability of acrylic resins polymerized with microwave energy is superior or similar to the dimensional stability of resins polymerized with heat. They also reported that the amount of water absorption and surface hardness values of the resins were similar (22). In terms of the porosity, acrylic resins polymerized with microwave energy were found to be similar or more risky than acrylic resins polymerized with heat (22,25,26).

2.1.6.2. Acrylic Resin Denture Base Materials According to Production Techniques

Until some time ago, denture base materials were only produced using the traditional method. Thanks to advances in science and technology, Computer Aided Design/Computer Aided Manufacturing (CAD/CAM) systems have started to be used in the production of denture bases. The production of acrylic resin-containing dentures base materials was also carried out using CAD/CAM systems as well as the traditional method (4,6).

2.1.6.2.1. Acrylic Resin Denture Based Materials Produced by Conventional Method

The use of conventional methods in the production of acrylic resin base materials involves many clinical and laboratory procedures. The polymerization stage takes place in a technician's laboratory or a dentist's office when production is carried out with this method (4,6).

In addition, complications such as fracture, wear, presence of porosity, presence of residual monomer and tissue irritation, loss of retention, inadequate esthetics and incorrect occlusal vertical dimension have been observed in acrylic resin base materials produced by the traditional method (4,6).

This poor performance of denture bases has been attributed to deficiencies in the resin materials used. Various methods have been used to improve the properties of acrylic resin base materials produced by the traditional method, such as changing the microstructure by adding different ingredients, adjusting the liquid-to-powder ratio, and improving the production steps. The development of new production techniques and new materials is considered as a potential solution to these problems (4,6).

2.1.6.2.2. Acrylic Resin Denture Based Materials Produced by CAD/CAM Systems

CAD/CAM systems have been produced to improve the properties of traditionally produced acrylic resin base materials. Commercially available CAD/CAM fabricated removable prostheses first reported in 2013 and have since grown in popularity (5,6).

In CAD/CAM technology, where the denture base is produced using acrylic discs, the polymerization process is carried out directly in the manufacturer's office. This system allows a prosthetic base to be manufactured from a single block (5,6).

In most CAD/CAM systems, after the denture base has been milled from the discs, the ready-made acrylic teeth are manually fixed using bonding materials in the spaces determined according to the digitally designed occlusion. In some systems, the teeth are milled from one block and the base plate from another. There are also systems in which the teeth and the base plate are in the same block (6).

Different techniques have been developed in the production of removable prostheses and researchers have used different methods. The 3D laser lithography (LL) technique was first used in 1994 by Maeda *et al*. The laser lithography technique was used to create a plastic model of the dentition and to record a base plate of light-cured

acrylic resin. The teeth in the prosthesis were formed using tooth-colored composite resin material and bonded to the base plate with self-curing composite resins (42).

The technique of duplication of removable prostheses was first developed by Kuwahata *et al.* in 1997. A computerized numerical control (CNC) machine and milling devices were used to mill the prosthesis from the modeling wax (43).

Sun *et al.* used 3D laser scanning and RP (rapid prototyping) to fabricate the prostheses (44).

In 2011, Wu *et al.* integrated a laser rapid shaping system and CAD/CAM technology to produce a titanium registration base for a total prosthesis (45).

Kanazawa *et al.* used cone beam computed tomography (CBCT) to scan a set of artificial teeth and the patient's existing denture to obtain records of the patient's mucosa and centric relationship. The prosthesis was then designed using a 3D CAD software, after which the transparent base plate was manufactured by milling using a CNC machine. There are spaces on the transparent base plate where the teeth will be placed. The teeth in the prosthesis were manually attached to the base plate (46).

Goodacre *et al.* published the first clinical case of a CAD/CAM proof-of-concept prosthesis made from prepolymerized PMMA using a five-axis milling machine and fine instruments. In this concept, the teeth were manually inserted into the cavities created when milling the prosthesis (47).

Inokoshi *et al.* produced the prostheses with rapid prototyping technology after scanning the try-in denture with CBCT (48).

Using CAD/CAM and rapid prototyping technology, Bilgin et *al.* fabricated artificial teeth from a one-piece monochromatic PMMA block and micro-hybrid nano-filled resin. They fixed their artificial teeth to the base plate with traditional methods (49).

In CAD/CAM systems using acrylic discs, the curing process is performed directly by the manufacturer. While some steps vary from company to company, certain practices are the same for every manufacturer. This involves mixing the powder and liquid in appropriate proportions and storing the resulting dough at a low temperature (-18°C) for about 24 hours. After storage, the dough is placed in molds, pressed under high pressure and gradually polymerized at increasingly higher temperatures (50).

The aim is to increase the degree of polymerization without creating voids in the material. Long polymer chains are formed as a result of this production process. As more

monomers are converted into polymers, fewer residual monomers are produced and a product with higher hardness is formed (50).

Acrylic resin materials produced with the CAD/CAM system are prepared by polymerizing under controlled high temperature and pressure. They are also called HPP (high performance polymers) because they contain a large number of cross-links in their chemical structure. Acrylic resins produced with the CAD/CAM system are prepolymerized and stored until use (47,51).

CAD/CAM acrylic resin materials have different mechanical properties and a highly cross-linked structure due to their monomer and chemical composition. Prepolymerized acrylic resin materials produced using an advanced five-axis milling system are less porous than conventional prosthetic acrylic resin materials. Bacteria and Candida Albicans retention is less in CAD/CAM acrylic resins (47,51).

CAD/CAM systems prevent low mechanical stability, reduced adaptation to supporting tissues and low clinical retention due to polymerization shrinkage in the structure of traditional acrylic resins. CAD/CAM acrylic resin materials are highly densified acrylic resins. This property leads to better flexural strength and stiffness (47,51).

Acrylic resins produced with this system polymerize under high temperature and pressure. High temperature and pressure trigger the formation of long polymer chains. This triggers the formation of long polymer chains. Since more monomers will be converted into polymers, there is less residual monomer formation and release. Color stability and optical properties of CAD/CAM acrylic resin materials have also improved (52).

CAD/CAM acrylic resin materials have several clinical advantages over conventionally produced ones. Reducing the number and duration of appointments and electronic archiving are clinical advantages. Prosthesis production is accelerated thanks to CAD/CAM systems and the production stages of the prosthesis are reduced (51).

2.2. CAD/CAM Systems

CAD/CAM means 'computer aided design, 'computer aided manufacturing' (53). CAD/CAM systems developed rapidly in a short time and began to be used in dentistry.

It has found a wide area of use, especially in the fields of prosthetic dental treatment and restorative dental treatment (54).

These systems, which play a role in the production of restorations such as orthodontic treatment, inlays, onlays, crowns, bridges, laminate veneers, maxillofacial prostheses , implant abutments, removable prostheses, infrastructure of hybrid prosthesis, maxillofacial prostheses, are used by dentists and technicians. (55,56).

Materials used in CAD/CAM systems in dentistry (57);

- Feldspathic ceramics
- Sintered oxide ceramics
- Oxide ceramics
- Glass infiltrated oxide ceramics
- Leucite reinforced glass ceramics
- Glass ceramics reinforced with lithium disilicate
- Lithium disilicate ceramics reinforced with zirconia
- Nanoceramics
- Hybrid ceramics
- Composites
- Metals
- Polymers
- Polymer infiltrated ceramics

With the development of CAD/CAM systems, it is aimed to produce restorations faster and to ensure standardization in production, to make more natural-looking and more aesthetic restorations and to produce high-strength restorations that can withstand pressure (58).

2.2.1. Development of CAD/CAM Systems

CAD (Computer Aided Design) is the design of an object in three dimensions with the help of computer systems. CAM (Computer Aided Manufacturing), on the other hand, is computer-aided manufacturing using this 3D design and all other data (59).

CAD/CAM systems were introduced in the early 1960s for use in the aerospace and automotive industries. The first CAD/CAM system used in dentistry was developed in early 1971, by Dr. Duret. He produced a crown for his wife to use in 1983 and

production time took less than an hour. Dr. Duret introduced this system in 1985 at the Congress of the French Dental Association (60). Dr. Duret also developed the Sopha Bioconcept system (Sopha Bioconceptt, Inc., Los Angeles, CA) but this system is not used today (61).

Dr. Moermann, and engineer Dr. Marco Bradestini developed the first commercial CAD/CAM system together in 1987 (61). The optical scanner in the system was manufactured by Dr. Marco Bradestini. The system is called CEREC, short for computer-assisted ceramic reconstruction. Dr. Rekow worked on a new CAD/CAM system in the 1980s to acquire data with a high-resolution scanner and perform restoration with a five-axis milling device. Dr. Andersson developed the Procera system (Nobel BioCare, Zurich, Switzerland) in 1983. Dr. Andersson was the first to use CAD/CAM in the manufacture of composite veneer restorations. (60,62).

2.2.2. Overview of CAD/CAM Components

CAD/CAM system consist of three main components (63).

Scanners that collect data from structures planned to be designed and manufactured (63). Scanners are divided into optical and mechanical scanners.

Optical scanner: It works with triangulation method to obtain a three-dimensional image of objects. Since it is very sensitive to movement, even the slightest movement can cause errors for obtaining of data (64). The most important advantage of optical scanners is that they collect data quickly and with high resolution (65).

Mechanical scanner: It scans with a pin, ball, or pinpoint. Three-dimensional scanning of the model to be used is done mechanically (66).

Software used to design restorations to be obtained from a working model in a virtual environment and to create the necessary arrangements for milling (54).

It is software that allows the user to design and edit by creating ready-made templates and a modification of ready-made templates. The restoration is designed using this software and a computer. When the design is finished, the virtual model created is made to be sent to the CAM with CAD software and the production phase starts (65).

Milling unit (54) connected to the computer where the restoration block or disc is used to produce the restoration.

The appropriate block/disc is selected for the production of the restoration and this block/disc is produced by milling with the help of the milling bur in the system. After the

production is finished, some corrections such as coloring and final polishing can be made by the technician if necessary (64).

Dr. Duret came up with an idea to make the design and production process practical. In CAD/CAM systems, it is not easy to see clearly all the boundaries of the part to be scanned due to the presence of saliva, neighboring teeth and gingiva. In this case, the traditional method is used to take an impression from the mouth and prepare a model. Then, a more comfortable scanning process can be performed on the model and the data can be transferred to the virtual environment (62,67).

2.2.3. Advantages of CAD/CAM Systems

The number of appointments for the patient and physician has decreased and time savings have been achieved thanks to CAD/CAM systems (54,68).

More durable and aesthetic restorations were made in a shorter time (54,60,67,69)

Preparation of the materials required for the measurement to be taken by the traditional method, selection of the impression tray, disinfection of impression and then sending to the laboratory, cast preparation, cast trimming, die preparation, waxing and casting have been eliminated. Problems that may occur such as the possibility that the impression may not be accurate and may need to be repeated, the impression tray may not fit properly, the impression and tray may separate from each other, air bubbles may form in the cast and the expansion of the gypsum cast have been eliminated (54).

Great convenience was provided to patients with gag reflex and physicians taking impression from these patients (54).

It has become possible to record the impression taken and store them in digital media (54).

The risk of cross-infection has also decreased as the prosthesis construction stages and the errors occurring during these stages have been significantly reduced (54,70).

The need for temporary restoration has been eliminated. Because the production of permanent restorations is realized in a short time (54,71).

During the scanning process, the scanned area can be checked on the screen and the missing areas can be scanned and completed again with the scanning device. In cases where the area to be studied is small, it may be sufficient to scan only the area to be studied and the opposite arch. All desired corrections can be made before the production phase. (54,60).

The previously produced restoration can be reproduced with the previously taken and recorded impression and standardization in production can be achieved (54,72). Fusion, condensation and sinterization processes applied to ceramic materials have been partially reduced (54).

CAD/CAM systems have eliminated the discomfort and time loss caused by the use of a facebow. The problems experienced during the stages of taking interocclusal recordings and transferring the recordings to the articulators have been eliminated (54).

This system can be used in implant applications. Adjustment of implant position, design and production of implant-supported restorations can be achieved with these systems (73).

The presence of state-of-the-art CAD/CAM devices in the clinic positively affects patients and increases the reputation of the doctor in the eyes of the patient (54,74).

CAD/CAM acrylic resins obtained under high temperature and pressure contain low residual monomers and a high degree of cross-linking. This makes the mechanical and biological properties of CAD/CAM acrylic resins superior to conventional acrylic resins (50).

Due to the high cross-links, CAD/CAM acrylic resins have high friction and crack resistance and hardness compared to conventional acrylic resins (47,51).

Thanks to the ability to store data in a digital environment, the prosthesis can be easily manufactured and reproduced. Reducing the production stages of the prosthesis is also an advantage of CAD/CAM system (51).

CAD/CAM acrylic resin materials are less porous than conventional dentures acrylic resin materials. This feature reduces bacterial contamination (47,51).

CAD/CAM systems prevent low mechanical stability, reduced adaptation to supporting tissues and low clinical retention due to polymerization shrinkage in the structure of traditional acrylic resins (47,51).

CAD/CAM acrylic resin materials are highly densified acrylic resins. This property leads to better flexural strength and stiffness. Acrylic resins produced with this system now have less monomer release, improved color stability and optical properties (47,51).

2.2.4. Disadvantages of CAD/CAM Systems

The high production cost is one of the disadvantages of the system (61).

Physicians spend a lot of time learning to use the system (68).

Meeting the patient's aesthetic expectations may not be possible using monochromatic blocks. Polychromatic blocks have been developed to solve this problem (72).

There may be problems in digital recording the subgingival margins of tooth preparations. Therefore, gingival retraction should be performed very well for accurate scanning (75).

Movement of the patient may cause errors during scanning and thus ill-fit restorations (65).

Stages such as impression taking, recording of occluso-vertical dimension, maxillomandibular relationship transfer and creation of the cheek lip support are also required for CAD/CAM systems and are performed as in the traditional method (67).

There may be difficulties in determining the mandibular occlusal plane (67).

The development of the system for taking impression of full arch restorations is ongoing. A study on *in-vitro*, full-arch models compared the accuracy of conventional and digital methods. In full arches, more deviation was observed in impression taken using digital systems (67).

Studies are also underway to use the system more effectively in edentulous patients. In a study, it was revealed that the data of patients with inadequate and abnormal anatomical structures could not be accurately transferred to the system (67).

2.2.5. Classification of CAD / CAM Systems

CAD/CAM systems are divided into two classes: laboratory systems and systems applied at the chair-side in the clinic (54).

1. Laboratory systems (54)

a. Companies that own scanning and milling units

- Amann Girbach
- 3M ESPE
- Sirona Dental Systems
- Zirkon Zahn
- Vhf camfacture AG

- Wieland Dental
- Pou-Yuen and U-Best Dental
- Planmeca
- KaVo Dental
- Dentsply Prosthetics
- Noritake Dental

b. Companies with only CAD system

- D2000,
- 3 Shape; Dental Wings 7 series,
- Dental Wings; IScan D104, I
- Metric 3D SA; Ceramill Map,
- AmannGirrbach; Activity 850 3D, Smart Optics)

c. Companies with only CAM system

- DWX-50
- Roland DGA Corporation; inLab MC X5
- Sirona; M5, Zirkonzahn; Tizian Cut 5 Smart, Schütz Dental
- S2 Model, vhf camfacture AG; Ceramill Motion 2, Amann Girrbach).

2. CAD/CAM systems applied at the chairside in the clinic (54)

a. CAD / CAM systems where the company has its own scanning and milling units (chairside)

- Sirona
- Planmeca

b. CAD/CAM systems where production is not possible and only a scanning system exists

- True Definition Scanner, 3M ESPE
- iTero, Align Technology, Inc
- Trios, 3Shape
- Apollo DI, Sirona;
- CS 3500, Carestream Dental LLC.

3. CAD / CAM, open and closed systems by data sharing (54)

a. Closed systems are systems where all procedures, including data collection, design and production of restoration, are carried out by the same company. All stages are integrated into one system. There is no data exchange between companies (54).

b. Open systems allow data in the virtual environment to be used by CAD and CAM devices belonging to different companies. The laboratory CAD systems are always open and once the restoration has been designed, all data is stored in an accessible STL format file. The laboratory where the restoration will be produced accepts the STL file from this open CAD system and sends it to a laboratory with an open CAM system. For the fabrication of complex restorations, the model can be scanned through open laboratory CAD/CAM or laboratory CAD systems and the generated STL file can be sent to another fabrication center (54,76).

2.2.6. CAD/CAM Systems Used in Dentistry

CAD/CAM systems have been used in dentistry in three categories (60,61,74).

- Office/Clinic Systems

The scan of the tooth preparation is done in the mouth and the restoration is prepared in the clinic.

CEREC (Sirona, Charlotte, NC, USA)

E4D DentistTM (D4D Technologies LLC, Dallas, TX)

- Laboratory Systems

The scanning process from the plaster model or impression is carried out in the laboratory. In most of these systems, substructure is produced. The technician adds porcelain on top of the substrate to finalize the restoration.

CEREC InLab

DCS Precident (Popp Dental Laboratory, Inc, Greendale, WI)

Everest (KaVo Dental Corp, Lake Zurich, IL)

CERCON

- Production Based Systems

Scanning of the model takes place in the laboratory. The data is then sent to the main production center via the internet. The restoration, whose substructure is prepared in the main production center, is sent back to the laboratory for porcelain to be added to it. Building all substructures in the same center ensures optimal quality control.

Procera" (Nobel Biocare USA, LLC, Yorba Linda, CA)

LavaTM {3MESPE, St Paul, MN)

TurboDent (U-Best Dental Technology, Anaheim, CA)

2.2.6.1. Cerec System (CEramic REConstruction system)

CEREC, the first commercial CAD/CAM system produced by Dr. Moermann and engineer Dr. Marco Bradestini, was launched in 1987 and was the first system to combine digital scanning with a milling unit (60,61).

This system is designed with the concept of "triangulation of light", where the intersection of three linear light beams is focused at a specific point in three-dimensional space. Surfaces with uneven light distribution reduced the accuracy of the scans. Therefore, the prepared tooth to be scanned is coated with a special titanium dioxide powder that opacifies translucent areas, ensuring uniform light distribution and allowing the camera to accurately record all tissues (77,78).

The image of the prepared tooth is then recorded with an optical scanner and the image is transferred to a computer. Margins and contours are determined on the computer screen and necessary adjustments are made. The ceramic block of the appropriate size is placed in the milling unit and the restoration is produced by milling the block. The system makes it possible for dentists to apply restorations made of ceramic blocks to the patient in a single session. Only inlays and onlays could be produced with the oldest CEREC systems (60,77).

Early CEREC systems have poor marginal integrity and surface shaping. In the following years, Cerec 2, Cerec 3, Cerec 3D, Cerec inLab, Cerec AC (Bluecam), Cerec 4, Cerec Omnicam and Cerec Primescan brought different innovations to the market. Launched in 2004, CEREC inLab is a laboratory system in which models are scanned by laser and the digital image of the virtual model created as a result of the scan is transferred to a computer. In the system, the substructure is designed, then the technician places the appropriate block in the machine for the production of the prosthesis and the milling is done. Thanks to its biogenic properties, restorations similar to the morphology of existing teeth or restorations can be designed. Thus, personalized design and production can be achieved (64).

In 2009, CEREC BlueCam was introduced as a scanner system. For easy and accurate scanning, the areas to be scanned are also coated with titanium dioxide powder in this system. In the CEREC BlueCam system, a single image is captured using visible blue light diodes (LEDs). In this system, while the impression of a single tooth or multiple teeth in a single arch can be taken clearly during scanning, there were problems with the impressions covering the entire arch (60).

In 2012, the CEREC OmniCam system was introduced, eliminating the use of titanium dioxide powder, which affects image quality and makes it difficult to obtain comfortable and accurate images. The CEREC Omnicam system does not use powder, allowing for obtaining of precise image and natural color image capture. This feature is also advantageous for scanning larger areas. A three-dimensional model is created from multiple data collected by sequential imaging. It has been reported that this system provides more accurate data in full-arch scans than the BlueCam system (79).

Most recently, in April 2019, Dentsply Sirona produced the CEREC Primescan system, which includes high-resolution sensors capable of deep dynamic scanning down to twenty millimeters. This system includes a touch screen, touch pad and the new Cerec 5 software (80).

The CEREC system is a closed system that operates using SIRONA's supporting CAM devices such as CEREC MC and CEREC In-Lab and exports the digitized data as a special format file. In other words, CAD and CAM operations are performed by the same company (SIRONA) (78).

2.2.6.2. E4D System (E4D Dentist System)

The E4D system, launched in 2008, includes a laser scanner called the Intra Oral Digitizer, a design unit and a milling unit. The high-speed laser technology in the system captures the image from every angle (60,81).

The use of powder is not necessary except in some cases when taking image. The design system automatically detects and marks the margin lines. Once the dentist confirms the limits, the computer suggests a restoration model. The E4D Dentist system has an "autogenesis" feature and is capable of personal design. Lithium disilicate, leucite-containing ceramic and nanoceramic blocks are available for use in the system. There are also blocks used for the production of temporary restorations. An advantage of E4D is that up to 16 restorations can be worked on at the same time. Once the restoration is

approved, the data is transmitted either to the milling machine available in the clinic or to a dental laboratory and fabrication is carried out (60,79).

A file in the E4D system can also be converted to an STL file by paying a fee to D4D technology. This means that the digital data can also be used by other CAD/CAM systems, so the E4D system can be considered as a semi-open system (60).

Like the CEREC BlueCam and Omnican systems, the E4D system allows the patient to be treated in a single session and to obtain high-strength ceramic or composite restorations even for minimally prepared teeth (82).

2.2.6.3. DCS Precident System (Digitizing Computer System)

The DCS Precident system was introduced in 1990. The DCS Precident system consists of a Precisan laser scanner and a Premill CAM multi-milling center. The system is designed to automatically determine the shapes of bridge pontic and connector sizes. Restoration substructures are manufactured from fully sintered Y-TZP blocks (DC-Zirconia) (61,83,84).

Materials used in the DCS system include porcelain, glass ceramics, zirconia, metals and fiber-supported composites. The DCS Precident system is one of the few CAD/CAM systems capable of milling titanium and fully sintered zirconia. In this system, a model of both jaws can be obtained and 14 prepared teeth can be scanned to produce up to 30 substructures (61,83,85).

2.2.6.4. Everest System

The Everest system was manufactured by Kavo in 2002. It consists of scanner, computer, milling and sintering unit. The plaster model obtained from the sample is placed on a turntable and scanned with a CCD (Charge Coupled Devices) camera. CCD cameras have high performance even in low light conditions and are not affected by vibration, high sensitivity and resolution. These cameras are charge-connected devices. The three-dimensional digital image of the scanned model is transferred to the computer. Three-dimensional design of the restoration is made using a Windows-based software (61,64).

After the design process is completed, the substructure blocks are milled in the milling unit with five-axes movement. The milling unit enables detailed morphological designs and precise production. With this system, leucite-reinforced glass ceramics,

partially and fully sintered zirconia ceramics and titanium can be etched. Inlay, onlay, crown and bridge restorations can be made with the Everest system (61,64).

2.2.6.5. Cercon System

This system was launched by Dentsply in 2002. In the early years, only the CAM system was available and there was no CAD unit. In 2005, a 3D optical scanner (Cercon eye) and CAD design software (Cercon Art) were added to the system. The limits of the restoration are automatically set and edited by the system. The system can mill semi-sintered zirconium blocks. Telescope crown, single crown, abutment and three-five unit bridge restorations can be produced from these blocks (61,64).

A wax sample is prepared on the die of the prepared tooth and this sample is placed on the main piece (Cercon Brain). After the sample is scanned with the laser system, the data obtained is transferred to the milling unit. The amount of shrinkage that may result from the final sintering is calculated and, taking this shrinkage into consideration, the substructures are designed larger than the desired size. Sintering takes place in the Cercon furnace at 1350^{o}C for six hours (22).

2.2.6.6. Procera System

The Procera system was introduced by Andersson in 1983. The Procera AllCeram system was developed by Andersson and Oden in collaboration with Nobel Biocare and Sandvik Hard Materials in 1993. Procera has been developed for pure and high strength, highly sintered aluminum oxide structures. With this system, aluminum oxide (Procera AllCeram) substructures, abutments, veneers, zirconium oxide (Procera AllZircon) crown and bridge substructures and titanium (Procera AllTitan) substructures, abutments and implant-based bridges can be produced. Full ceramic crown production is also possible with these systems (60,62,85,86,87).

The scanning of the model is performed in the system's own laboratory. The data is sent by e-mail to the production center in Sweden or the USA. The sintered and prepared substrates are returned to the screening laboratory. Porcelain application and final procedures are performed by the technician (86,88).

This system follows a different method to form alumina and zirconia copings. First, a 3D image of the mold is taken with the scanner and the data obtained is sent to the CAM center. A wider mold is produced in the center of the CAM, designed to

compensate for the 15-20% shrinkage of the ceramic material. Copings are produced by sintering high purity aluminum oxide powder at temperatures higher than 1550°C in larger than normal molds hardened by dry pressing technique. These substructures are then milled to the desired thickness. The resulting restoration substructure is returned to the laboratory. After the low-resolution surface porcelain is processed on the substructure and its anatomical form is given, the porcelain is fired and the restoration is completed (86,87,88).

2.2.6.7. Lava System

The Lava system was launched by 3M ESPE in 2002. The system consists of Lava Scan three-dimensional optical scanner, Lava Form computer-aided milling machine and Lava Term sintering furnace (65,40).

In this system, the model obtained with the impression taken is scanned with an optical scanner without touching the model. The data obtained is digitized. The restoration margins are automatically determined by the system's software. In order to prevent %20-25 shrinkage of the substructure of the restoration due to sintering, the system automatically prepares the substructures wider. After the substructure design is completed, the tetragonal, semi-sintered zirconium polycrystalline material stabilized with yttrium is selected in appropriate dimensions for substructure production and the restoration is produced in the milling device. Finally, porcelain is added to the sintered substructure and the restoration is completed (63,65,89,90).

The Lava Chairside Oral Scanner (COS) was launched in February 2008. With Lava COS, three-dimensional data can be obtained from a single-lens imaging system. Three sensors simultaneously capture clinical images from different angles. Lava COS has the smallest scanner tip at 13.2 mm wide. The scanner sends pulsating visible blue light as a light source and works in conjunction with a computer and touchscreen. Titanium oxide powder is also used in this method for screening. If a critical missing or blurred area is detected during scanning, it is sufficient to re-scan this area and the software automatically corrects it (63,79,91).

Once the scan has been performed and the data validated, the collected data is sent wirelessly to the laboratory. The data is then sent to 3M ESPE. Thus, the technician examines the images without creating a model and then the gypsum cast is sent to the laboratory. The substructure is designed by calculating a shrinkage rate of %20 and

manufactured from semi-sintered zirconia blocks. Lava COS is considered a semi-open system as it can work with different software (60,61,63,79).

2.2.6.8. Turbodent System

This system was introduced in 2005. In the system, the gypsum cast is scanned with Turbodent Scanner and designed with Turbodent Designer. The system has a five-axis milling unit and inlays, onlays, veneers, copings, bridge substructures, customized abutments and implant bars are produced from ceramic and titanium (61).

2.3. Color

Color is one of the most important aesthetic parameters in dentistry (92). Color is a form of perception formed by reflection, diffusion, transmission or absorption of light from objects (Figure 2.3). In other words, color is the perception of light of different wavelengths when it reaches the retinal layer of the eye (93).

Light is necessary for us to talk about color. If there is light, there is color (94).

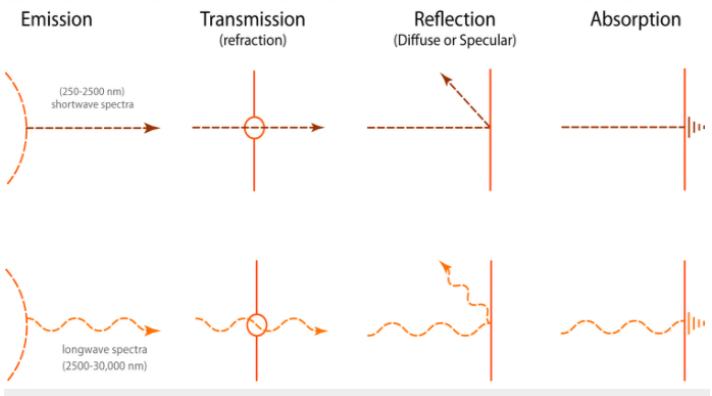

Figure 2.3. Emission, transmission, reflection, absorption

Light is an electromagnetic radiation generated by wavelengths and is expressed in nanometers. Isaac Newton discovered that a beam of white light can be divided into its component colors or wavelengths when it passes through a prism (Figure 2.4). White light passing through the prism is refracted and separated into the seven colors of the rainbow.

Light in the region with the longest wavelengths is perceived by the human eye as red, while light in the region with the shortest wavelengths is perceived as violet (22,94).

This distribution of colors is called a spectrum. This spectrum includes radio waves and gamma between the waves of the rays. A small part of this electromagnetic spectrum (Figure 2.5), 380-780 nm, between infrared and ultraviolet rays, is defined as the visible light spectrum. Only the energy carried by waves with wavelengths in this range can stimulate the cone cells in the retina of the eye and allow us to perceive color. Isaac Newton states that the interaction of incoming light with the object creates the perception of color (22,94,95,96).

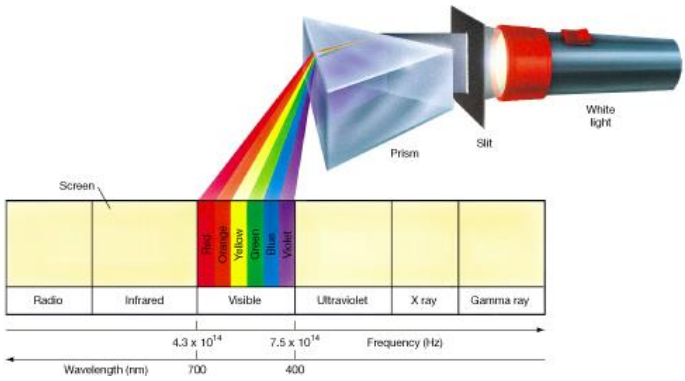

Figure 2.4. Components of electromagnetic spectrum

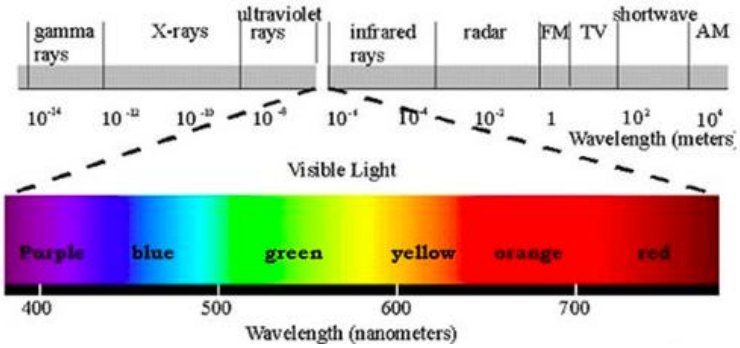

Figure 2.5. Electromagnetic spectrum

2.3.1. Color Perception

The perception of color occurs by absorption and reflection of different wavelengths of visible light. Light emitted and emitted from a source can reach our eyes directly, hit an object and be reflected or pass through it. In some cases, light can be absorbed by the object. The object absorbs all colors in its spectrum except its own color and reflects only its own color. An object is perceived as green if it absorbs all wavelengths except green and reflects only green wavelengths. Black color absorbs all wavelengths of light, while white color reflects all wavelengths (22,93,94,97)

Light comes from the source and enters the eye.There are 2 types of photoreceptors in the eye, called cone and basil. The bacilli are responsible for twilight vision, night vision and peripheral vision and their number increases rapidly towards the periphery and decreases slightly in the extreme periphery. The cones are responsible for bright light vision, color vision and sharp vision. The cones in the human retina absorb photons in three light spectra: 419 nm (blue), 531 nm (green) and 558 nm (red). A given color can stimulate these three types of receptors at different rates and the response to this stimulation affects the perception of the color. Receptors in the retina detect wavelengths that are not absorbed and these electrical impulses are transmitted via the optic nerve to the visual center at the back of the brain (Figure 2.6) (22,24,94,95,96,98,99).

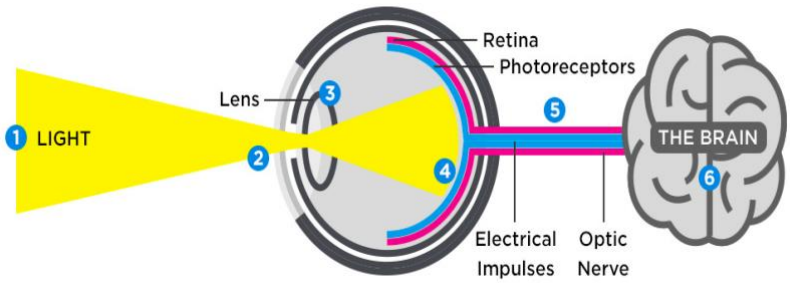

Figure 2.6. Perception of light

2.3.1.1. The Factors Affecting Perception of Color

Observer Differences: One factor that makes a difference in the perception of color is the eye of the observer. Light from a source is reflected, emitted or transmitted by the object. Light is then perceived by the basil and cone cells in the eye. Basil cells are sensitive to black and white color, while cone cells are sensitive to red, blue or green color. Electrical impulses from these cells are transmitted to the visual center in the brain and color perception occurs (98,99).

The sensitivity of the eye varies from person to person. And with age, physical and cognitive changes occur in the individual. One organ affected by these changes is the eye. The lens in the eye becomes opaque over time, the cornea degenerates and the pupil shrinks (96).

This leads to reduced visual acuity and contrast sensitivity, reduced night vision and increased sensitivity to brightness. As a result of age-related changes in the lens and cornea, the person has difficulty distinguishing between yellow and white color. Blindness, the difference between the two eyes, eye diseases such as cataracts, glaucoma, macular degeneration, diabetic retinopathy, dry eye, and drugs such as viagra, morphine, oral contraceptives also cause differences in the perception of color. Alcohol, caffeine and smoking also affect color perception (96,100,101).

Eye strain is another factor affecting color perception. The color perception of a tired eye and a non-tired eye is different (96).

An individual's mood can affect the diameter of the pupil, which in turn will cause a change in the amount of light entering the eye, affecting color discrimination (96,102).

Nutrition varies from person to person. While nutrients such as vitamins C and E, zinc and lutein have a positive effect on the eye, a diet rich in saturated foods and excess sugar consumption have a negative effect (96,103).

Light: Light from a source interacts with the object it reaches and is perceived as color by the observer. Since the wavelengths and intensities of light emitted from different light sources are different, color perception varies. The International Light Source Commission (Commission International de I'Éclairage-CIE) has standardized the light source so that color measurement is not affected by the light source. This standard light is expressed in Kelvin (K). Daylight corresponds to a light temperature of 6500 K and is expressed as D65 (23,104).

In low light, the basil photoreceptors in the eye are more dominant than the cones, resulting in reduced color perception. For the most accurate color detection, the light intensity should be 150-200 foot candles. The color also changes if the brightness and angle of incidence of light changes (96).

Object: The color of an object is determined by the amount of light it transmits, absorbs and reflects. The size of the object also changes the perception of color. Colors in a wider area appear brighter and more vivid than colors located in a narrow area (23).

Conditions: The brightness and dryness of the background changes the perception of color. An object appears duller against a bright background than against a dark background. This is called the contrast effect and negatively affects the correct color decision (99).

2.3.2. Color Spaces

The definition of color varies from person to person. For this reason, color identification systems have been developed to clearly define color. There are many color identification systems available today. Munsell color system and CIE L*a*b* (ΔE_{ab}) color system are generally used. The Ostwald color system is another option. In addition, the CIE (Commission Intérnationale de l'éclairage) found and proposed an updated system, CIEDE2000 (ΔE_{00}), in 2001 (105,106).

2.3.2.1. Munsell Color System

Albert H. Munsell developed the Munsell color system in 1905. This is the oldest known color system. In the Munsell color system, colors are located on three-dimensional coordinates (Figure 2.7). This color scheme can be likened to a sphere or cylinder. The colorless rays are located in the center of the cylinder (Figure 2.8). The basic principle of this system is based on visual spacing equality between neighboring samples (94,105,107).

The system refers to three different dimensions of color: hue, value and chroma. Value is displayed on the vertical axis of the roller and shows shades of gray from white to black. Hue is on the ring around the cylinder. Chroma extends horizontally and increases from the center to outwards (23,108).

Hue: It means the name of the color and color variant. In other words, it is the color perceived by light of a certain wavelength that stimulates the retina. Munsell defined

the concept of hue as 'the value used to distinguish one color family from another' (93,94,109).

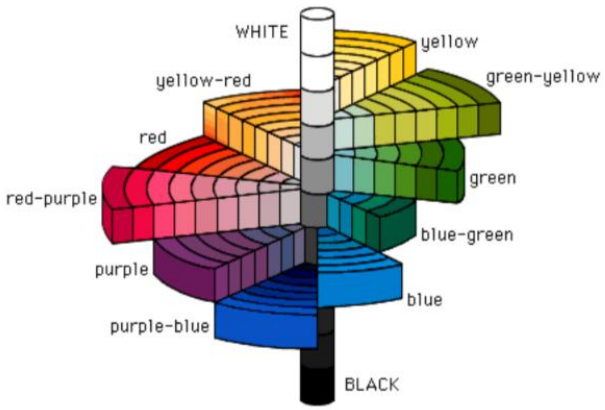

Figure 2.7. The three dimensions of color space

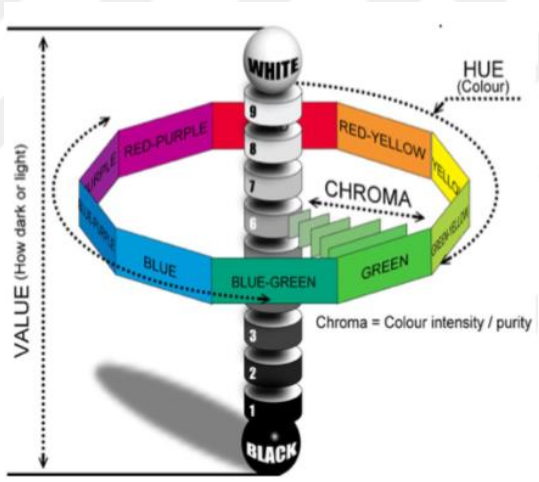

Figure 2.8. The three parameters of color in Munsell color system

This concept is determined by the wavelength at which light dominates in the visible light spectrum. As the wavelength gets shorter, the hue moves closer to the violet part of the spectrum, and as the wavelength gets longer, the hue moves closer to the red part of the spectrum. In the Munsell color system, there are 5 primary colors (red, yellow,

green, blue, purple) and 5 intermediate colors (yellow-red, green-yellow, blue-green, purple-blue, red-purple) (Figure 2.9) (22,23,109).

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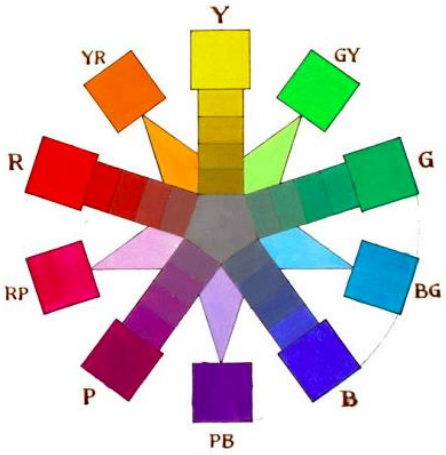

Figure 2.9. Arrangement of hue in the Munsell system

Value: Brightness, i.e. brightness value. It is located on the vertical axis in the center of the cylinder in the Munsell system. The color is black at the bottom of the system and white at the top. The black part is numbered 0 and the white part is numbered 10. Among these values, the shades of gray from black to white form the value values. In the Munsell system, pure white was set as 10 and pure black was set as 0 and considered unattainable. Therefore, there are 9 different value values in the system (Figure 2.10). It describes the lightness and darkness of the color. As the value increases, an object appears brighter and brighter, and as the value decreases, it appears darker and darker (23,110).

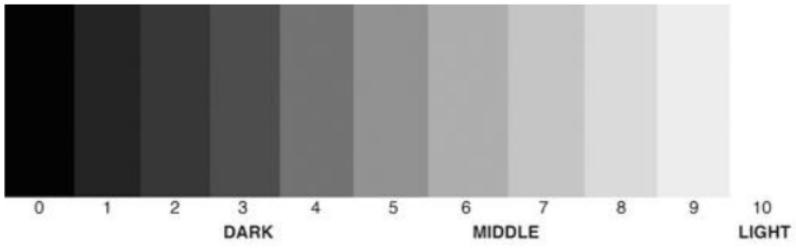

Figure 2.10. Arrangement of value in the Munsell system

Chroma: It refers to the saturation, intensity of a color. The color becomes purer as you move towards the outside of the cylinder. The intensity of each hue decreases in the horizontal direction from the outside to the center. As you move to the center, the colors become grayer (Figure 2.11). The more saturated a color is, the more vivid it appears. When its saturation decreases, the color approaches gray. White, black, white shades between black and white are called achromatic colors. Achromatic colors do not have color characteristics and saturation (23). All parameters of color in Munsell color system is shown in the Figure 2.12.

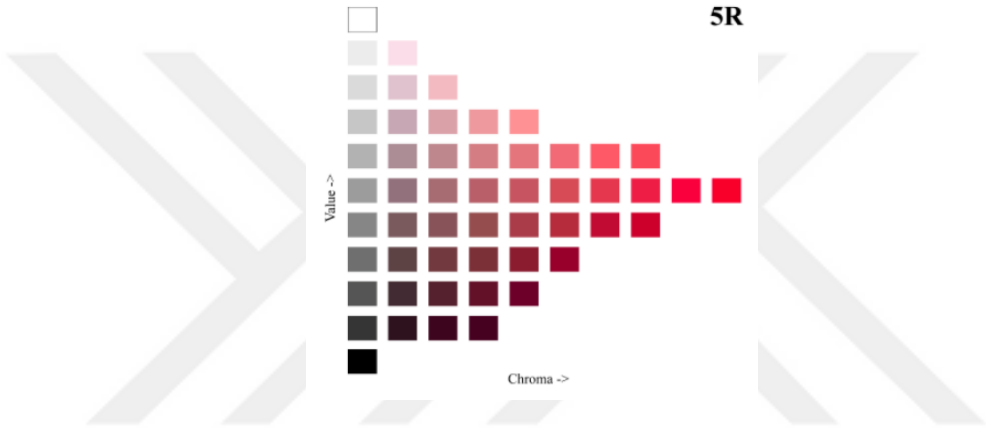

Figure 2.11. Arrangement of value and chroma in the Munsell system

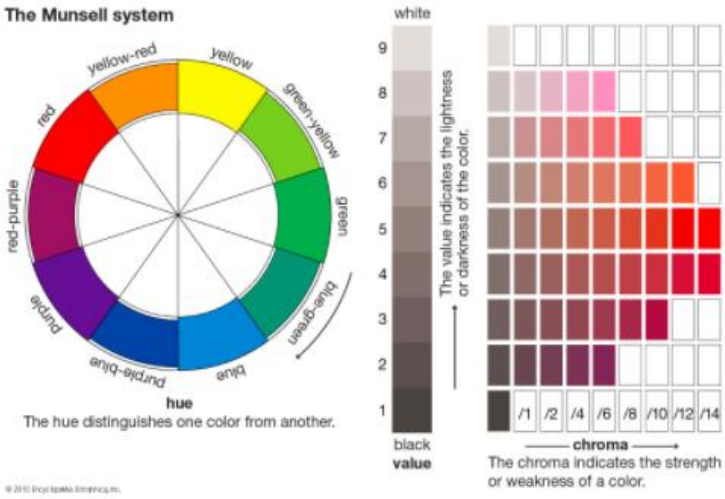

Figure 2.12. All parameters of color in Munsell color system

2.3.2.2. Ostwald Color System

This system was invented and developed by the German scientist Wilhelm Ostwald in 1914. Colors are shown on a circle (Figure 2.13a). Towards the middle of the circle the color becomes gray (Figure 2.13b). In this circle, located at the intersection of two diametrically opposed cones, the colors get darker to downwards and lighter to upwards. This system has 4 primary colors and 8 different gradations. The main colors are yellow, teal, red and sea green. These are placed in a circle to form 24 colors with different shades (111).

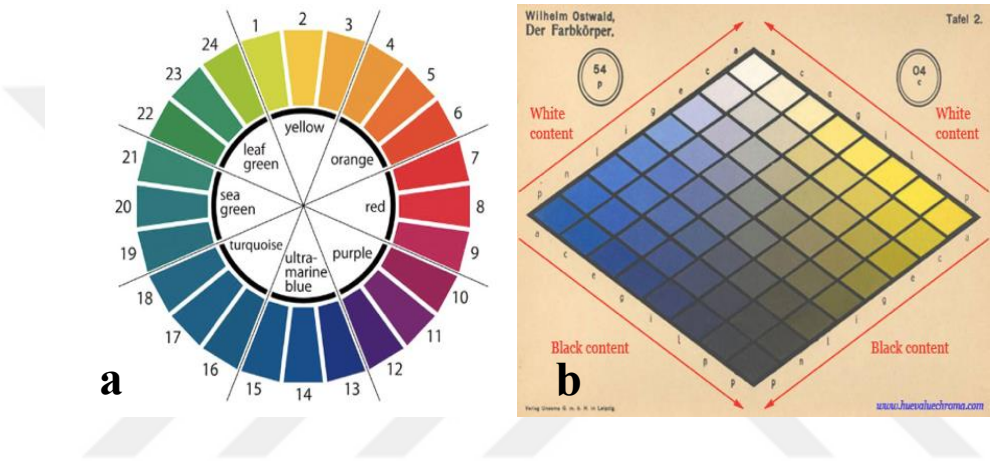

Figure 2.13a. Ostwald color wheel, **b.** Three dimensions of Ostwald color system

2.3.2.3. CIE Color Spaces

Founded in Vienna, Austria in 1913, the International Commission on Illumination (usually abbreviated as CIE, the first letters of the French name Commission internationale de l'éclairage) is the international authority on light, illumination, color and color spaces (95).

CIE XYZ Tristimulus Values and CIE Yxy Color Space: As mentioned earlier, there are 2 types of photoreceptors in the eye, namely cones and bacilli. The bacilli are responsible for twilight vision, night vision and peripheral vision. The cones are responsible for bright light vision, color vision and sharp vision. There are 4 types of cone receptors in the human retina. 3 of these 4 types detect three different wavelengths: 420 nm – 440 nm (short), 530 nm – 540 nm (medium), 560 nm – 580 nm (long). The three

parameters corresponding to the excitation levels of these three types of cone cells sensing different wavelengths are called LMS tristimulus values **(**Figure 2.14**)** (95).

The LMS color space is widely used when performing chromatic adaptation (predicting the appearance of a sample under a different illuminant). It is also useful in color blindness studies when there is a defect in one or more cone types. In this system, the wavelength ranges overlapped, which caused problems in the calculation of LMS tristimulus values (95).

The International Commission on Illumination (CIE) has developed a new value system that is equivalent to the LMS tristimulus values and includes XYZ tristimulus values where the wavelength ranges do not overlap (Figure 2.15). The sum of the X, Y and Z values is equal to the sum of the impulses of the three primary colors (red, green, blue) perceived by the cone cells and transmitted to the brain. Y represents brightness, Z mostly blue wavelengths and X the remaining wavelengths. The XYZ value system created by the "Commision de l'Eclairage" (CIE) is a two-dimensional representation of color space (95).

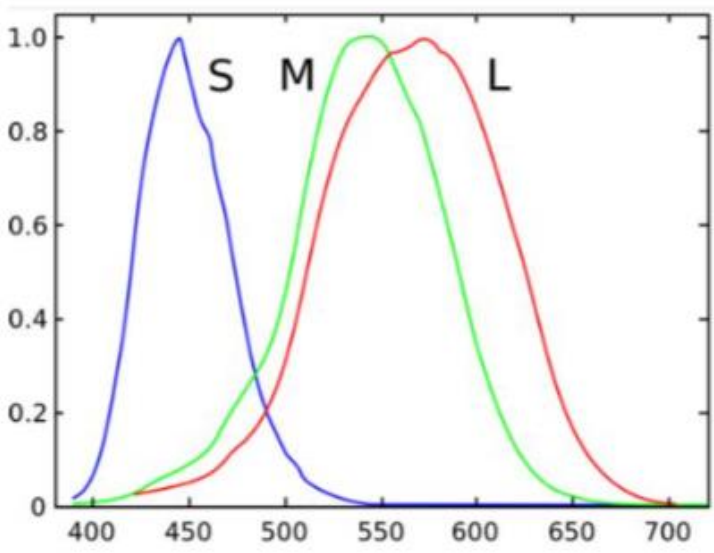

Figure 2.14. The normalized spectral sensitivity of human cone cells of short, middle, and long wavelength types.

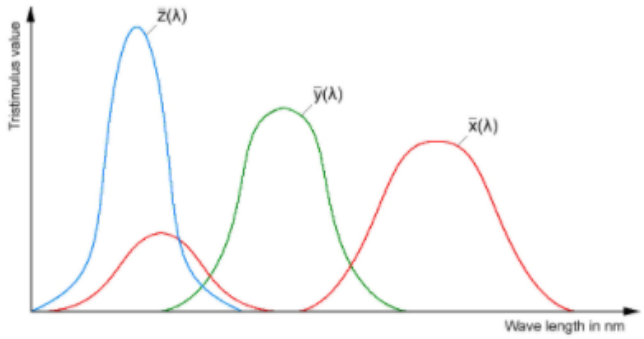

Figure 2.15. Spectral sensitivity corresponding to the human eye (color-matching functions of the CIE 1931 standard observer)

The use of XYZ tristimulus values is useful when describing a color, but the results are difficult to visualize. Therefore, in 1931, the CIE introduced another definition of color space to create a visual color chart that is luminance independent and in two dimensions. This definition is the Yxy color space. Y corresponds to the brightness or reflexion coefficient (and is the same as the LMS tristimulus Y value) and the tristimulus values x and y are the chromaticity coordinates calculated using XYZ (95).

The CIE Yxy chromaticity diagram is shown in the Figure 2.16. In this diagram, achromatic colors are located in the center of the diagram, while chromaticity increases as one moves towards the edges of the diagram (95).

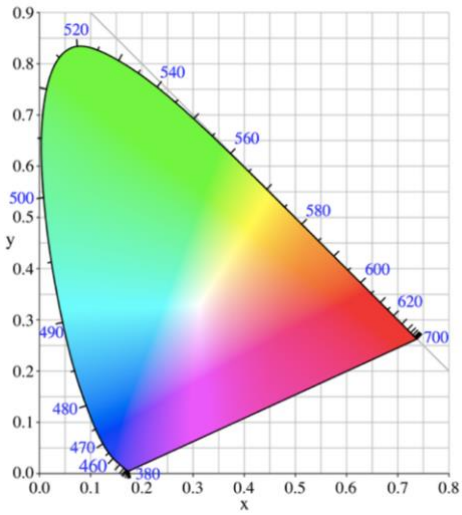

Figure 2.16. Chromaticity diagram of Yxy color space.

CIE L*a*b* Color Space: Introduced in 1976 by the Commission Intérnationale de l'éclairage, the L*a*b* color space, also known as CIELAB, is one of the most commonly used color spaces in color measurement today and is often used in all fields, including dentistry research. The CIE L*a*b* color system is based on the Munsell color system, meaning that the system includes three dimensions of color. In the system, all colors are located in a sphere where the intersection point of three different axes is the center (Figure 2.17) (112,113).

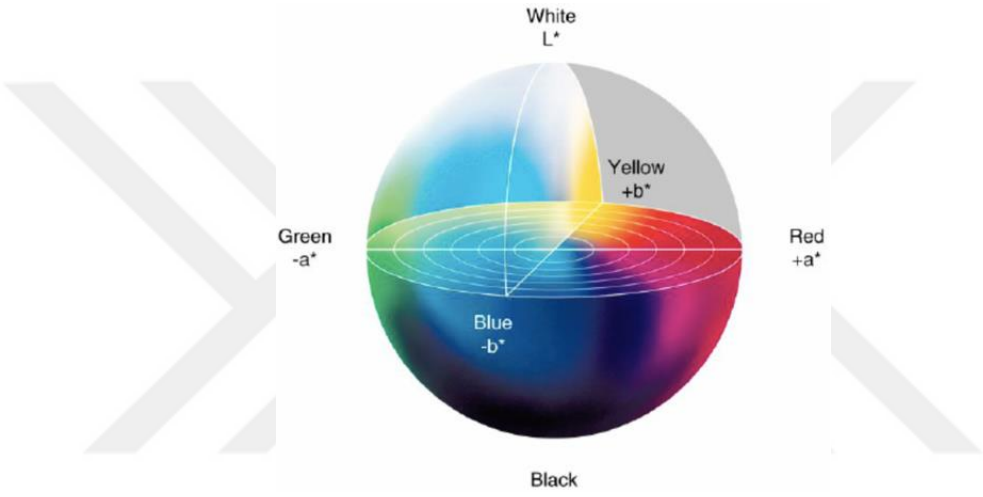

Figure 2.17. Representation of color solid for L*a*b* color space

The axes in question are the L, a and b axes (Figure 2.18). The intersection of these three coordinates gives the value of that color (114) .

"L" is the vertical axis, the parameter indicating the lightness, darkness or brightness of the color. It shows the lightness-darkness coordinates of an object between white (+) and black (-). The L* value is higher for light objects and lower for dark objects (114).

Pure black is 0 and pure white is 100 (114).

The horizontal axis "a" shows the chroma coordinates of an object between red (+) and green (-). As the value increases, the color of the object approaches red, and as the value decreases, it approaches green (114).

The horizontal axis "b" shows the chroma coordinates of an object between yellow (+) and blue (-). As the value increases, the color of the object approaches yellow, and as the value decreases, it approaches blue (114).

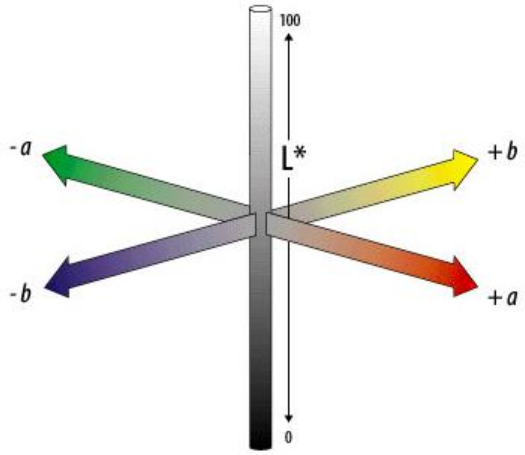

Figure 2.18. L*a*b* color space diagram

As mentioned, the intersection of the three coordinates gives the color value of the object. So a color can be defined with a single value. In this system, the difference between two color measurement values can be calculated. This difference is expressed as the color change value or ΔE and is expressed by the following formula. The ΔL*, Δa* and Δb* values in this formula are the difference between the CIE L*a*b* color variables of the sample whose color is measured (24,94,114,115).

The difference of color (ΔE) before and after staining was calculated using the following formula (116):

$$\Delta E_{2\text{-}1} = [\ (\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2} = [\ (L_2\text{-}L_1{}^2 + (a_2\text{-}a_1)^2 + (b_2\text{-}b_1)^2]^{1/2}$$

Clinical interpretation of the ΔE value can be classified as follows according to O'Brien (Table 2.1) (23).

Table 2.1. Clinical interpretation of the ΔE value according to O'Brien

ΔE	**Clinical Color Matching**
0	Perfect
0.5-1	Very good
1-2	Good
2-3.5	Clinically acceptable
>3.5	Clinically unacceptable

To make a correlation between the amount of color change measured by the spectrophotometer and the amount of color change (ΔE) recorded in the clinical setting, the data were converted to National Bureau of Standards (NBS) units using the following formula:

NBSunits = ΔE×0.92

Table 2.2 explains the National Bureau of Standards (NBS) ratings (117).

Table 2.2. National Bureau of Standards (NBS) ratings

NBS UNIT	Critical remarks of color differences
0.0－0.5	Trace: Extremely slight change
0.5－1.5	Slight: Slight change
1.5－3.0	Noticeable: Perceivable change
3.0－6.0	Appreciable: Marked change
6.0－12.0	Much: Extremely marked change
12.0 or more	Very much: Change to other color

CIEDE2000 color system: The CIEDE2000 system, which was developed to overcome the shortcomings of the CIE L*a*b* system, was adopted in 2001. A strong

correlation was found between the calculated CIE L*a*b* (ΔE) color difference and CIEDE2000 (ΔE00) color difference (114,118,119).

The equation formulating the color difference (ΔE00) in the CIEDE2000 system is as follows (114,118).

$$\Delta E_{00} = \left[\left(\frac{\Delta L'}{K_L S_L} \right)^2 + \left(\frac{\Delta C'}{K_c S_C} \right)^2 + \left(\frac{\Delta H'}{K_H S_H} \right)^2 + R_T \left(\frac{\Delta C'}{K_C S_c} \right) \left(\frac{\Delta H'}{K_H S_H} \right) \right]^{1/2}$$

ΔL′, ΔC′ and ΔH′ are the difference in color, illuminance and intensity between two measurements. The rotation function, is a function that calculates the color and tone differences in the blue region. Weighting functions, is a function for brightness, color and hue at the coordinates. The parametric factors are correction symbols for experimental conditions (120).

2.3.3. Measurement of Color

The assessment of color can be done visually or using color measurement devices. Color measurement using colorimeters has some advantages over visual color determination. The use of color measurement devices eliminates subjective interpretation in color selection. Measuring instruments can also express the numerical value of a color in accordance with international standards. Color measurement devices can be classified as colorimeters, spectrocolorimeters, spectrophotometers, spectroradiometers and digital cameras (107,121,122).

2.3.3.1.Visual Measurement

Color perception varies from person to person (123). Studies have shown that factors such as old age, fatigue, the distance of the object to the eye, the duration of the eye's examination of the object, ambient conditions and light source differences create differences in visual color detection (96,100,124,125).

There are different methods for visual measurement. The Munsell color system is one of the most commonly used. Another method is the use of a color scale (126).

2.3.3.2. Instrumental Measurement

The color measurement using the device takes place by analyzing the light reflected from the object. The use of a device in color measurement enables standardized, objective and mathematical results to be obtained quickly in the expression of color (107,126).

Colorimeters, spectroradiometers, spectrophotometers and digital cameras are used in color analysis today. In instrumental color measurement, factors such as failure to calibrate the device, improper calibration or improper use of the device may lead to incorrect measurements (127).

Color measurement devices are divided into two according to the area they measure on the surface. These are single point measurement devices (SM-Spot measurement) and complete tooth measurement devices (CTM-Complete tooth measurement). When the color parameters of the tooth are to be determined, it is necessary to measure from more than one region with SM devices, whereas CTM devices are a more advantageous option because they can determine all color parameters in a single measurement (128).

Colorimeters

Colorimeters were the first standard color measuring devices (Figure 2.19a,b). They are designed for measuring on flat surfaces. In measurements with colorimeters, the light source and visual angle are fixed and only X, Y, Z tristimulus values can be measured (122).

Colorimeters are smaller instruments than spectroradiometers and spectrophotometers. They are easier to use, and their prices are also more affordable. However, colorimeters also have some disadvantages. Some of these are that their filters wear out in a short time, give inaccurate results on sloping surfaces, and fail to evaluate metamerism. In the determination of the colors of translucent materials with colorimeters, problems such as refraction of the light reflected from the object at different angles and edge-loss are experienced (107,122,129).

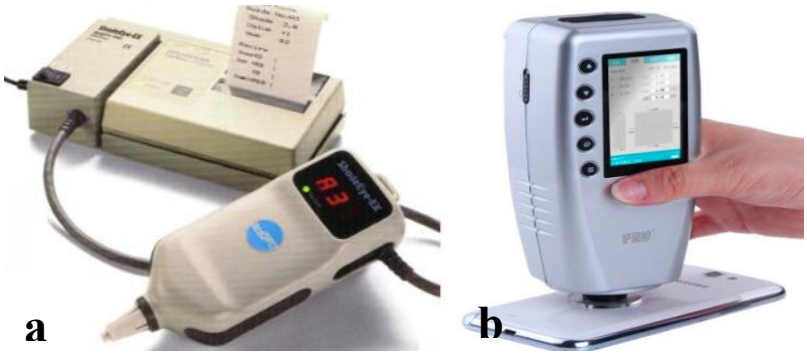

Figure 2.19a,b. Colorimeters

Spectrocolorimeters

The spectrocolorimeter is a hybrid instrument capable of providing colorimetric data such as X, Y, Z or CIE L*a*b* values for various CIE standard Illuminants (Figure 2.20a,b). Therefore, they have the ability to provide much better quality control compared to colorimeters. Their price is slightly higher compared to tristimulus colorimeters, but lower than most spectrophotometers. Spectrocolorimetry can only be used for quality control applications (122).

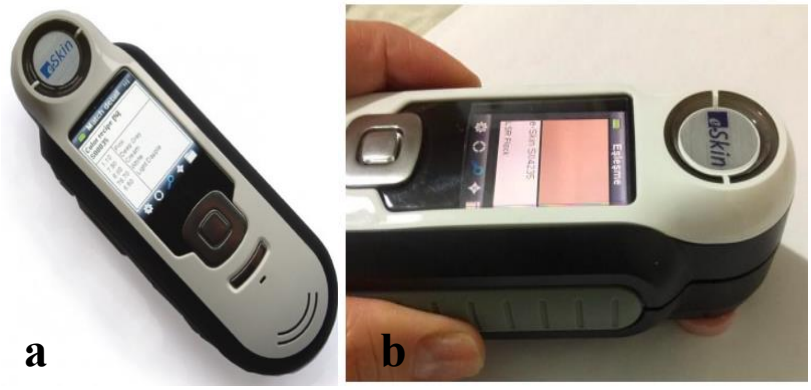

Figure 2.20a,b. Spectrocolorimeters

Spectrophotometers

Devices that can measure the amount of light reflected at many wavelengths (Figure 2.21a,b). It works by measuring the ratio of light reflected from a sample to light reflected from a white surface and comparing this reflected light to a reference parameter. They are also capable of measuring the amount of light energy reflected from the object in the visible spectrum range from 1-25 nm wavelength (122,130).

With the help of a spectrophotometer, numerical data on color and spectral reflectance graphs can be obtained. It is also preferred for complex color analysis due to its high-precision sensor and its ability to make precise measurements under different lighting conditions. Another advantage is that it can evaluate metamerism. There are studies showing that spectrophotometers are more reliable than colorimeters in terms of reliability and reproducibility. Its high cost can be considered as a disadvantage (107,130,131).

Figure 2.21a,b. Spectrophotometers

Spectroradiometers

Spectroradiometers are used to measure radiometric values (Figure 2.22a,b). They include a spectrometer calibrated to the wavelength scale and radiometric scale.

With a spectroradiometer, color can be measured under the same observation conditions as visual color determination and without touching the sample. The spectroradiometer also allows color measurements of shiny objects and surfaces. The slightest change in the angle during measurement may cause a difference in the results (107).

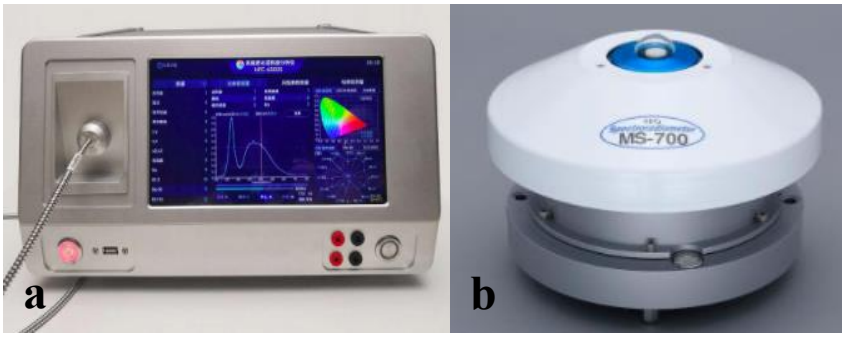

Figure 2.22a,b. Spectroradiometers

Digital Cameras

The use of digital cameras for color measurement has recently become popular. These devices are also called RGB devices (Figure 2.23). This system consists of a digital camera, computer, driver, computer software and color sensor. The image of the desired sample is taken with a digital camera and transferred to a computer to which the camera is connected. And thanks to the software on the computer, the values are obtained in CIE L*, a*, b* (107,132).

The advantage of the system is that the color appearance of the whole object can be obtained as an image, rather than measuring a single point (107).

Digital cameras are practical to use, but factors such as angle and light can alter the assessment of color. Their use alone is not sufficient. Another limitation of digital cameras is that gray card and Adobe Photoshop literature-based protocols are required to know the shade matching protocol (96).

Figure 2.23. A digital camera used in dental photography

2.3.4. Terms Related to Color and Light

2.3.4.1. Metamerism

Metamerism is when two objects that appear the same color under the same light source appear different colors under different light sources (Figure 2.24). The spectral reflectance properties of the colors of metameric objects are different. Under the same light source, the tristimulus values of two objects may be the same, but under a different light source they are measured differently. This problem usually occurs because the objects contain different pigments, their content is different. In order to prevent metamerism, the color should be selected by checking under different light sources (96,97).

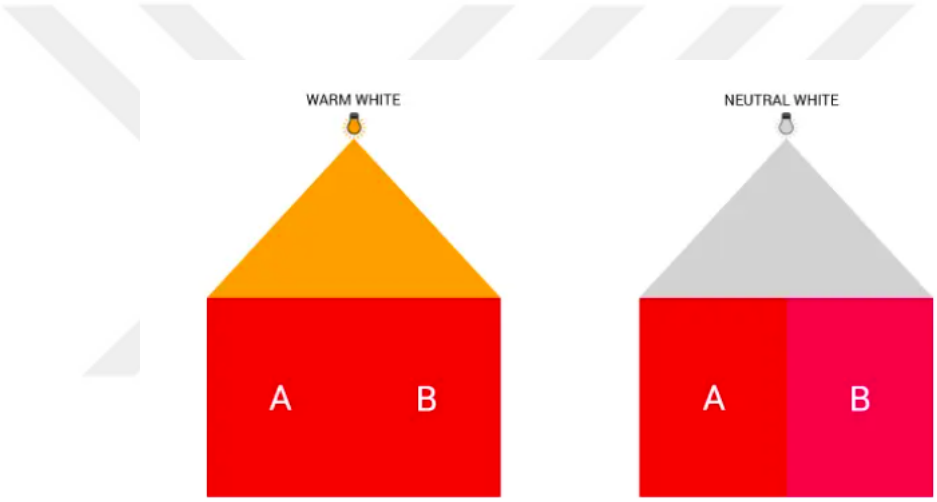

Figure 2.24. The phenomenon of metamerism

2.3.4.2. Refraction and Reflection

Refraction is the separation of the beam of light as it passes obliquely through the transparent layer at different speeds. However, light never passes completely through a transparent or translucent object; some of it is reflected from the surface. When rays hit a surface and return back, this is called reflection (Figure 2.25a,b) (133).

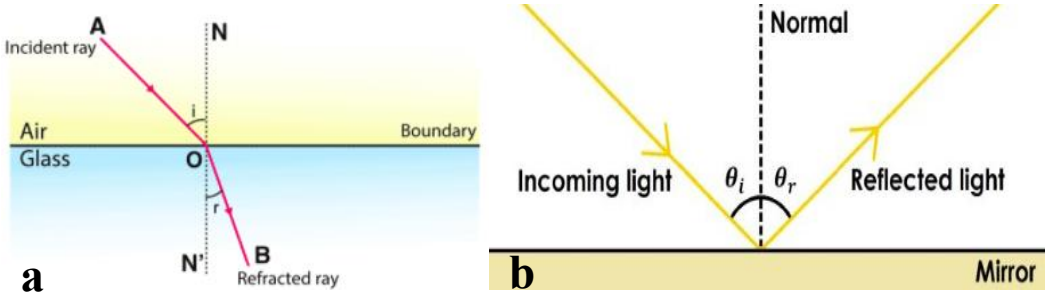

Figure 2.25a,b. a. Refraction, **b.** Reflection

2.3.4.3. Transparency

Transparency is the property of a material to completely transmit light through it. The object behind a transparent object can be seen clearly (22,107).

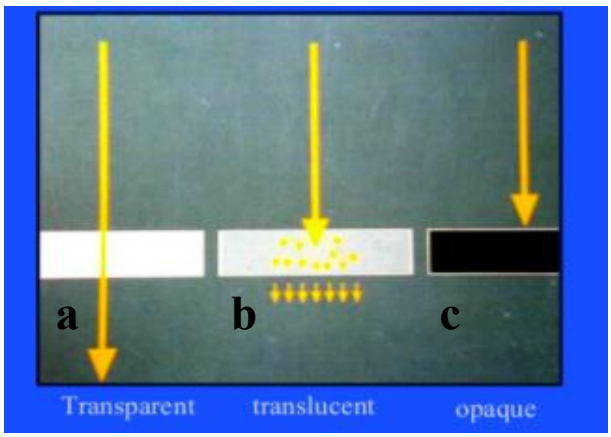

Figure 2.26a-c. a. Transparent, **b.** Translucent, **c.** Opaque

2.3.4.4. Opacity and Translucency

Translucency is the ability of the material to transmit light (Figure 2.26b). Light cannot pass completely through translucent objects, some of it is diffused, refracted and

scattered. Opacity is the ability of the material to prevent light transmission (Figure 2.26c). Therefore, translucency can be expressed as a value between transparency and opacity (107,133).

2.3.4.5. Opelasans Feature

The appearance of the material in different colors when light is absorbed and reflected is called the opalescence property of light (134).

2.3.4.6. Radiation (Fluorescence) Property

The absorption of light by an object and its emission at a long wavelength is called light fluorescence. Fluorescent materials appear brighter and more vivid (133).

2.3.4.7. Pigmentation

The small particles that make up the color are called pigment and the coloration in an object is called pigmentation (22).

3. MATERIALS AND METHOD

This study was conducted in Optimal Dental laboratory, FEBE Dental Laboratory, Hard and Soft Tissue Laboratories at the Faculty of Dentistry, Yeditepe University, Turkey.

3.1. Preparation of Acrylic Resin Materials

Three CAD/CAM (Merz-M-PM Disc, Dentsply Sirona-Lucitone 199 Denture Base Disc, Ondent-Tempo-Cad Pink PMMA Disc) and one heat-cured (Heraeus Kulzer-Paladent 20) acrylic resin materials were tested. The acrylic resin materials tested are shown in the Table 3.1.

Table 3.1. The acrylic resin materials tested in the study

Material	Shade	Manufacturer	Lot No.
Paladent 20 (Heat-cured) (**HC.H**)	41	Kulzer Mitsui Chemicals Group, Hanau, Germany	K010036
Lucitone 199 (Milled) (**CC1.D**)	Original	Dentsply, Surrey, United Kingdom	200319
M-PM (Milled) (**CC2.M**)	Pink	Merz Dental GmbH, Lütjenburg, Germany	22820 41216
Tempo-Cad (Milled) (**CC3.O**)	Pink	Ondent Medical Equipment Ltd., İzmir, Turkey	17069

3.1.1. Preparation of Heat-Cured Acrylic Resin Materials

For the preparation of heat-cured acrylic resin samples, a 3D drawing of a dis-shaped sample with a diameter of 15 mm and a thickness of 2.5 mm was first made. With the help of a laser, stainless steel plates were cut and molds were obtained using the prepared three-dimensional drawing as a guide. While creating the samples, grooves were cut with a milling cutter on the mold plates to allow the excess acrylic to flow **(**Figure 3.1a, and Figure 3.1b). As a results, a total of 40 heat-cured acrylic resin samples were prepared.

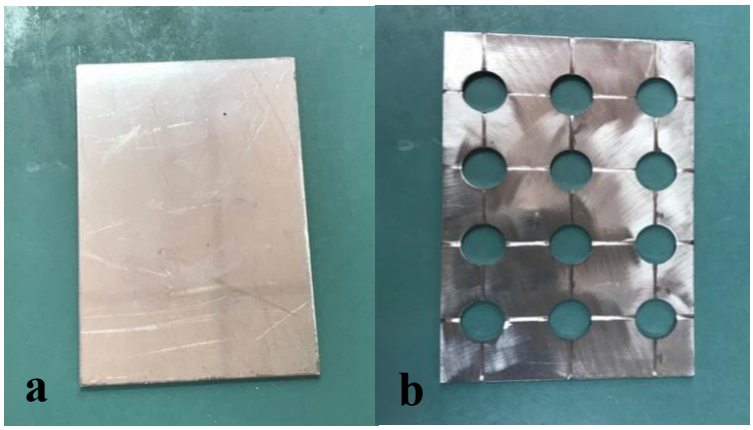

Figure 3.1a. Stainless steel plates

b. Stainless steel plates with grooves and holes

Heat-cured acrylic resin material was mixed in the ratios recommended by the manufacturer (70 g/ 28 ml) and the cavities in the mold plate were filled (Figure 3.2a, and Figure 3.2b). This plate was placed between two flat plates and then placed in the press machine. Excess acrylic was allowed to flow from the plates compressed by the press machine. According to the recommendation of manufacturer, the plates were then immersed in boiling water, the heat source was turned off and left for 20 minutes. Than the plates were left to cool after polymerization and the samples were removed from the mold.

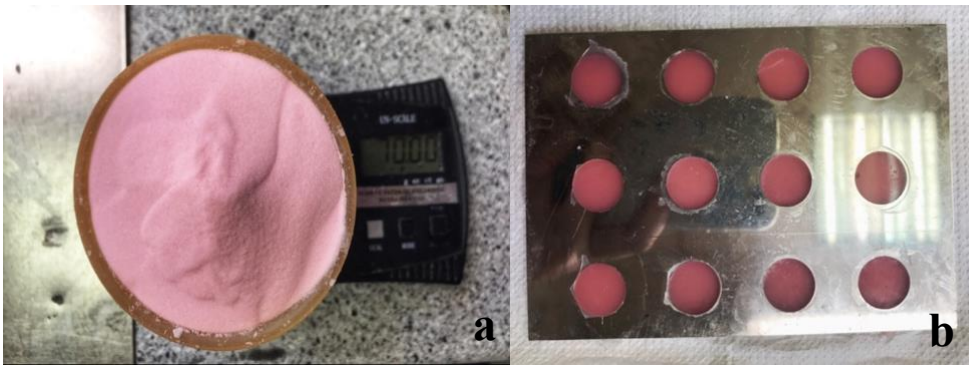

Figure 3.2a. Recommended ratio of heat-cured acrylic resin material powder

b. Mold plate with cavities

3.1.2. Preparation of CAD/CAM Acrylic Resin Materials

To obtain CAD/CAM acrylic resin samples, a 3D drawing of a disc-shaped sample with a diameter of 15 mm and a thickness of 2.5 mm was made Figure 3.3. This 3D drawing was transferred to the milling machine (D43 New, Yenadent, Turkey) (Figure 3.4.) in the laboratory. The CAD/CAM acrylic samples were obtained by milling from the acrylic blocks (Figure 3.5a-c). An example of milled CAD/CAM acrylic blocks and samples obtained are shown in Figure 3.6a,b, and Figure 3.7. As a result, 40 samples were prepared from each CAD/CAM acrylic resin brand.

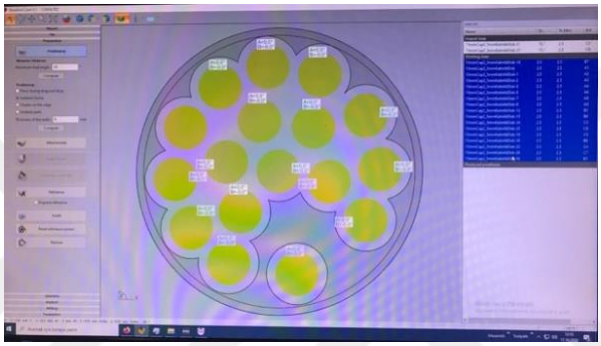

Figure 3.3. A 3D drawing of a disc-shaped sample

Figure 3.4. The milling machine

Figure 3.5a-c. CAD/CAM acrylic blocks

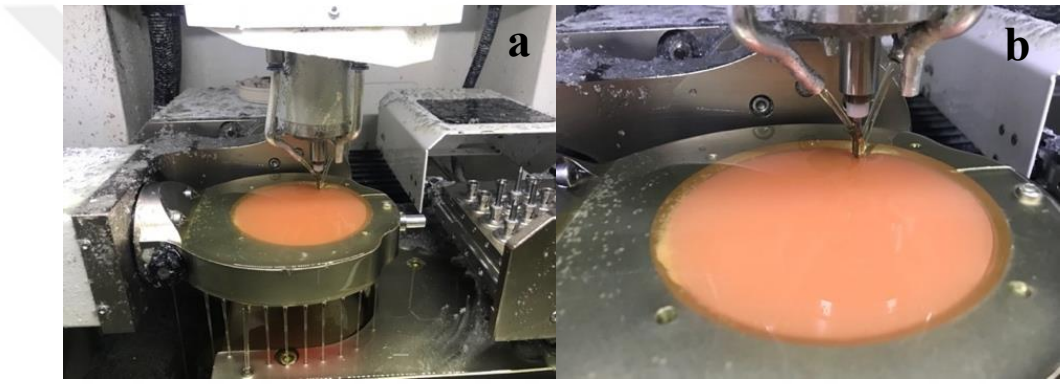

Figure3.6a,b. Milled CAD/CAM acrylic blocks

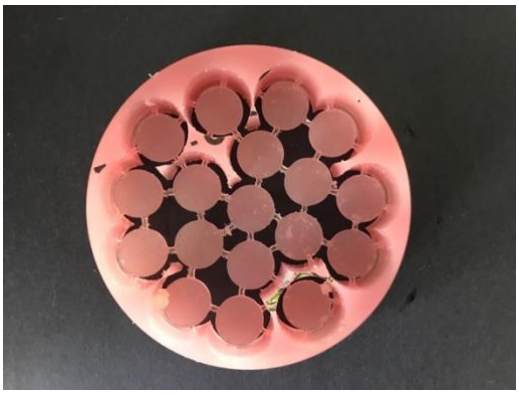

Figure 3.7. Milled CAD/CAM acrylic resin samples

Before sanding, the surface that will not be abraded (corresponding to the impression surface), was marked with a milling notch (Figure 3.8).

Figure 3.8. A sample notched

3.2. Grinding and Polishing of Samples

A grinding and polishing device (Phoenix Beta Twin Whell, Buehler, Illinois, USA) was used to grind the samples to their final thickness (2 mm) (Figure 3.9). The samples were abraded with the polishing device under water cooling with 1000 grit silicone carbide abrasive papers (Atlas, Turkey). The surface on which the grinding and polishing process is applied (corresponding to the polished surface) is the surface on which the color measurement will be made.

Figure 3.9. A grinding and polishing machine

The final thickness of the samples was checked to be 2 mm with a digital caliper and the degree of precision of the thickness values was determined as 0.01 (Figure 3.10).

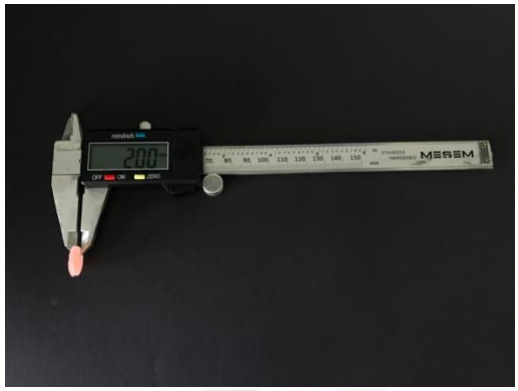

Figure 3.10. Checking of the final thickness of the samples

The samples were then treated with diluted pumice and a brush for 90 seconds with the polishing device (Rotaks TRD, Turkey) (Figure 3.11a). For fine polishing, the samples were then polished on the same instrument for 90 seconds using Ivoclar Vivadent Universal Polishing Paste (Figure 3.11b). All grinding and polishing was done on the non-notched polished surface.

Figure 3.11a. The polishing device (Rotaks TRD, Turkey),

b. Universal polishing paste (Ivoclar Vivadent)

3.3. Pre-staining Procedure

All of the 160 samples prepared for the experiment were placed in containers filled with distilled water and kept in an incubator (Memmert GmbH+ Co, Germany) at 37°C for 24 hours (Figure 3.12).

Figure 3.12. Incubator (Memmert GmbH+ Co, Germany)

The samples were then removed from their containers and washed under running water (Figure 3.13) for 10 seconds and dried with paper towels without pressure. Following the drying process, baseline (T0) color measurements were made.

Figure 3.13. Washing of the sample

3.4. Grouping of the Samples

Each acrylic group was randomly divided into 4 groups according to the solution in which they would be immersed. Therefore, 16 groups were obtained (n=10 per group). The solutions used in the study were distilled water, tea, coffee and red wine (Table 3.2 and Figure 3.14).

Table 3.2. The solutions used in the study

Solution Type	Trade Name	Manufacturer	Lot No.
Tea	Lipton Yellow Label	UNILEVER, United Kingdom	50099/11-5
Coffee	Nescafe Classic	Nestle, Turkey	L02620289A2
Red Wine	Doluca	DOLUCA, Turkey	01113-201113
Distilled Water	Pure Water	Aqua Medical Tools and Equipment, ind.trade.co.ltd., Turkey	1564

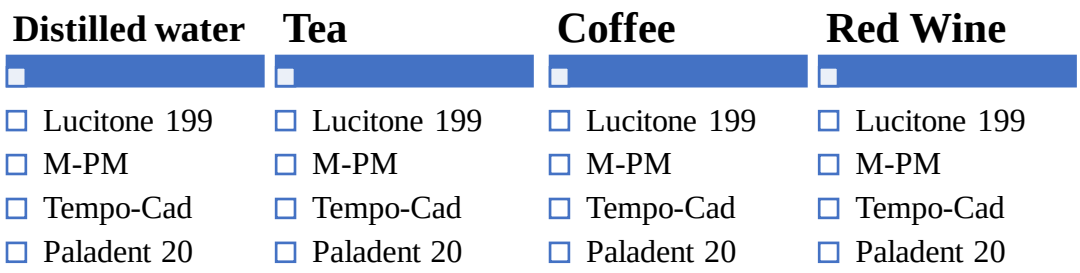

Figure 3.14. Grouping of samples

3.5. Staining Procedure

Coffee solution (Nescafe Classic, Nestle, Turkey) was prepared by adding 4 g of coffee powder to 400 ml of water. For the tea solution, two tea bags (black tea bag, Lipton Yellow Label, United Kingdom) were soaked in 400 ml of water and steeped for 10 minutes. Both solutions were stirred for 10 seconds every 30 minutes to cool down to 37°C and filtered through a filter paper before use. The other solutions (distilled water and red wine) were ready for use. The solutions were placed in 5 ml containers. All solutions were renewed every 24 hours. All solutions used in the study are shown in Figure 3.15.

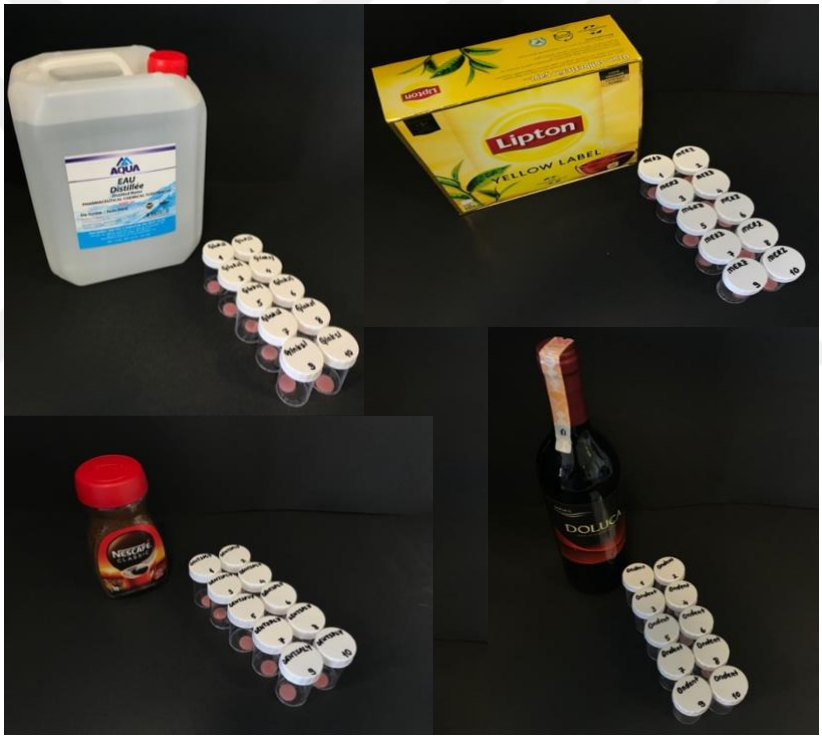

Figure 3.15. Grouping of all acrylic samples according to the sollutions used in the study (distilled water, tea, coffee, red wine)

Samples were placed in containers with the solution. All containers were placed in an incubator set at 37°C to mimic the oral environment (Figure 3.16). The samples were exposed to the solution for a total of 840 hours.

Figure 3.16. Samples placed in containers with the solutions in the incubator

3.6. Spectrophotometric Measurements

Samples were kept in distilled water at 37±1°C for 24 hours before immersion in the solutions. After 24 hours of immersion, target color measurements of each sample (T0) were performed by a spectrophotometer (CM-2600d, Konica Minolta Sensing Inc., Japan). The samples were then kept in the containers with the test solutions until further color measurement were performed.

It was stated that the average consumption duration of 1 cup of coffee is 15 minutes and an average of 3.2 cups of coffee is consumed per day. Therefore, given that 3.2 cups of coffee are consumed per day, the exposure time to coffee was found to be 48 minutes a day. Calculations showed that 24 hours in solution lasted 1440 minutes, which corresponds to 30 days of coffee consumption (116).

As a result of the calculations, measurements were made for 24 hours (T1: 1 month of beverage consumption), 1 week (T2: 7 months of beverage consumption), 2 weeks (T3: 14 months of consumption), 3 weeks (T4: 21 months of consumption), 4 weeks (T5: 28 months of consumption), and 5 weeks (T6: 35 months of consumption). The 5 weeks of immersion in the solution immersion yielded results equivalent to approximately 3 years of clinical use of the prosthesis.

Before each color measurement, samples were rinsed under running water for 10 seconds and gently dried with a paper towel without pressure. After each color

measurement, the samples were soaked in artificial saliva for 30 minutes before getting stored in the immersion solutions again. Artificial saliva was produced in the Laboratory of Yeditepe University Faculty of Pharmacy.

Color measurements of the samples were performed using a spectrophotometer (CM-2600d, Konica Minolta Sensing Inc., Japan) (Figure 3.17a-c). The results were evaluated with Commission Internationale de L'eclairage L*a*b* (CIE L*a*b*) color space and "Spectra-Magic 3.1" computer program.

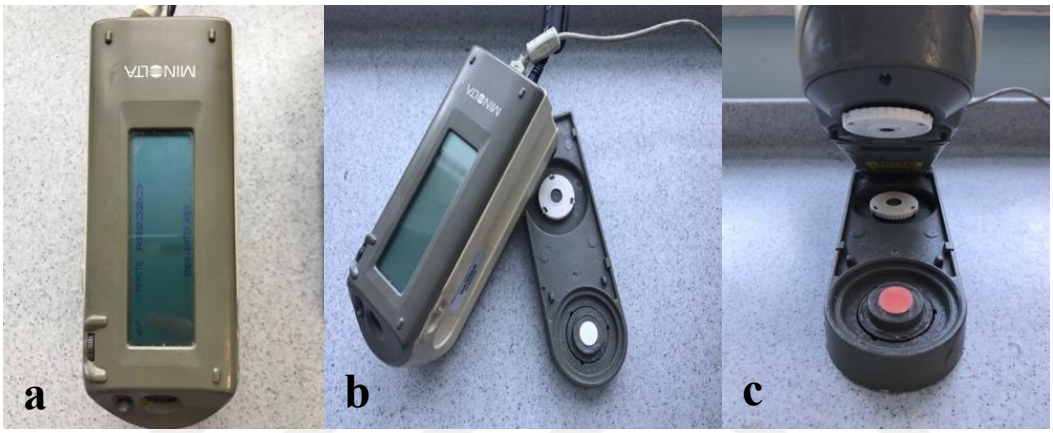

Figure 3.17a-c. The spectrophotometer

Three different measurements were taken from each sample and the mean values of these measurements were recorded. Measurements were performed using a white background and in daylight. The color difference before and after staining (ΔE) was calculated using the following formula (116).

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{½}$$

In this formula "L" stands for luminosity and brightness; larger "L" values indicate brighter brightness.

The "A" coordinate is a measure of color along the red-green axis; a positive "a" stands for redness, while a negative "a" represents green.

The "B" coordinate is a measure of color along the yellow-blue axis; a positive "b" represents yellowness, while a negative "b" means blueness.

ΔL, Δa, Δb are the differences in L*, a*, b* values before (T0) and after immersion in the solution at each time interval (T1, T2, T3, T4, T5, T6).

To correlate between the amount of color change (ΔE) measured in the clinical setting with the spectrophotometer and the clinical environment, the data of color measurement were converted to National Bureau of Standards (NBS) units using the following formula:

$$\text{NBS units} = \Delta E \times 0.92$$

According to these system, the NBS value of more than 3 shows "marked" discoloration (Table 2.2). In the present study, NBS = 3 was accepted as the threshold value and values above 3 (ΔE = 3.3) were considered clinically unacceptable as in other similar studies (18,135).

The final version of some randomly selected samples from each group at the end of the study is shown in Figure 3.18.

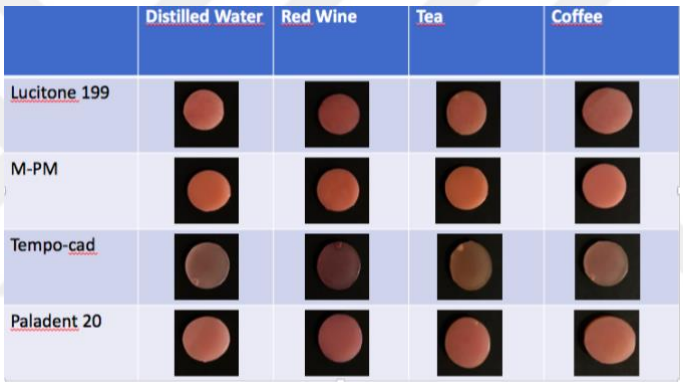

Figure 3.18. The final version of randomly selected samples from each group at the end of the study

3.7. Statistical Analysis

Statistical analysis was calculated with the statistical package (IBM SPSS Statistics for Windows v26.0; IBM Corp).

The normal distribution status of the color measurement data for each acrylic resin group was evaluated with the help of the kurtosis and skewness values. Kalaycı (136) declared that the skewness-kurtosis coefficients within the range of ± 3 can be evaluated within the normal limits. It was observed that the assumption of normal distribution was met in every single group. Therefore, parametric tests were used in this study.

First of all, data of color measurement obtained from color stability tests were analyzed with One-Way ANOVA Repeated Measures for analyzing differences between 6 color measurements on different measuring times for each brand&solution pair. When sphericity assumption is not met greenhouse-geisser results were interpreted in analysis. Pairwise comparisons for time were completed by EMMeans contrasts with sequential Bonferroni adjustments for multiple comparisons.

3 Way Repeated Measure ANOVA test was then used to investigate the main and interaction effects of time, brand and solution on the color measurements. When sphericity assumption is not met greenhouse-geisser results were interpreted in analyze. Pairwise comparisons for brand and solution groups were completed with the EMMeans contrasts with sequential Bonferroni adjustments for multiple comparisons.

The statistical significance was adjusted at the 0.05 level.

4. RESULTS

The mean value of color difference (ΔE) with standard deviations and corresponding NBS grading unit values of all acrylic resin groups tested after 24 hours (T1), 1 week (T2), 2 weeks (T3), 3 weeks (T4), and 4 weeks (T5) and 5 weeks (T6) of immersion in all solutions tested are shown in Table 4.1 and Table 4.2. These tables are also presented the results of statistical analysis to compare of mean ΔE values with different variables.

Pairwise comparisons of color change of each acrylic resin group for different solution groups are presented in Table 4.4. Descriptive color change statistics (ΔE) for each acrylic resin materials and results of the comparison between color measurements are also presented in Tables 4.3 to 4.7. Also, pairwise comparisons of color change of acrylic resin groups by brand are shown in the Table 4.8.

According to the results of the statistical analysis, it was determined that the acrylic resins and solution factors had a significant effect on color change over time. Therefore, the null hypothesis that the type of acrylic resin denture base material and immersion solution have no effect on the stainability of acrylic resin denture base materials was rejected. Statistical analysis presented that the color stability of all acrylic resin materials was significantly affected by the immersion period ($p=0.000$) (Table 4.2). In other words, the color change (ΔE) of acrylic resin materials increased as the immersion period increased (Table 4.1).

When the samples were immersed in **distilled water** solution, a statistical significance ($p<0.05$) was found in paired comparisons between all acrylic resin materials except between CC1.D and HC.H ($p=0.724$), CC2.M and HC.H ($p=0.091$) groups. CC3.O group has a high statistical significance ($p=0.000$) in paired comparisons between the other groups (Table 4.8).

When the samples were immersed in **coffee** solution, a high statistical significance ($p=0.000$) was found in paired comparisons between all acrylic resin materials except between CC2.M and HC.H ($p=1.000$) groups (Table 4.8).

When the samples were immersed in **tea** and **red wine** solutions, a high statistical significance ($p=0.000$) was found in paired comparisons between all materials (Table 4.8).

All acrylic resin samples (coffee, tea, red wine) in tested groups showed higher ΔE values compared to the control group samples (distilled water), at the end of the study (after 5 weeks: approximately equal to 3 years of clinical use of the prosthesis) (Table 4.1).

Among all the immersion solutions for each time immersion period (T1 to T6), **coffee** caused the least discoloration for all acrylic resin groups except in CC1.D group (at T1), CC2.M group (at T1,T2), HC.H group (at T1). Coffee caused the highest discoloration in the CC3.O group (ΔE=5.25, NBS unit=4.83) among all acrylic resin groups, and this values was obtained in the color measurements after 5 weeks of immersion (T6) (Table 4.1).

Red wine generally caused the most marked color change in all acrylic resin groups, except in CC3.O group (at T1,T2,T3,T4) where at these color measurements the samples presented the highest ΔE value when immersed in tea (Table 4.1). The highest color change was presented in the CC1.D samples immersed in red wine at the end of the 5 weeks immersion (T6), (ΔE=6.28, NBS unit=5.77) (Table 4.1).

After the the obtaining and evaluating of **NBS unit** values in this study, NBS values of all acrylic resin groups immersed in **distilled water** ranged from 0.21 (ΔE=0.23 – CC3.O group at T1) to 2.45 (ΔE=2.67 – CC1.D group at T6). In addition, the NBS unit values of all acrylic resin samples immersed in the **coffee** ranged from 0.75 (ΔE=0.82 – HC.H group at T1) to 4.83 (ΔE=5.25 – CC3.O group at T6). According to these values, there was no clinically marked change in all acrylic resin materials immersed in distilled water, and CC3.O group in coffee solution showed “marked” discoloration starting from T3 (Table 4.1).

NBS unit values of all acrylic resin groups immersed in **tea** ranged from 0.67 (ΔE=0.73 – HC.H group at T1) to 5.09 (ΔE=5.54 – CC3.O group at T6). CC3.O group showed the earliest “marked” discoloration starting from T2 (Table 4.1).

The samples in CC1.D group immersed in **red wine** showed earliest “marked” color changes starting from T3 in the staining solutions compared to the other groups. NBS values of all acrylic resin groups immersed in red wine were ranged from 1.39 (ΔE=1.52 – CC2.M at T1) to 5.77 (ΔE=6.28 – CC1.D at T6). Besides, there is no “Extremely marked” discoloration (NBS>6) at the end of the present study in each solution groups for all immersion period (T1-T6) (Table 4.1).

Table 4.1 Means and standard deviations of color difference (ΔE) and corresponding NBS values for acrylic resin materials after staining at different testing intervals, and descriptive statistics of color change for acrylic resin materials and results of comparison between measurements.

MATERIAL	SOLUTION	T1		T2		T3		T4		T5		T6		ANOVA		Post[a] Hoc
		ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	F	P	
HC.H	Distilled water	0.31 (±0.07)	0.28	0.70 (±0.13)	0.64	1.59 (±0.08)	1.46	1.90 (±0.15)	1.74	2.26 (±0.09)	2.07	2.46 (±0.11)	2.26	718.927	**0.000**	T1-T2, T1-T3, T1-T4, T1-T5, T1-T6, T2-T3, T2-T4, T2-T5, T2-T6, T3-T4, T3-T5, T3-T6, T4-T5, T4-T6, T5-T6
	Tea	0.73 (±0.12)	0.67	2.11 (±0.19)	1.94	2.71 (±0.17)	2.49	3.45 (±0.08)	3.17	4.20 (±0.12)	3.86	4.45 (±0.15)	4.09	870.191	**0.000**	T1-T2, T1-T3, T1-T4, T1-T5, T1-T6, T2-T3, T2-T4, T2-T5, T2-T6, T3-T4, T3-T5, T3-T6, T4-T5, T4-T6, T5-T6
	Coffee	0.82 (±0.17)	0.75	1.62 (±0.12)	1.49	2.11 (±0.08)	1.94	2.79 (±0.08)	2.56	3.25 (±0.20)	2.99	3.64 (±0.21)	3.34	833.800	**0.000**	T1-T2, T1-T3, T1-T4, T1-T5, T1-T6, T2-T3, T2-T4, T2-T5, T2-T6, T3-T4, T3-T5, T3-T6, T4-T5, T4-T6, T5-T6
	Red wine	1.83 (±0.15)	1.68	2.44 (±0.16)	2.24	3.14 (±0.07)	2.88	3.90 (±0.15)	3.58	4.61 (±0.15)	4.24	5.85 (±0.19)	5.38	1442.683	**0.000**	T1-T2, T1-T3, T1-T4, T1-T5, T1-T6, T2-T3, T2-T4, T2-T5, T2-T6, T3-T4, T3-T5, T3-T6, T4-T5, T4-T6, T5-T6
CC1.D	Distilled water	0.26 (±0.14)	0.23	0.64 (±0.09)	0.58	1.47 (±0.17)	1.35	2.13 (±0.11)	1.95	2.39 (±0.13)	2.11	2.67 (±0.19)	2.45	320.425	**0.000**	T1-T2, T1-T3, T1-T4, T1-T5, T1-T6, T2-T3, T2-T4, T2-T5, T2-T6, T3-T4, T3-T5, T3-T6, T4-T5, T4-T6, T5-T6
	Tea	0.89 (±0.13)	0.81	1.98 (±0.12)	1.82	2.82 (±0.11)	2.59	3.80 (±0.12)	3.49	4.49 (±0.30)	4.13	4.88 (±0.19)	4.48	669.800	**0.000**	T1-T2, T1-T3, T1-T4, T1-T5, T1-T6, T2-T3, T2-T4, T2-T5, T2-T6, T3-T4, T3-T5, T3-T6, T4-T5, T4-T6, T5-T6
	Coffee	1.28 (±0.08)	1.17	1.96 (±0.06)	1.80	2.47 (±0.10)	2.27	3.37 (±0.22)	3.10	3.72 (±0.16)	3.42	4.22 (±0.19)	3.88	621.773	**0.000**	T1-T2, T1-T3, T1-T4, T1-T5, T1-T6, T2-T3, T2-T4, T2-T5, T2-T6, T3-T4, T3-T5, T3-T6, T4-T5, T4-T6, T5-T6
	Red wine	2.15 (±0.24)	1.97	2.75 (±0.14)	2.53	3.48 (±0.20)	3.20	4.46 (±0.14)	4.10	5.35 (±0.23)	4.92	6.28 (±0.24)	5.77	992.280	**0.000**	T1-T2, T1-T3, T1-T4, T1-T5, T1-T6, T2-T3, T2-T4, T2-T5, T2-T6, T3-T4, T3-T5, T3-T6, T4-T5, T4-T6, T5-T6

	NBS: 0.5－1.5	Slight: Slight change	
	NBS: 1.5－3.0	Noticeable: Perceivable change	
	NBS: 3.0－6.0	Appreciable: Marked change	
	NBS: 6.0－12.0	Much: Extremely marked change	
	NBS: 12.0 or	Very much: Change to other color	

Table 4.1. Continued.

MATERIAL	SOLUTION	T1		T2		T3		T4		T5		T6		ANOVA		Post[a] Hoc
		ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	F	P	
CC2.M	Distilled water	0.44 (±0.14)	0.40	0.61 (±0.15)	0.56	0.91 (±0.11)	0.83	2.04 (±0.16)	1.87	2.16 (±0.12)	1.98	2.52 (±0.16)	2.31	869.931	**0.000**	T1-T2, T1-T3, T1-T4, T1-T5, T1-T6, T2-T3, T2-T4, T2-T5, T2-T6, T3-T4, T3-T5, T3-T6, T4-T5, T4-T6, T5-T6
	Tea	0.88 (±0.06)	0.80	1.62 (±0.14)	1.49	2.42 (±0.24)	2.22	3.22 (±0.11)	2.96	3.89 (±0.15)	3.57	4.23 (±0.14)	3.89	1667.974	**0.000**	T1-T2, T1-T3, T1-T4, T1-T5, T1-T6, T2-T3, T2-T4, T2-T5, T2-T6, T3-T4, T3-T5, T3-T6, T4-T5, T4-T6, T5-T6
	Coffee	1.14 (±0.07)	1.04	1.66 (±0.12)	1.52	2.09 (±0.07)	1.92	2.69 (±0.12)	2.47	3.00 (±0.14)	2.76	3.48 (±0.18)	3.20	524.920	**0.000**	T1-T2, T1-T3, T1-T4, T1-T5, T1-T6, T2-T3, T2-T4, T2-T5, T2-T6, T3-T4, T3-T5, T3-T6, T4-T5, T4-T6, T5-T6
	Red wine	1.52 (±0.12)	1.39	2.18 (±0.14)	2.00	2.85 (±0.16)	2.62	3.74 (±0.16)	3.44	4.39 (±0.13)	4.03	5.24 (±0.18)	4.82	1112.488	**0.000**	T1-T2, T1-T3, T1-T4, T1-T5, T1-T6, T2-T3, T2-T4, T2-T5, T2-T6, T3-T4, T3-T5, T3-T6, T4-T5, T4-T6, T5-T6
CC3.O	Distilled water	0.23 (±0.08)	0.21	0.53 (±0.12)	0.48	1.13 (±0.11)	1.03	1.38 (±0.12)	1.26	1.53 (±0.18)	1.40	1.92 (±0.1)	1.76	626.708	**0.000**	T1-T2, T1-T3, T1-T4, T1-T5, T1-T6, T2-T3, T2-T4, T2-T5, T2-T6, T3-T4, T3-T5, T3-T6, T4-T5, T4-T6, T5-T6
	Tea	1.63 (±0.16)	1.49	3.67 (±0.23)	3.37	4.42 (±0.21)	4.06	4.65 (±0.22)	4.27	5.06 (±0.13)	4.65	5.54 (±0.2)	5.09	1270.354	**0.000**	T1-T2, T1-T3, T1-T4, T1-T5, T1-T6, T2-T3, T2-T4, T2-T5, T2-T6, T3-T4, T3-T5, T3-T6, T4-T5, T4-T6, T5-T6
	Coffee	1.14 (±0.18)	1.04	3.16 (±0.13)	2.90	3.49 (±0.09)	3.21	4.11 (±0.26)	3.78	4.63 (±0.21)	4.25	5.25 (±0.2)	4.83	886.982	**0.000**	T1-T2, T1-T3, T1-T4, T1-T5, T1-T6, T2-T3, T2-T4, T2-T5, T2-T6, T3-T4, T3-T5, T3-T6, T4-T5, T4-T6, T5-T6
	Red wine	1.54 (±0.21)	1.41	2.45 (±0.33)	2.25	2.69 (±0.18)	2.72	4.58 (±0.22)	4.21	5.57 (±0.23)	5.12	6.16 (±0.1)	5.66	1059.880	**0.000**	T1-T2, T1-T3, T1-T4, T1-T5, T1-T6, T2-T3, T2-T4, T2-T5, T2-T6, T3-T4, T3-T5, T3-T6, T4-T5, T4-T6, T5-T6

	NBS: 0.5−1.5	Slight: Slight change	
	NBS: 1.5−3.0	Noticeable: Perceivable change	
	NBS: 3.0−6.0	Appreciable: Marked change	
	NBS: 6.0−12.0	Much: Extremely marked change	
	NBS: 12.0 or	Very much: Change to other color	

According to results of Table 4.2, there is a significant difference between ΔE means of 6 repeated color measurements (F=12892.64, p=0.000). This results can be correlated to the color stability of the acrylic resin samples increasingly change over time regaredless of brand or solution type.

Otherwise, the difference between ΔE means of 6 repeated color measurements by acrylic resin groups was found significant (F=44.650, p=0.000). In other words, it was obtained that the color stability of the acrylic resins which made of 4 different materials were used differed significantly from before to after the experiment, that is, the common effects of being in different acrylic resin groups (CC1.D, CC2.M, CC3.O, HC.H) and factors on the color stability levels of repeated color measurements were found to be significant.

Table 4.2. Analysis results of the effect of the brand, solutions and time factors on color change

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Between-Subjects Effect						
Brand	93.031	3	31.010	649.191	.000*	.917
Solution	848.359	3	282.786	5920.032	.000*	.990
Brand * Solution	106.437	9	11.826	247.579	.000*	.927
Error	8.407	176	.048			
Within-Subjects Effect						
Time	1426.630	4.530	314.912	12892.644	.000*	.987
Time * Brand	14.822	13.591	1.091	44.650	.000*	.432
Time * Solution	94.623	13.591	6.962	285.039	.000*	.829
Time * Brand * Solution	28.995	40.772	.711	29.114	.000*	.598
Error (Time)	19.475	797.324	.024			

3 Way Repeated Measure ANOVA
*significant p-value at 0.05 level
a. Computed using alpha = 0.05

Furthermore, the difference between ΔE means of 6 repeated color measurements by solution groups is found significant (F=285.039, p=0.000). In other words, it was obtained that the color stability of the acrylic resins immersed in 4 different solutions changed significantly from before to after the our experiment that is correlated to the common effects of being in different solution groups (distilled water, tea, coffee, red wine) and repeated color measurement factors found to be significant on color stability levels.

In addition, the difference between ΔE means by brand*solution blocks was found significant (F=247.579, p=0.000). In other words, the color stability of the acrylic resins immersed in 4 different solutions and 4 brands differed significantly in general, that is, the common effect of brand and solution factors on color stability were significant.

Finally, it was found that the color stability of 4 different brand groups where 4 different solutions were applied showed a significant difference (F=29.114, p=0.000) between before and after the experiment, that is correlated to the common effect of time, type of acrylic resin and solution factors on color stability levels were significant (Table 4.2).

4.1. Results of the Color Changes of HC.H Groups after Immersion Procedure

ΔE values of the HC.H samples immersed in distilled water, tea, coffee, and red wine presented statistically significant increase, throughout repeated color measurements over-time (p=0.000) (Table 4.3 and Figure 4.1).

Among all solutions tested for each time immersion period (T1 to T6), distilled water caused the least discoloration in the HC.H samples (Table 4.3). There was statistically significant difference (p=0.000) between ΔE values of the HC.H samples in all solution groups (Table 4.4).

The HC.H samples immersed in distilled water presented statistically different ΔE values between repeated color measurements (p=0.000). The ΔE value of the HC.H samples was 0.31 in distilled water ,in the measurement at T1, this ΔE value increased to 2.46 in the measurements at T6 (Table 4.3 and Figure 4.1).

ΔE values of HC.H samples immersed in the coffee were statistically significantly lower than in tea and red wine (except at the T1 measurement) which ΔE values of HC.H samples in tea solution is lower than the coffee solution (Table 4.3)

Table 4.3. Descriptive statistics of color change (ΔE) for HC.H groups

MATERIAL	SOLUTION	T1		T2		T3		T4		T5		T6		ANOVA	Post[a] Hoc
		ΔE (±Sd)	NBS	ΔE (±Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	P	
HC.H	Distilled water	0.31 (±0.07)	0.28	0.70 (±0.13)	0.64	1.59 (±0.08)	1.46	1.90 (±0.15)	1.74	2.26 (±0.09)	2.07	2.46 (±0.11)	2.26	0.000	T1-T2, T1-T3, T1-T4, T1-T5, T1-T6, T2-T3, T2-T4, T2-T5, T2-T6, T3-T4, T3-T5, T3-T6, T4-T5, T4-T6, T5-T6
	Tea	0.73 (±0.12)	0.67	2.11 (±0.19)	1.94	2.71 (±0.17)	2.49	3.45 (±0.08)	3.17	4.20 (±0.12)	3.86	4.45 (±0.15)	4.09		
	Coffee	0.82 (±0.17)	0.75	1.62 (±0.12)	1.49	2.11 (±0.08)	1.94	2.79 (±0.08)	2.56	3.25 (±0.20)	2.99	3.64 (±0.21)	3.34		
	Red wine	1.83 (±0.15)	1.68	2.44 (±0.16)	2.24	3.14 (±0.07)	2.88	3.90 (±0.15)	3.58	4.61 (±0.15)	4.24	5.85 (±0.19)	5.38		

	NBS: 0.5—1.5	Slight: Slight change
	NBS: 1.5—3.0	Noticeable: Perceivable change
	NBS: 3.0—6.0	Appreciable: Marked change
	NBS: 6.0—12.0	Much: Extremely marked change
	NBS: 12.0 or more	Very much: Change to other color

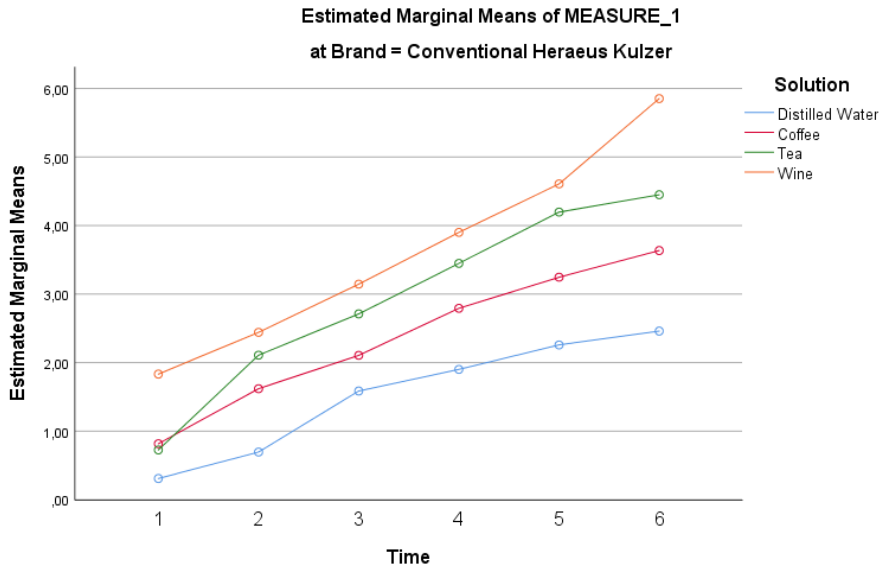

Figure 4.1. Color stability of HC.H groups after immersion in different solutions

Table 4.4. Pairwise comparisons of color change of each acrylic resin group for different solution groups

Acrylic resin materials	Solution (I)	Solution (J)	Mean Difference (I-J)	Std. Error	P*	95% Confidence Interval for Difference[a]	
						Lower Bound	Upper Bound
CC1.D	Distilled Water	Coffee	-1.245*	.036	.000*	-1.342	-1.148
		Tea	-1.553*	.036	.000*	-1.650	-1.455
		Red Wine	-2.487*	.036	.000*	-2.584	-2.390
	Coffee	Distilled Water	1.245*	.036	.000*	1.148	1.342
		Tea	-.308*	.036	.000*	-.405	-.210
		Red Wine	-1.242*	.036	.000*	-1.339	-1.145
	Tea	Distilled Water	1.553*	.036	.000*	1.455	1.650
		Coffee	.308*	.036	.000*	.210	.405
		Red Wine	-.934*	.036	.000*	-1.031	-.837
	Red Wine	Distilled Water	2.487*	.036	.000*	2.390	2.584
		Coffee	1.242*	.036	.000*	1.145	1.339
		Tea	.934*	.036	.000*	.837	1.031
CC2.M	Distilled Water	Coffee	-.897*	.036	.000*	-.994	-.800
		Tea	-1.264*	.036	.000*	-1.361	-1.166
		Red Wine	-1.875*	.036	.000*	-1.972	-1.778
	Coffee	Distilled Water	.897*	.036	.000*	.800	.994
		Tea	-.367*	.036	.000*	-.464	-.269
		Red Wine	-.978*	.036	.000*	-1.075	-.881
	Tea	Distilled Water	1.264*	.036	.000*	1.166	1.361
		Coffee	.367*	.036	.000*	.269	.464
		Red Wine	-.611*	.036	.000*	-.708	-.514
	Red Wine	Distilled Water	1.875*	.036	.000*	1.778	1.972
		Coffee	.978*	.036	.000*	.881	1.075
		Tea	.611*	.036	.000*	.514	.708

Based on estimated marginal means
*The mean difference is significant at the .05 level
a. Computed using alpha = 0.05
b.Adjustment for multiple comparisons: Bonferroni

Table 4.4. Continued

Acrylic resin materials	Solution (I)	Solution (J)	Mean Difference (I-J)	Std. Error	P*	95% Confidence Interval for Difference[a]	
						Lower Bound	Upper Bound
CC3.O	Distilled Water	Coffee	-2.508*	.036	.000*	-2.606	-2.411
		Tea	-3.042*	.036	.000*	-3.139	-2.945
		Red Wine	-2.755*	.036	.000*	-2.852	-2.658
	Coffee	Distilled Water	2.508*	.036	.000*	2.411	2.606
		Tea	-.533*	.036	.000*	-.631	-.436
		Red Wine	-.246*	.036	.000*	-.344	-.149
	Tea	Distilled Water	3.042*	.036	.000*	2.945	3.139
		Coffee	.533*	.036	.000*	.436	.631
		Red Wine	.287*	.036	.000*	.190	.384
	Red Wine	Distilled Water	2.755*	.036	.000*	2.658	2.852
		Coffee	.246*	.036	.000*	.149	.344
		Tea	-.287*	.036	.000*	-.384	-.190
HC.H	Distilled Water	Coffee	-.834*	.036	.000*	-.932	-.737
		Tea	-1.405*	.036	.000*	-1.502	-1.308
		Red Wine	-2.095*	.036	.000*	-2.192	-1.998
	Coffee	Distilled Water	.834*	.036	.000*	.737	.932
		Tea	-.571*	.036	.000*	-.668	-.473
		Red Wine	-1.261*	.036	.000*	-1.358	-1.163
	Tea	Distilled Water	1.405*	.036	.000*	1.308	1.502
		Coffee	.571*	.036	.000*	.473	.668
		Red Wine	-.690*	.036	.000*	-.787	-.593
	Red Wine	Distilled Water	2.095*	.036	.000*	1.998	2.192
		Coffee	1.261*	.036	.000*	1.163	1.358
		Tea	.690*	.036	.000*	.593	.787

Based on estimated marginal means
*The mean difference is significant at the .05 level
a. Computed using alpha = 0.05
b.Adjustment for multiple comparisons: Bonferroni

When comparing solutions for HC.H samples, there is a statistically significant difference between all solutions (p=0.000). Accordingly, ranking from high to low of ΔE was **Wine > Tea > Coffee > Distilled water** at the end of the last immersion period (Table 4.3, Figure 4.1).

When HC.H samples were immersed in **distilled water, tea**, **coffee**, and **red wine**, the ΔE values of the samples between repeated measurements (from T1 to T6) differed statistically (p=0.000). While the ΔE value of HC.H samples in **distilled water** was 0.31 in the measurement at T1, this value increased to 2.46 in the measurements at T6 (Table 4.4 and Figure 4.1).While the ΔE value of HC.H samples in **tea** was 0.73 in the measurement at T1, this ΔE value increased to 4.45 in the measurements at T6 (Table 4.4 and Figure 4.1). While the ΔE value of HC.H samples in **coffee** was 0.82 in the measurement at T1, this ΔE value increased to 3.64 in the measurements at T6. While the ΔE value of the HC.H samples in **red wine** was 1.83 at T1, this value increased to 5.85 in the measurements at T6. In pairwise comparisons between these 4 solutions (distilled water, tea, coffee, red wine), there was statistically significant difference between ΔE values of HC.H samples in all solutions (p=0.000) (Table 4.3, Table 4.4).

Red wine was found to be the most staining solution for the HC.H samples among all the solutions tested, followed by tea, and coffee, for each time immersion period (T1 to T6). The difference between the ΔE values of the HC.H samples immersed in tea, coffee and red wine were statistically significant (p=0.000) (Table 4.4).

According to NBS rating system, no clinically “Extremely marked” color change (NBS unit > 6) was observed in the HC.H samples immersed in all sollutions at the 6 different color measurements. A“marked” color change (6 > NBS unit > 3) was observed in the HC.H samples immersed in tea at T4,T5,T6; in coffee at T6; in red wine at T4,T5,T6. Even at the end of the immersion period (T6, namely 35 months of consuming), the highest NBS value (at T6) of the HC.H samples immersed in distilled water was below 3. In other words, after 35 months consuming, distilled water did not cause a clinically "marked" color change in HC.H samples (Table 4.3).

A “marked” color change (6 > NBS unit > 3) was observed in HC.H samples in tea after 3 weeks of immersion (T4, namely 21 months of tea consumption), in coffee after 5 weeks (T6, namely 35 months of coffee consumption), in red wine after 3 weeks (T4, namely 21 months of red wine consumption) (Table 4.3).

After 5 weeks, the highest color changes were observed in HC.H samples immersed in red wine (ΔE= 5.85 and NBS unit=5.38) and that is thought to be a "marked" color change which NBS unit is more than 3 according to the NBS rating system (Table 4.3).

At the end of the 5 weeks immersion, a “perceivable” color change observed in distilled water; “marked” color change observed in tea, coffee and red wine for HC.H samples (Table 4.3).

4.2. Results of the Color Changes of CC1.D Groups after Immersion Procedure

ΔE values of the CC1.D samples immersed in distilled water, tea, coffee, and red wine presented statistically significant increase, throughout repeated color measurements over-time (p=0.000) (Table 4.5 and Figure 4.2).

Among all the solutions tested for each time immersion period (T1 to T6), distilled water caused the least discoloration in CC1.D samples than tea, coffee, and red wine (Table 4.5). There was statistically significant difference (p=0.000) between ΔE values of CC1.D samples in all solution groups (Table 4.4).

Table 4.5. Descriptive statistics of color change (ΔE) for CC1.D groups

MATERIAL	SOLUTION	T1		T2		T3		T4		T5		T6		ANOVA	Post[a] Hoc
		ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	P	
CC1.D	Distilled water	0.26 (±0.14)	0.23	0.64 (±0.09)	0.58	1.47 (±0.17)	1.35	2.13 (±0.11)	1.95	2.39 (±0.13)	2.11	2.67 (±0.19)	2.45	0.000	T1-T2, T1-T3, T1-T4, T1-T5, T1-T6, T2-T3, T2-T4, T2-T5, T2-T6, T3-T4, T3-T5, T3-T6, T4-T5, T4-T6, T5-T6
	Tea	0.89 (±0.13)	0.81	1.98 (±0.12)	1.82	2.82 (±0.11)	2.59	3.80 (±0.12)	3.49	4.49 (±0.30)	4.13	4.88 (±0.19)	4.48		
	Coffee	1.28 (±0.08)	1.17	1.96 (±0.06)	1.80	2.47 (±0.10)	2.27	3.37 (±0.22)	3.10	3.72 (±0.16)	3.42	4.22 (±0.19)	3.88		
	Red wine	2.15 (±0.24)	1.97	2.75 (±0.14)	2.53	3.48 (±0.20)	3.20	4.46 (±0.14)	4.10	5.35 (±0.23)	4.92	6.28 (±0.24)	5.77		

	NBS: 0.5−1.5	Slight: Slight change
	NBS: 1.5−3.0	Noticeable: Perceivable change
	NBS: 3.0−6.0	Appreciable: Marked change
	NBS: 6.0−12.0	Much: Extremely marked change
	NBS: 12.0 or more	Very much: Change to other color

The CC1.D samples immersed in distilled water presented statistically different ΔE values between repeated color measurements (p=0.000). The ΔE value of CC1.D samples was 0.26 in distilled water, in the measurement at T1, this ΔE value increased to 2.67 in the measurements at T6 (Table 4.5 and Figure 4.2).

ΔE values of CC1.D samples immersed in coffee were significantly lower than in tea and red wine except at the T1 measurement which ΔE values of CC1.D samples in tea solution is lower than the coffee solution (Table 4.5).

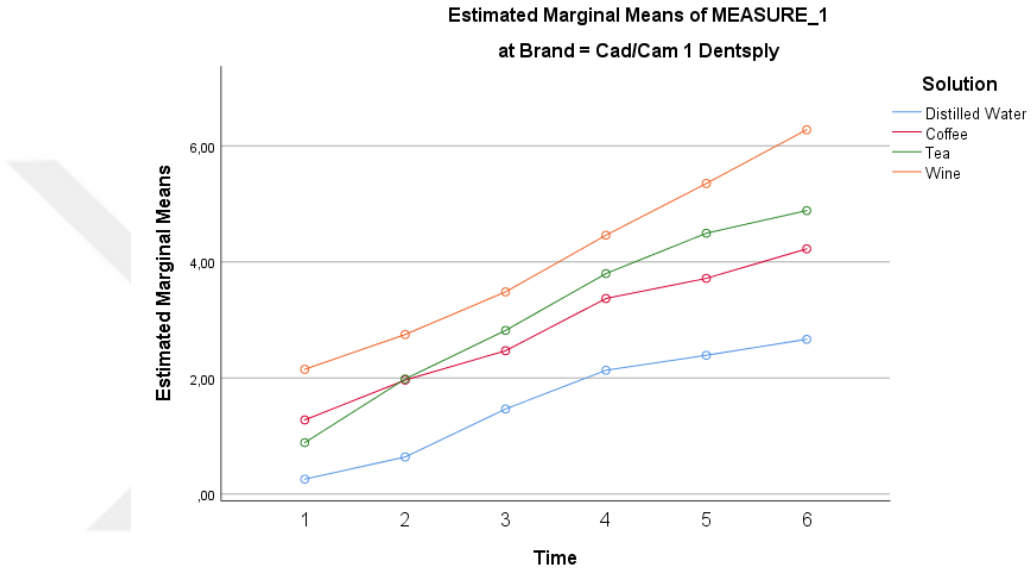

Figure 4.2. Color stability of CC1.D groups after immersion in different solutions

When comparing solutions for CC1.D samples, ranking from high to low of ΔE value was **Red Wine > Tea > Coffee >Distilled Water** at the end of the last immersion period (Table 4.5, Figure 4.2).

When CC1.D samples were immersed in **distilled water**, **tea**, **coffee**, and **red wine**, ΔE values of the samples between repeated measurements (from T1 to T6) differed statistically (p=0.000). While the ΔE value of CC1.D samples in **distilled water** was 0.26 in the measurement at T1, this value increased to 2.67 in the measurements at T6. While the ΔE value of CC1.D samples in **tea** was 0.89 in the measurement at T1, this value increased to 4.88 in the measurements at T6. While the ΔE value of the CC1.D samples in **coffee** was 1.28 at T1, this value increased to 4.22 in the measurements at T6. While

the ΔE value of the CC1.D in **red wine** was 2.15 at T1, this value increased to 6.28 in the measurements at T6 (Table 4.5 and Figure 4.2).

Red wine was found to be the most staining solution for the CC1.D samples among all the solutions tested, followed by tea and coffee, for each time immersion period. The difference between the ΔE values of the CC1.D samples immersed in red wine, tea and coffee were statistically significant (p= 0.000) (Table 4.4).

According to NBS rating system, no clinically "Extremely marked" color change (NBS>6) was observed in the CC1.D samples immersed in all solutions at the 6 different color measurements. The highest NBS value was 2.45, less than 3, and belonged to distilled water. Even at the end of the 5 weeks immersion, the highest NBS value of the CC1.D samples immersed in distilled water was below 3. In other words, after 5 weeks, distilled water did not cause a "marked" color change in CC1.D samples (Table 4.5).

For CC1.D samples, a "marked" color change (6 >NBS > 3) was observed in red wine after 2 weeks of immersion (T3, which is equal to 14 months of consuming), in tea after 3 weeks of immersion (T4, which is equal to 21 months of consuming), in coffee after 3 weeks of immersion (T4, which is equal to 21 months of consuming) (Table 4.1).

After 5 weeks, the highest color changes were observed in CC1.D samples immersed in red wine (ΔE= 6.28 and NBS unit= 5.77), which is considered to be "marked" color change, as it is more than 3 according to the NBS rating system (Table 4.5).

At the end of immersing for 5 weeks, a "perceivable" color change occurred in distilled water; and "marked" color change was observed in tea, coffee, and red wine for CC1.D samples (Table 4.5).

4.3. Results of the Color Changes of CC2.M Groups after Immersion Procedure

The CC2.M samples exposed to distilled water, tea, coffee, and red wine showed statistically significance increase in the ΔE values throughout measurements repeated over-time (p=0.000) (Table 4.6 and Figure 4.3).

Among all the solutions tested for each time period (T1 to T6), distilled water produced significantly less discoloration in the CC2.M samples than tea, coffee, and red wine (Table 4.6). There was statistically significant difference (p=0.000) between ΔE values of CC2.M samples in all solution groups (Table 4.4). The CC2.M samples

exposed to distilled water showed statistically different ΔE values between repeated measurements (p=0.000). While the ΔE of CC2.M samples in distilled water was 0.44 in the measurement at T1, this value increased to 2.52 in the measurements at T6 (Table 4.6 and Figure 4.3).

Table 4.6. Descriptive statistics of color change (ΔE) for CC2.M groups

MATERIAL	SOLUTION	T1		T2		T3		T4		T5		T6		ANOVA	Post[a] Hoc
		ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	P	
CC2.M	Distilled water	0.44 (±0.14)	0.40	0.61 (±0.15)	0.56	0.91 (±0.11)	0.83	2.04 (±0.16)	1.87	2.16 (±0.12)	1.98	2.52 (±0.16)	2.31	0.000	T1-T2, T1-T3, T1-T4, T1-T5, T1-T6, T2-T3, T2-T4, T2-T5, T2-T6, T3-T4, T3-T5, T3-T6, T4-T5, T4-T6, T5-T6
	Tea	0.88 (±0. 06)	0.80	1.62 (±0.14)	1.49	2.42 (±0.24)	2.22	3.22 (±0.11)	2.96	3.89 (±0.15)	3.57	4.23 (±0.14)	3.89		
	Coffee	1.14 (±0.07)	1.04	1.66 (±0.12)	1.52	2.09 (±0.07)	1.92	2.69 (±0.12)	2.47	3.00 (±0.14)	2.76	3.48 (±0.18)	3.20		
	Red wine	1.52 (±0.12)	1.39	2.18 (±0.14)	2.00	2.85 (±0.16)	2.62	3.74 (±0.16)	3.44	4.39 (±0.13)	4.03	5.24 (±0.18)	4.82		

	NBS: 0.5−1.5	Slight: Slight change
	NBS: 1.5−3.0	Noticeable: Perceivable change
	NBS: 3.0−6.0	Appreciable: Marked change
	NBS: 6.0−12.0	Much: Extremely marked change
	NBS: 12.0 or more	Very much: Change to other color

When CC2.M samples were immersed in **distilled water**, **tea**, **coffee**, and **red wine**, they displayed significantly different ΔE values between repeated measurements (from T1 to T6) (p=0.000). While the ΔE of CC2.M samples in **tea** was 0.88 in the measurement at T1, this value increased to 4.23 in the measurements at T6. While the ΔE of CC2.M samples in **coffee** was 1.14 in the measurement at T1, this value increased to 3.48 in the measurements at T6. While the ΔE of the samples in **red wine** was 1.52 at T1, this value increased to 5.24 in the measurements at T6. (Table 4.6 and Figure 4.3).

Among all the staining solutions for all measurement period, the red wine was the most staining solution for the CC2.M samples. ΔE values of samples in the coffee

solution were statistically significantly lower than ΔE values of CC2.M samples exposed to tea and red wine except at the T1 and T2 measurements which ΔE values of CC2.M samples in tea solution is lower than the coffee solution, as was the case with other brands of resins (p=0.000) (Table 4.4, Table 4.6).

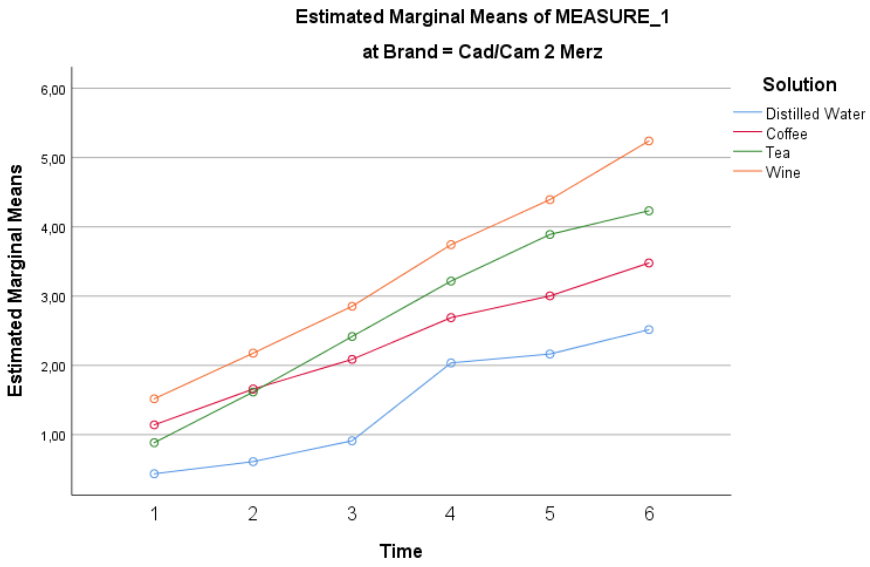

Figure 4.3. Color stability of CC2.M groups after immersion in different solutions

When comparing solutions for CC2.M samples, ranking from high to low of ΔE was **Red Wine > Tea > Coffee > Distilled Water** at the end of the last immersion period (Table 4.6, Figure 4.3).

When color changes are evaluated **according to NBS rating system**, no clinically "Extremely marked" color change was observed in the CC2.M samples immersed in all solutions at the 6 different color measurements. The highest NBS unit value was 2.31, which is less than 3, and belonged to distilled water.

While a clinically "marked" color change (6>NBS>3) occurred in CC2.M samples in tea after 4 weeks of immersion (T5, which is equal to 28 months of consuming tea), a "marked" color change occurred in CC2.M samples immersed in coffee after 5 weeks of immersion (T6, which is equal to 35 months consuming), a "marked" color change occurred in CC2.M samples immersed in red wine after 3 weeks of immersion (T4, which is equal to 21 months consuming).

After 5 weeks, the highest color changes were seen in CC2.M samples immersed in red wine (ΔE= 5.24 and NBS unit= 4.82), which is considered to be "marked" color change, as it is more than 3 according to the NBS rating system (Table 4.6).

At the end of immersing for 5 weeks, a “perceivable” color change occurred in distilled water; “marked” color change occurred in tea, coffee, and red wine for CC2.M samples (Table 4.6).

4.4. Results of the Color Changes of CC3.O Groups after Immersion Procedure

The CC3.O samples exposed to distilled water, tea, coffee, and red wine showed statistically significance increase in terms of ΔE values throughout measurements repeated over-time (p=0.000) (Table 4.7 and Figure 4.4).

Among all the staining solutions for each time period (T1 to T6), distilled water was the solution that caused the least color change on CC3.O samples (Table 4.7). There were statistically significant differences between all solution groups in their staining effects (p=0.000) (Table 4.4).

Table 4.7. Descriptive statistics of color change (ΔE) for CC3.O groups

MATERIAL	SOLUTION	T1		T2		T3		T4		T5		T6		ANOVA	Post[a] Hoc
		ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	ΔE (± Sd)	NBS	P	
CC3.O	Distilled water	0.23 (±0.08)	0.21	0.53 (±0.12)	0.48	1.13 (±0.11)	1.03	1.38 (±0.12)	1.26	1.53 (±0.18)	1.40	1.92 (±0.18)	1.76	0.000	T1-T2, T1-T3, T1-T4, T1-T5, T1-T6, T2-T3, T2-T4, T2-T5, T2-T6, T3-T4, T3-T5, T3-T6, T4-T5, T4-T6, T5-T6
	Tea	1.63 (±0.16)	1.49	3.67 (±0.23)	3.37	4.42 (±0.21)	4.06	4.65 (±0.22)	4.27	5.06 (±0.13)	4.65	5.54 (±0.21)	5.09		
	Coffee	1.14 (±0.18)	1.04	3.16 (±0.13)	2.90	3.49 (±0.09)	3.21	4.11 (±0.26)	3.78	4.63 (±0.21)	4.25	5.25 (±0.25)	4.83		
	Red wine	1.54 (±0.21)	1.41	2.45 (±0.33)	2.25	2.96 (±0.18)	2.72	4.58 (±0.22)	4.21	5.57 (±0.23)	5.12	6.16 (±0.16)	5.66		

	NBS: 0.5−1.5	Slight: Slight change
	NBS: 1.5−3.0	Noticeable: Perceivable change
	NBS: 3.0−6.0	Appreciable: Marked change
	NBS: 6.0−12.0	Much: Extremely marked change
	NBS: 12.0 or more	Very much: Change to other color

The CC3.O samples exposed to distilled water showed significantly different ΔE values between repeated measurements (p=0.000). While the ΔE of CC3.O samples in distilled water was 0.23 in the measurement at T1, this value increased to 1.92 in the measurements at T6 (Table 4.7 and Figure 4.4).

ΔE values of CC3.O samples in the tea solution were statistically significantly higher than ΔE values of CC3.O samples exposed to coffee in all measurement period. ΔE values of CC3.O samples in the tea solution were statistically significantly higher than ΔE values of CC3.O samples exposed to red wine in the most of all measurement period except T5, T6 (p = 0.000) (Table 4.4, Table 4.7).

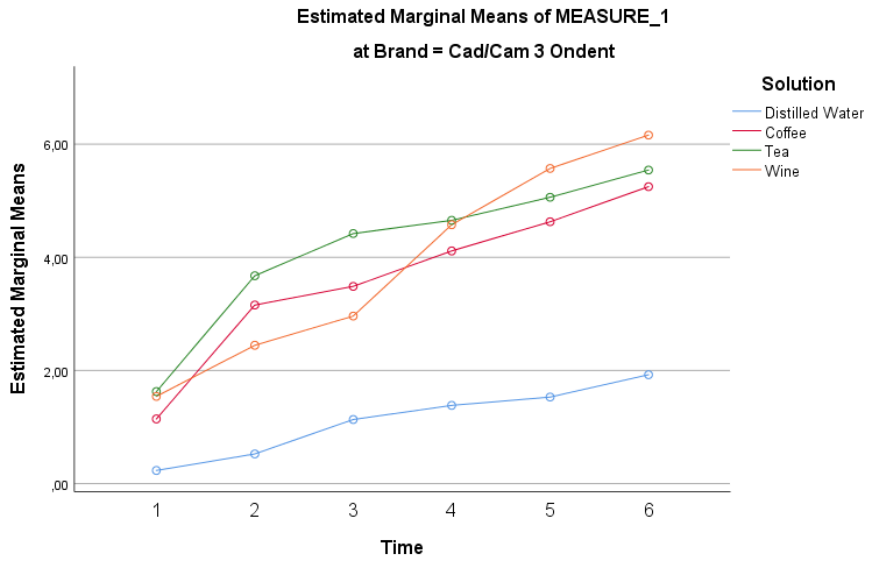

Figure 4.4. Color stability of CC3.O groups after immersion in different solutions

When CC3.O samples were immersed in **distilled water**, **tea**, **coffee**, and **red wine**, they showed statistically different ΔE values between repeated measurements (from T1 to T6) (p=0.000). While the ΔE of CC3.O samples in **distilled water** was 0.23 in the measurement at T1, this value increased to 1.92 in the measurements at T6. While the ΔE of CC3.O samples in **tea** was 1.63 in the measurement at T1, this value increased to 5.54 in the measurements at T6. While the ΔE of CC3.O samples in **coffee** was 1.14 in the measurement at T1, this value increased to 5.25 in the measurements at T6. While the ΔE value for **red wine** was 1.54 at T1, this value increased up to 6.16 in T6 (Table 4.7 and Figure 4.4).

When comparing all solutions tested for CC3.O samples, there was a statistically significant difference between all solutions (p= 0.000). Ranking from high to low of ΔE was **Red Wine > Tea> Coffee > Distilled Water** at the end of the last immersion period (Table 4.4, Table 4.7).

When color changes are evaluated **according to NBS rating system**, no clinically "Extremely marked" color change was observed in the CC3.O samples immersed in all solutions at the 6 different color measurements. The highest NBS value was 1.76, which is less than 3, and belonged to distilled water. Even at the end of the 5 weeks immersion, the highest NBS value of the CC3.O samples immersed in distilled water was below 3. In other words, after 5 weeks, distilled water did not cause a clinically "marked" color change in CC3.O samples (Table 4.7).

Tea caused clinically "marked" color change in CC3.O samples from the second measurement (T2, which is equal to 7 months consuming) to the end of the last measurement (T6, which is equal to 35 months consuming) (Table 4.7).

Coffee caused clinically "marked" color change in CC3.O samples from the third measurement (T3, which is equal to 14 months consuming) to the end of the last measurement (T6, which is equal to 35 months consuming) (Table 4.7).

Red wine caused clinically "marked" color change in CC3.O samples from the fourth measurement (T4, which is equal to 21months consuming) to end of the last measurement (T6, which is equal to 35 months consuming) (Table 4.7).

At the end of the 5 weeks immersion, among all the staining solutions, the red wine was the most staining solution for the CC3.O samples (ΔE= 6.16 and NBS unit= 5.66), which is considered to be "marked" color change, as it is more than 3 according to the NBS rating system.

At the end of immersing for 5 weeks, a “perceivable” color change occurred in distilled water; “marked” color change occurred in tea, coffee and red wine for CC3.O samples (Table 4.7).

Multiple comparisons for all acrylic resin materials between solution groups for dependent variable of ΔE values are shown in Table 4.8. In addition, Figure 4.5 demonstrates the color difference (ΔE) of acrylic resin materials after immersion in distilled water, tea, coffee, and red wine.

Table 4.8. Pairwise comparisons of color change of acrylic resin groups by brand

Solution	Brand (I)	Brand (J)	Mean Difference (I-J)	Std. Error	p	95% Confidence Interval for Difference[a]	
						Lower Bound	Upper Bound
Distilled Water	CC1.D	CC2.M	.146	.036	.001*	.049	.243
		CC3.O	.470	.036	.000*	.373	.568
		HC.H	.057	.036	**.724**	-.040	.154
	CC2.M	CC1.D	-.146	.036	.001*	-.243	-.049
		CC3.O	.324	.036	.000*	.227	.422
		HC.H	-.089	.036	**.091**	-.187	.008
	CC3.O	CC1.D	-.470	.036	.000*	-.568	-.373
		CC2.M	-.324	.036	.000*	-.422	-.227
		HC.H	-.414	.036	.000*	-.511	-.316
	HC.H	CC1.D	-.057	.036	**.724**	-.154	.040
		CC2.M	.089	.036	**.091**	-.008	.187
		CC3.O	.414	.036	.000*	.316	.511
Coffee	CC1.D	CC2.M	.494	.036	.000*	.397	.591
		CC3.O	-.793	.036	.000*	-.890	-.696
		HC.H	.468	.036	.000*	.370	.565
	CC2.M	CC1.D	-.494	.036	.000*	-.591	-.397
		CC3.O	-1.287	.036	.000*	-1.384	-1.190
		HC.H	-.027	.036	**1.000**	-.124	.071
	CC3.O	CC1.D	.793	.036	.000*	.696	.890
		CC2.M	1.287	.036	.000*	1.190	1.384
		HC.H	1.260	.036	.000*	1.163	1.358
	HC.H	CC1.D	-.468	.036	.000*	-.565	-.370
		CC2.M	.027	.036	**1.000**	-.071	.124
		CC3.O	-1.260	.036	.000*	-1.358	-1.163

Based on estimated marginal means
*The mean difference is significant at the 0.05 level
a. Computed using alpha = 0.05
b. Adjustment for multiple comparisons: Bonferroni

Table 4.8. Continued

Solution	Brand (I)	Brand (J)	Mean Difference (I-J)	Std. Error	p	95% Confidence Interval for Difference[a]	
						Lower Bound	Upper Bound
Tea	CC1.D	CC2.M	.435	.036	.000*	.338	.532
		CC3.O	-1.019	.036	.000*	-1.116	-.922
		HC.H	.205	.036	.000*	.107	.302
	CC2.M	CC1.D	-.435	.036	.000*	-.532	-.338
		CC3.O	-1.454	.036	.000*	-1.551	-1.357
		HC.H	-.231	.036	.000*	-.328	-.133
	CC3.O	CC1.D	1.019	.036	.000*	.922	1.116
		CC2.M	1.454	.036	.000*	1.357	1.551
		HC.H	1.223	.036	.000*	1.126	1.321
	HC.H	CC1.D	-.205	.036	.000*	-.302	-.107
		CC2.M	.231	.036	.000*	.133	.328
		CC3.O	-1.223	.036	.000*	-1.321	-1.126
Red Wine	CC1.D	CC2.M	.758	.036	.000*	.661	.855
		CC3.O	.202	.036	.000*	.105	.300
		HC.H	.449	.036	.000*	.352	.546
	CC2.M	CC1.D	-.758	.036	.000*	-.855	-.661
		CC3.O	-.556	.036	.000*	-.653	-.458
		HC.H	-.309	.036	.000*	-.407	-.212
	CC3.O	CC1.D	-.202	.036	.000*	-.300	-.105
		CC2.M	.556	.036	.000*	.458	.653
		HC.H	.246	.036	.000*	.149	.343
	HC.H	CC1.D	-.449	.036	.000*	-.546	-.352
		CC2.M	.309	.036	.000*	.212	.407
		CC3.O	-.246	.036	.000*	-.343	-.149

Based on estimated marginal means
*The mean difference is significant at the 0.05 level
a. Computed using alpha = 0.05
b. Adjustment for multiple comparisons: Bonferroni

4.5. Comparison of Color Stability between All Acrylic Resin Test Groups

4.5.1. Comparison of Color Stability between HC.H and CC1.D Acrylic Resins

When comparing the ΔE values of HC.H and CC1.D groups, there were no statistically significant differences between both materials immersed in distilled water ($p>0.05$). However, when immersed in tea, coffee, and red wine, there was a statistically significant difference between HC.H and CC1.D samples ($p=0.000$) (Table 4.8). The mean ΔE values of CC1.D samples immersed in tea, coffee and red wine were higher than those of the HC.H samples in the all color measurements except at T2 in tea solution (Table 4.1, Figure 4.5).

The ΔE values observed in HC.H and CC1.D samples immersed in **distilled water** varied between 0.26 and 2.67. Hence, both materials in distilled water did not present a "marked" discoloration (NBS<3) through the whole immersion period (Table 4.1).

On the other hand, the samples immersed in **tea** for HC.H and CC1.D groups demonstrated a "marked" discoloration (NBS unit>3) after 3 weeks of immersion (T4) (Table 4.1).

The samples immersed in **coffee** for HC.H group demonstrated a "marked" discoloration (NBS unit>3) after 5 weeks of immersion (T6), and their ΔE values was 3.64; and the samples immersed in **coffee** for CC1.D group demonstrated a "marked" discoloration (NBS unit>3) after 3 weeks of immersion (T4), and their ΔE values were 3.37 (Table 4.1).

The samples immersed in **red wine** for HC.H groups demonstrated a "marked" discoloration (NBS unit>3) after 3 weeks of immersion (T4), and their ΔE values was 3.90; and the samples immersed in **red wine** for CC1.D group demonstrated a clinically "marked" discoloration (NBS unit>3) after 2 weeks of immersion (T3), and their ΔE values were 3.48 (Table 4.1).

At the end of 5 weeks (35 months consumption) HC.H samples showed lower ΔE values than CC1.D samples when immersed in all solutions. According to Table 4.1, it can be concluded that CC1.D samples undergoes more discoloration than the HC.H samples in last 4 measurements (T3-T6).

In summary, HC.H and CC1.D samples in distilled water indicated "perceivable" color change (NBS<3) at the end of the 5 weeks (35 months consumption). However, HC.H and CC1.D samples demonstrated "marked" color change when immersed in tea, coffe and red wine at the end of the 5 weeks (35 months consumption) (Table 4.1).

4.5.2. Comparison of Color Stability between HC.H and CC2.M Acrylic Resins

When comparing the ΔE values of HC.H and CC2.M groups, there were no statistically significant differences between both materials immersed in distilled water and coffee ($p>0.05$). However, when immersed in tea and red wine, there was a statistically significant difference between HC.H and CC2.M samples ($p=0.000$) (Table 4.8). The mean ΔE values of HC.H samples immersed in tea, coffee and red wine were higher than those of the CC2.M samples in the last 4 measurements (T3-T6) (Table 4.1, Figure 4.5).

The ΔE values observed in HC.H and CC2.M samples immersed in **distilled water** varied between 0.31 and 2.52. Hence, both materials in distilled water did not present a "marked" discoloration (NBS<3) through the whole immersion period (Table 4.1).

On the other hand, the samples immersed in **tea** for HC.H group demonstrated a "marked" discoloration (NBS unit>3) after 3 weeks of immersion (T4), and their ΔE values was 3.45, the samples immersed in **tea** for CC2.M group demonstrated a "marked" discoloration (NBS unit>3) after 4 weeks of immersion (T5), and their ΔE values were 3.89 (Table 4.1).

The samples immersed in **coffee** for HC.H group demonstrated a "marked" discoloration (NBS unit>3) after 5 weeks of immersion (T6), and their ΔE values was 3.64; and the samples immersed in **coffee** for CC2.M group demonstrated a "marked" discoloration (NBS unit>3) after 5 weeks of immersion (T6), and their ΔE values was 3.48 (Table 4.1).

The samples immersed in **red wine** for HC.H groups demonstrated a "marked" discoloration (NBS unit>3) after 3 weeks of immersion (T4), and their ΔE values was 3.90; and the samples immersed in **red wine** for CC2.M group demonstrated a clinically "marked" discoloration (NBS unit>3) after 3 weeks of immersion (T4), and their ΔE values was 3.74 (Table 4.1).

At the end of 5 weeks (35 months consumption) CC2.M samples showed lower ΔE values than HC.H samples when immersed in all solutions tested except distilled water. According to Table 4.1, it can be concluded that HC.H samples undergoes more discoloration than the CC2.M samples in last 4 measurements (T3-T6).

In summary, HC.H and CC2.M samples in distilled water indicated “perceivable” color change (NBS<3) at the end of the 5 weeks (35 months consumption). However, HC.H and CC2.M samples demonstrated “marked” color change when immersed in tea, coffee and red wine at the end of the 5 weeks (35 months consumption) (Table 4.1).

4.5.3. Comparison of Color Stability between HC.H and CC3.O Acrylic Resins

When comparing the ΔE values of HC.H and CC3.O groups, there were statistically significant differences between both materials immersed in distilled water, tea, coffee and red wine (p=0.000) (Table 4.8). The mean ΔE values of HC.H samples immersed in tea, coffee and red wine were lower than those of the CC3.O samples in the last 3 measurements (T4-T6) (Table 4.1, Figure 4.5).

The ΔE values observed in HC.H and CC3.O samples immersed in **distilled water** varied between 0.23 and 2.46. Hence, both materials in distilled water did not present a “marked” discoloration (NBS<3) through the whole immersion period (Table 4.1).

On the other hand, the samples immersed in **tea** for HC.H group demonstrated a “marked” discoloration (NBS unit>3) after 3 weeks of immersion (T4), and their ΔE values was 3.45; the samples immersed in **tea** for CC3.O group demonstrated a “marked” discoloration (NBS unit>3) after 1 week of immersion (T2), and their ΔE values were 3.67 (Table 4.1).

The samples immersed in **coffee** for HC.H group demonstrated a “marked” discoloration (NBS unit>3) after 5 weeks of immersion (T6), and their ΔE values was 3.64; and the samples immersed in **coffee** for CC3.O group demonstrated a “marked” discoloration (NBS unit>3) after 2 weeks of immersion (T3), and their ΔE values was 3.49 (Table 4.1).

The samples immersed in **red wine** for HC.H groups demonstrated a “marked” discoloration (NBS unit>3) after 3 weeks of immersion (T4), and their ΔE values was 3.90; and the samples immersed in **red wine** for CC3.O group demonstrated a “marked” discoloration (NBS unit>3) after 3 weeks of immersion (T4), and their ΔE values was 4.58 (Table 4.1).

At the end of 5 weeks (35 months consumption), HC.H samples showed lower ΔE values than CC3.O samples when immersed in tea, coffee and red wine. According to Table 4.1, it can be concluded that CC3.O samples undergoes more discoloration than the HC.H samples in last 3 measurements (T4-T6).

In summary, HC.H and CC3.O samples in distilled water indicated "perceivable" color change at the end of the 5 weeks (35 months consumption) (NBS<3). However, HC.H and CC3.O samples demonstrated "marked" color change when immersed in tea, coffee and red wine at the end of the 5 weeks (35 months consumption) (Table 4.1).

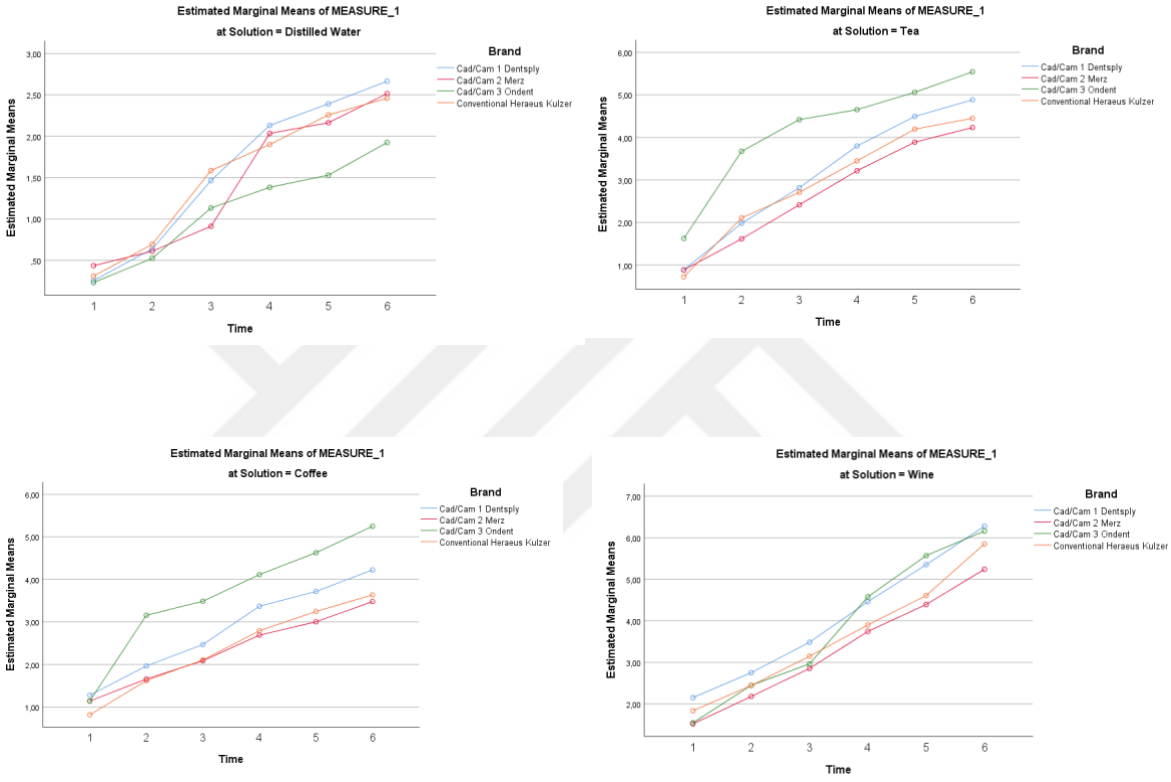

Figure 4.5. Color difference (ΔE) of acrylic resins after immersion in all solutions

4.5.4. Comparison of Color Stability between CC1.D and CC2.M Acrylic Resins

When comparing the ΔE values of CC1.D groups and the CC2.M groups, there were statistically significant differences between both materials immersed in all immersion solutions ($p<0.05$) (Table 4.8 and Figure 4.5).

The ΔE values observed in CC1.D and CC2.M acrylic resin materials immersed in **distilled water** varied between 0.26 and 2.67. Hence, both materials in distilled water

did not show a “marked” discoloration (NBS<3) through the whole immersion period (Table 4.1).

On the other hand, the samples immersed in **tea** for CC1.D demonstrated a “marked” discoloration (NBS unit>3) after 3 weeks of immersion (T4), CC2.M samples demonstrated a clinically “marked” discoloration (NBS unit>3) after 4 weeks of immersion (T5).

When immersed in **coffee** for CC1.D samples demonstrated a “marked” discoloration (NBS unit>3) after 3 weeks of immersion (T4), CC2.M samples demonstrated a clinically “marked” discoloration (NBS unit>3) after 5 weeks of immersion (T6).

When immersed in **red wine** for CC1.D samples demonstrated a “marked” discoloration (NBS unit>3) after 2 weeks of immersion (T3), CC2.M samples demonstrated a clinically “marked” discoloration (NBS unit>3) after 3 weeks of immersion (T4).

At the end of 5 weeks (35 months of consumption), CC2.M samples presented lower ΔE values than CC1.D samples when immersed in distilled water, tea, coffee, and red wine. According to Table 4.1, it can be concluded that the CC1.D samples undergoes more discoloration than CC2.M samples.

In summary, CC1.D and CC2.M groups in distilled water demonstrated “perceivable” color change at the end of the 5 weeks (35 months consumption) (NBS<3). However, these acrylic resin groups demonstrated “marked” color change when immersed in other 3 immersion solutions at the end of the 5 weeks (35 months consumption) (Table 4.1).

4.5.5. Comparison of Color Stability between CC1.D and CC3.O Acrylic Resins

When comparing the ΔE values of CC1.D groups and the CC3.O groups, there were statistically significant differences between both materials immersed in all immersion solutions ($p<0.05$) (Table 4.8 and Figure 4.5).

The ΔE values observed in CC1.D and CC3.O acrylic resin materials immersed in **distilled water** varied between 0.23 and 2.67. Hence, both materials in distilled water did not show a “marked” discoloration (NBS<3) through the whole immersion period (Table 4.1).

On the other hand, the samples immersed in **tea** for CC1.D demonstrated a "marked" discoloration (NBS unit>3) after 3 weeks of immersion (T4), CC3.O samples demonstrated a "marked" discoloration (NBS unit>3) after 1 week of immersion (T2).

When immersed in **coffee** for CC1.D samples demonstrated a "marked" discoloration (NBS unit>3) after 3 weeks of immersion (T4), CC3.O samples demonstrated a "marked" discoloration (NBS unit>3) after 2 weeks of immersion (T3).

When immersed in **red wine** for CC1.D samples demonstrated a "marked" discoloration (NBS unit>3) after 2 weeks of immersion (T3), CC3.O samples demonstrated a "marked" discoloration (NBS unit>3) after 3 weeks of immersion (T4).

At the end of 5 weeks (35 months of consumption) CC1.D samples presented lower ΔE values than CC3.O samples when immersed in tea and coffee solutions.

In summary, CC1.D and CC3.O groups in distilled water demonstrated "perceivable" color change at the end of the 5 weeks (35 months consumption) (NBS<3). However, these acrylic resin groups demonstrated "marked" color change when immersed in other 3 immersion solutions at the end of the 5 weeks (35 months consumption) (Table 4.1).

4.5.6. Comparison of Color Stability between CC2.M and CC3.O Acrylic Resins

When comparing the ΔE values of CC2.M groups and the CC3.O groups, there were statistically significant differences between both materials immersed in all immersion solutions ($p<0.05$) (Table 4.8 and Figure 4.5).

The ΔE values observed in CC2.M and CC3.O acrylic resin materials immersed in **distilled water** varied between 0.23 and 2.52. Hence, both materials in distilled water did not show a "marked" discoloration (NBS<3) through the whole immersion period (Table 4.1).

On the other hand, the samples immersed in **tea** for CC2.M samples demonstrated a "marked" discoloration (NBS unit>3) after 4 weeks of immersion (T5), CC3.O samples demonstrated a "marked" discoloration (NBS unit>3) after 1 week of immersion (T2).

When immersed in **coffee** for CC2.M samples demonstrated a "marked" discoloration (NBS unit>3) after 5 weeks of immersion (T6), CC3.O samples demonstrated a "marked" discoloration (NBS unit>3) after 2 weeks of immersion (T3).

When immersed in **red wine** for CC2.M samples demonstrated a "marked" discoloration (NBS unit>3) after 3 weeks of immersion (T4), CC3.O samples demonstrated a "marked" discoloration (NBS unit>3) after 3 weeks of immersion (T4).

At the end of 5 weeks (35 months of consumption) CC2.M samples presented lower ΔE values than CC3.O samples when immersed in tea, coffee, and red wine. According to Table 4.1, it can be concluded that the CC3.O samples undergoes more discoloration than CC2.M samples.

In summary, CC2.M and CC3.O groups in distilled water demonstrated "perceivable" color change at the end of the 5 weeks (35 months consumption) (NBS<3). However, these acrylic resin groups demonstrated "marked" color change when immersed in other 3 immersion solutions at the end of the 5 weeks (35 months consumption) (Table 4.1).

4.6. Comparison of Color Stability between the Four Acrylic Resin Groups

At the end of the last immersion period, when comparing acrylic resin groups for **distilled water** solution, ranking from high to low of ΔE value was **CC1.D > CC2.M > HC.H > CC3.O** (Table 4.1, Figure 4.5).

According to **Table 4.1**; when comparing color stability of acrylic resin materials for **distilled water**, there is no statistically significant difference between CC1.D and HC.H also between CC2.M and HC.H groups ($p>0.05$). For all other pairwise comparisons, a significant difference between acrylic resin groups was determined.

When comparing acrylic resin groups for **tea and coffee** solution, ranking from high to low of ΔE value was **CC3.O > CC1.D > HC.H > CC2.M** at the end of the last immersion period (Table 4.1, Figure 4.5). According to **Table 4.1;** when comparing color stability of acrylic resin materials for **tea**, there is a statistically significant difference between all acrylic resin groups ($p<0.05$).

When comparing color stability of acrylic resin materials for **coffee** solution, there is no statistically significant difference between CC2.M and HC.H groups ($p>0.05$). For all other pairwise comparisons, a significant difference between acrylic resin groups was determined.

When comparing acrylic resin groups for **red wine** solution, ranking from high to low of ΔE value was **CC1.D > CC3.O > HC.H > CC2.M** at the end of the last immersion period (Table 4.1, Figure 4.5). When comparing color stability of acrylic resin materials for **red wine**, there is a statistically significant difference between all acrylic resin groups ($p<0.05$).

When comparing solutions for **all acrylic resin groups** tested, ranking from high to low of ΔE value was **Red Wine > Tea > Coffee > Distilled Water** at the end of the last immersion period (Table 4.1, Figure 4.2).

According to the analysis results, it was seen that the color change in the acrylic resin groups increased over time in the comparison between the measurements in 6 different time periods.

As a result of the 3 Way ANOVA test, it was seen that both brand and solution factor had a significant effect on color change over time. Therefore, the null hypothesis that the type of acrylic resin denture base material and immersion solution have no effect on the stainability of acrylic resin denture base materials was rejected.

When CC1.D, CC2.M and HC.H acrylic resins were used, it was observed that the lowest color change was in distilled water and the highest color change was in red wine. Red wine was followed by tea and then coffee. When CC3.O acrylic resin was used, the lowest color change was in distilled water and the highest color change was tea throughout the experiments. Tea was followed by red wine and then coffee.

When binary comparisons were made on the basis of acrylic resin groups, in the experiments which distilled water solution was used, it was observed that the lowest color change was in CC3.O group (ΔE=1.92). Also, in the experiments using coffee (ΔE=5.25) and tea (ΔE=5.54) solution, it was observed that the highest color change was in CC3.O groups. The lowest color change was in CC2.M group for tea (ΔE=4.23), and both HC.H (ΔE=3.64) and CC2.M (ΔE=3.48) brand dentures for coffee. Besides, in the experiments which red wine solution was used, it was observed that the lowest color change was in CC2.M (ΔE=5.24) and the highest color change was in CC1.D (ΔE=6.28). CC1.D was followed by CC3.O (ΔE=6.16) and then HC.H (ΔE=5.85) .

When comparing the ΔE values of CAD/CAM acrylic resin materials tested, there were statistically significant differences between all CAD/CAM acrylic resin materials immersed in distilled water, tea, coffee and red wine (p=0.000) (Table 4.8 and Figure 4.5).

In summary, at the end of the 5 weeks (35 months consumption), all acrylic resin groups in distilled water did not show any discoloration that could be detected clinically (NBS<3). However, all acrylic resin groups demonstrated “marked” color change (3<NBS unit<6) when immersed in the 3 staining solutions (tea, coffee and red wine) (Table 4.1).

5. DISCUSSION AND CONCLUSION

In the past, removable dental prostheses were produced only by conventional methods, but with the advancement of technology, they started to be produced from acrylic resin based on polymethylmethacrylate, which is prepolymerized under high temperature and pressure. It is aimed to produce acrylic denture bases with CAD/CAM acrylic resin materials that are thought to have less monomer release and less porosity, thus showing less discoloration and having a better dimensional stability and fracture strength (3,5,7). Although current scientific data show the clinical superiority of CAD/CAM acrylic resin materials over conventional acrylic resin materials, data on properties of these materials are still limited (21).

The appearance of the denture base in harmony with the tissues and color stability are important for the esthetics of the prosthesis. The color of acrylic resin materials may change by intrinsic or extrinsic factors. Intrinsic factors are related to material itself (15). External factors are related to the absorption and adsorption of coloring agents (16,17). Daily consumed beverages such as coffee, tea and red wine may cause discoloration of the prosthesis and it leads to esthetic problems (18,19). Color changes in the prosthesis may result in dissatisfaction of the patient and it may be necessary to replace the prosthesis (8,9,10).

The discoloration of acrylic resin denture bases and/or acrylic teeth with different beverage and food colorants has been the subject of researches (14,18,20,117,135,137)

Therefore, the aim of the study was to determine and compare of the color stability of the previously mentioned CAD/CAM and heat-cured acrylic resin denture base materials by exposing to distilled water, tea, coffee and red wine solutions, as these are frequently consumed by humans.

According to the results of the present study, immersion of the acrylic resin denture base materials in some drinks affected the color of acrylic resin denture base materials, therefore, the null hypothesis that the type of acrylic resin denture base material and immersion solutions have no effect on the stainability of acrylic resin denture base materials was rejected.

Color changes can be measured with the help of different methods. It can be measured visually or using color measurement devices. The devices used for color measurement, which are previously mentioned, can eliminate the factors that affect the color perception and interpretation (18). Colorimeters, spectrocolorimeters,

spectroradiometers, spectrophotometers and digital cameras were used in color analysis in researches (127).

Measurements with colorimeters use the same light source, illumination methodology and viewing angle, and the measurement conditions are the same whether day or night, indoors or outdoors. In addition, colorimetric measurements can give inaccurate results on inclined surfaces and cannot evaluate metamerism. In the determination of the colors of translucent materials with colorimeters, there are problems such as the light reflected from the object being refracted and scattered at different angles and not fully returning to the device (107,122,129).

The spectrocolorimeter is a hybrid instrument capable of providing colorimetric data such as X, Y, Z or CIE L*a*b* values for various CIE standard Illuminants (Figure 2.20a,b). Therefore, they have the ability to provide much better quality control compared to colorimeters. Their price is slightly higher compared to tristimulus colorimeters, but lower than most spectrophotometers. Spectrocolorimetry can only be used for quality control applications (122).

Spectroradiometers are used to measure radiometric values (Figure 2.22a,b). They include a spectrometer calibrated to the wavelength scale and radiometric scale (64). With a spectroradiometer, color can be measured under the same observation conditions as visual color determination and without touching the sample. The spectroradiometer also allows color measurements of shiny objects and surfaces. The slightest change in the angle during measurement may cause a difference in the results (107).

The use of digital cameras for color measurement has recently become popular. These devices are also called RGB devices (Figure 2.23). This system consists of a digital camera, computer, driver, computer software and color sensor. The image of the desired sample is taken with a digital camera and transferred to a computer to which the camera is connected. The values are obtained in CIE L*, a*, b* thanks to the software on the computer (107,132). The advantage of the system is that the color appearance of the whole object can be obtained as an image, rather than measuring a single point (107). Digital cameras are practical to use, but factors such as angle and light can alter the assessment of color. Their use alone is not sufficient. Another limitation of digital cameras is that gray card and Adobe Photoshop literature-based protocols are required to know the shade matching protocol (96).

With the help of a spectrophotometer, numerical data on color and spectral reflectance graphs can be obtained. Spectrophotometers also have the advantage of calculating tristimulus values for CIE standard observer functions (122). In addition, spectrophotometers are equipped with high-precision sensors and can perform precise measurements under different lighting conditions. They are frequently used in research because they provide the most accurate spectral analysis of the data reflected by the object by placing the object inside the spectrophotometer and exposing it to light from different angles and directions. These devices can also assess metamerism. Some studies (11,137) stated that spectrophotometers are more successful than colorimeters in terms of reliability, reproducibility and accuracy. Therefore, due to all these advantages a spectrophotometer was used to color measurement for determining the color differences in present research to overcome subjective errors may occur in color evaluation.

The Commission Internationale d'Eclairage L*a*b* color space (CIE L*a*b*) was used to explain the analysis of color changes in our study. The CIE L*a*b* color space is one of the most widely used and preferred color spaces today and is very convenient and sufficient for determining even the smallest differences in color (138).

Providing color differences to be stated in units that can be associated with visual perception and clinical significance is the advantage of the CIE L*a*b* color space (121).

Visual perceptible and acceptable thresholds can be digitized by combination of visual and instrumental methods of color measurement (96). When the color difference between compared objects is visible to 50% of observers (the remaining 50% will not see the difference), this corresponds to the perceptible threshold of 50:50%. When the color difference is visible to 50% of observers (the remaining 50% would think that it is unacceptable), this corresponds to the acceptable threshold of 50:50%. Color difference at or below the perceptible threshold is named as a perceptible color change; color difference at or below the acceptable threshold is named an acceptable color change. (107).

$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{½}$ formula was used to calculate the color differences in this study (116).

In dental literature, ΔE values were used by many researchers (135,137,139) to evaluate the acceptable and perceptible color difference. On the other hand, the acceptable and perceptable treshold preferred by each researcher may differ. Al-Qarni *et al.* (137), Alfouzan *et al.* (139) and Gruber *et al.* (140) suggested that above the ΔE value of 2.7 is

unacceptable that it is clinically perceptible. Dayan *et al.* (135) stated that, if the ΔE value is up to 3.3, it is considered acceptable; if the ΔE value is more than 3.3, it is considered unacceptable. The NBS grading system is commonly used to determine the degree of color difference, to avoid such situations that vary from person to person. NBS grading system provides absolute criteria by converting ΔE values to define the clinical significance (141). Therefore, NBS units were calculated to explain the color differences caused by immersion time and/or solution types in our study. According to this system, the NBS value of more than 3 shows "marked" discoloration and it is clinically unacceptable. In the NBS grading system, NBS = 3 was accepted as the threshold value and values over 3 were thought clinically unacceptable in our study similar to study of Dayan *et al*. (135) (NBS units = ΔE × 0.92 = 3.3 × 0.92 = 3.036).

With the CIEDE2000 system, some new terms such as weighing and rotation function and parametric factors were introduced in new formula. These terms allow the detection of variabilities in texture, separations and background etc. for the components of color (hue, value and chroma). The aim of these new terms and the new formula was to increase the relationship between instrumental and visual color differences. Even though CIEDE2000 system is used to evaluate the differences in hue, chroma and lightness; both ΔEab and ΔE00 formulas can be used to evaluate the color differences (118). Significant correlation was detected between ΔEab and ΔE00 formulas. With the involvement of weighting functions, ΔE00 formula was started to recommended for evaluation. Use of ΔE00 formula is more complex. Therefore, although ΔE00 formula has some advantages, ΔEab formula is more commonly used in recent studies (118). In addition, the use of the ΔEab formula will provide convenience in comparing and discussing the results with previous studies using the ΔEab formula.

10 or 15 mm diameter and 2 or 3 mm thickness of the disc-shaped samples were used in some studies which evaluate the color stability of acrylic resins (135,137,139, 140,142). In the present study, diameter of 15 mm and a thickness of 2 mm was used. The purpose of preparing samples with a diameter of 15 mm is to ensure that the sample is fit properly in the slot of the spectrophotometer. General recommendations suggest that the thickness of the acrylic resin denture base is between 1.5 to 3 mm which is considerably suitable compare to clinical circumstances (143). Therefore, in our study the thickness of the samples was adjusted to be 2.5 mm to reach 2 mm thickness after grinding and polishing.

Similar to previous studies (137,139,140), before making the baseline (target) color measurements, the samples were immersed in distilled water at 37°C in the incubator for 24 hours in our study.

Before each color measurement was performed, the samples were then removed from their containers and washed under running water without brushing for 10 seconds and then dried with paper towel without pressure similar to the study performed by Bagheri *et al.* (144). Then, the baseline (T0) color measurements of each samples were performed by using a spectrophotometer.

In previous studies, the color stability of acrylic resin denture base materials in various drinks such as distilled water as a control group (20,135,137,140), and some colorant drinks such as tea (11,20,145), coffee (11,20,135,137,139,140,142,145), and red wine (11,20,135,137,140) were investigated. These colorant beverages are constantly consumed by people on a daily basis and they have the possibility of causing staining in dental restorative materials. Therefore, distilled water, tea, coffee, and red wine were used in this study. All the samples in solutions were stored in an incubator at 37°C to imitate the intraoral environment. In addition, similar to the study of Al-Qarni *et al.* (137) and Alfouzan *et al.* (139), all solutions tested were changed every 24 hours.

It is stated that the consumption time for a person to drink 1 cup of coffee is 15 minutes and it is assumed that a person drinks 3.2 cups of coffee per day on average (116,146). Thus, it is assumed that consumption of 3.2 cups of coffee per day, 48 minutes is calculated for the exposure time to coffee each day. Therefore, it can be calculated that the samples will be immersed to coffee for 1440 minutes (24 hours), that is equal to 30 days (1 month) of consumption. In the current study, consumption time was calculated similarly, color measurements of samples were indicated as 24 hours (T1: 1 day: 30 day (1 month) consumption), 168 hours (T2: 7 days: 210-day (7 months) consumption), 336 hours (T3: 14 days: 420-day (14 months) consumption), 504 hours (T4: 21 days: 630-day (21 months) consumption), 672 hours (T5: 28 days: 840 day (28 months) consumption), and 840 hours (T6: 35 days: 1.050 day (35 months) consumption) after the samples were immersed in solutions tested. The immersion time was set to 840 hours of immersion (nearly 3 year consumption) in our study.

As for the results of our study, the ΔE values of heat-cured acrylic resin samples immersed in tea and red wine**,** which caused the highest discoloration, ranged from 4.45 to 5.85, while the ΔE values of CAD/CAM acrylic resin samples ranged from 4.23 to

6.28. Therefore, when comparing the color change, it cannot be concluded that the heat-cured acrylic resin materials have more susceptibility to staining compared to CAD/CAM acrylic resin materials in our study similar to the study of Al-Qarni *et al.* (137), Gruber *et al.* (140) and Alp *et al.* (142). In addition, the calculated ΔE values of all acrylic resin groups (3 < NBS unit < 6) found above clinically acceptable threshold at the end of our study. This result was not consistent with the results of some studies (135,137,140) which investigated the color stability of acrylic resin denture base materials. This finding may be associated with the longer immersion time in our study compared to the other studies.

In the study performed by Al-Qarni *et al.* (137) samples were divided into 3 acrylic resin groups; which were produced by compression molding, injection molding and CAD/CAM system. They soaked the samples in three different agents: coffee, red wine and distilled water (control group) for 7 days, and compared the stainability of the samples. All solutions were renewed every 24 hours over 7 days. All color measurements were performed by using spectrophotometer and results were expressed by the CIE L*a*b* (ΔE_{ab}) formula. Similar to the study of Al-Qarni *et al.*, Dentsply Lucitone 199 was used for CAD/CAM acrylic resin in our study. As a result of the Al-Qarni *et a*l's study (137), the degree of color change was dependent on the immersion solution and the type of material. Red wine produced the highest discoloration for the denture base resins in the study of Al-Qarni *et al.*, regardless of what the processing method was used. In addition, denture base samples immersed in coffee and red wine had significantly more color change than the samples which immersed in distilled water for denture base acrylics ($p<0.001$). CAD/CAM samples immersed in coffee and red wine had less color difference than compression molded samples, but the differences were not statistically significant (all $p>0.05$). CAD/CAM acrylic resins underwent a color change that is comparable with that of the conventionally fabricated acrylic resins similar to our study. ΔE value of 2.3 in coffee and ΔE value of 3.2 in red wine were found for compression molding acrylic resin group at the end of the 7 days of immersion period. In addition, ΔE value of 2.01 in coffee and ΔE value of 3.0 in red wine for CAD/CAM acrylic resin group at the end of the 7 days of immersion period. In our study, ΔE values were 1.62 in coffee and 2.44 in red wine at the end of the 7 days of immersion period for the heat-cured acrylic resin group. For the CC1.D (Luccitone 199) group, ΔE values were 1.96 in coffee and 2.75 in red wine at the end of the 7 days of immersion period. These values are close to those of Al-Qarni *et al.'*s study and these values were below than the threshold which we used in

our study (NBS<3) at the end of the 7 days of immersion period. Similar to study of Al-Qarni *et al.* (137), red wine was found to be the most colorant solution after 7 days immersion for all acrylic resin groups except CC3.O (Tempo-cad) group in our study.

In the study of Dayan *et al.* (135), the color stability of acrylic resin samples produced by heat-polymerization, autopolymerization, light-polymerization, microwave-polymerization and CAD/CAM system were evaluated when exposed to different agents (distilled water, coffee, coke, red wine). The samples were stored in solutions for 15 minutes twice per day, the solutions were renewed on a daily basis for 30 days. The color measurement data was recorded before and after immersion at 1 week and 1 month in different colorant agents with the CIE L*a*b* color scale by using a spectrophotometer. Similar to the study of Dayan *et al.*, Heraeus Kulzer Paladent 20 was used for heat-cured acrylic resin and Merz M-PM was used for CAD/CAM acrylic resin in our study. Dayan *et al.* (135) reported that the acrylic resin denture base materials showed significantly different discoloration after immersion in the beverages at both evaluation stages. Color change increased over time in all acrylic resin groups. The color stability of the acrylic resin blocks produced by using the CAD/CAM system was better than the other groups. At the end of the test period (30 days) the highest color change was observed in the samples in red wine for both heat-cured and CAD/CAM acrylic resin groups compared to other storage media ($p<0.001$). Similar to our study, a ΔE value of up to 3.3 was established as acceptable in the study of Dayan *et al.*. Contrary to our study, it was stated that the discoloration was clinically acceptable level ($\Delta E<3.3$) after 30 days in both heat-polymerized and CAD/CAM acrylic resin groups stored in distilled water, coffee and red wine in the study of Dayan *et al.*. In our study, the ΔE values of the heat-cured Heraeus Kulzer Paladent 20 samples were 3.25 in coffee and 4.61 in red wine at the end of 336 hours (nearly 1 month immersion). In addition the ΔE values of the CAD/CAM Merz M-PM samples were 3.00 in coffee and 4.39 in red wine. In addition, all acrylic resin samples tested showed clinically marked discoloration at the end of the 1 month of immersion period in our study ($\Delta E>3.3$). In other words, , Dayan *et al.* obtained lower ΔE values compared to the color measurement results of our study at the end of the same immersion period (30 days). This result can be attributed to some differences in the research methodology. All samples were stored in solutions for 15 minutes twice per day in the study of Dayan *et al.* (135). However, unlike the study of Dayan *et al.* , the samples were exposed to colorant solutions except during the washing of the samples for 10 seconds

and keeping the samples in artificial saliva for 30 minutes after color measurement in our study. Therefore, the higher ΔE values in our study can be attributed to the longer immersion time of the samples in colorant solutions compared to the study of Dayan *et al.*.

In the study of Alfouzan *et al.* (139), the color stability of 2 groups of 3D-printed and 1 group of heat-cured acrylic resin samples were evaluated when immersed in different solutions (coffee, lemon juice, coke, and artificial saliva). The samples were thermocycled, brushed mechanically and later immersed in solutions between color measurements. Color measurements of the samples were evaluated with 3D-CIE $L^*a^*b^*$ color space by using a UV light visible spectrophotometer at target (T0), and after one year (T1) and two years (T2) of simulation. The samples were immersed in solutions for 288 hours corresponding to one year of oral exposure (T1). As a result of the Alfouzan *et al.*'s study, regarding the material type, the highest ΔE value was observed in the heat-cured acrylic resin group, and the difference in the ΔE values between all materials tested was statistically significant ($p<0.001$). In addition, the ΔE values of all samples decreased at T2 when comparing to T1, and this difference was statistically significant ($p<0.001$). However, unlike Alfouzan *et al*'s study, the ΔE values of all samples increased in direct proportionally to time in our study. This different result can be attributed to some differences in the research methodology. In the Alfouzan *et al*'s study (139), the samples were subjected to mechanical brushing between color measurements which could have inhibited the adsorption of colorants onto the acrylic resin surfaces.However, no brushing or polishing procedures were performed in our study.

In the study of Alp *et al.* (142), the effect of coffee thermocycling on the color stability of 3 CAD/CAM acrylic resin groups and 1 heat-cured acrylic resin group was evaluated. The color measurements of the samples were calculated by using a noncontact spectroradiometer and color differences were evaluated by using CIEDE2000 color difference formula. Similar to the study of Alp *et al.*, Merz M-PM was used for CAD/CAM acrylic resin in our study. In the study of Alp *et al.* (142), the color change between the heat-polymerized and different prepolymerized CAD/CAM acrylic resin samples were not significantly different in coffee. This finding may be attributed to the 8 hours of polymerization of the heat-polymerized group, which improves physical properties. In addition, all acrylic resins tested demonstrated imperceptible color changes after coffee thermocycling corresponding to 6 months of coffee consumption in Alp *et*

al's study. This different result can be attributed to some differences in the research methodology. In Alp *et al*'s study, the samples were subjected to mechanical brushing 10 times by using toothpaste. While in our study, the samples were kept in artificial saliva for 30 minutes only after each color measurement and no brushing or polishing procedures were performed between the immersion periods.

In the study performed by Gruber *et al.* (140), the color stability of 1 heat-polymerized, 4 CAD/CAM subtractively manufactured and 1 CAD/CAM additively manufactured (3D-printed) groups were analyzed and compared. They made treats the samples to 4 aging processes: thermal cycling, coffee, red wine and distilled water for 30 days. A spectrophotometer was used to evaluate the color change (ΔE). Color measurements were recorded at baseline (T_0) and at the end of the 30 day (T_{30}). Similar to the study of Gruber *et al.*, Merz M-PM was used for CAD/CAM acrylic resin in our study. As a result of the Gruber *et al.*'s study (140), CAD/CAM subtractively manufactured acrylic resin groups were not inferior to the heat-polymerized acrylic resin group in terms of color stability and the ΔE values of the samples tested were below the perceptible threshold which they accepted ($\Delta E \leq 1.2$). No significant differences in the color change were found between the heat-polymerized group and CAD/CAM substractively manufactured groups in coffee and red wine in Gruber *et al.*'s study. In our study, there was significant difference between the heat-cured and the CAD/CAM 2 Merz (CC2.M) acrylic resin group in red wine but not in coffee. In addition, we found out that the ΔE values of both heat-cured and CC2.M group were below the unacceptable threshold in distilled water and coffee solutions ($\Delta E<3.3$, NBS<3).

In our study, it was found that the type of acrylic resin, type of solution and time factor had a significant effect on color change ($p=0.000$). Similar to other studies (11,135,137,142,145), the color change (ΔE) of acrylic resins increased with the progress of immersion time in our study.

In addition, similar to other studies (135,137), the least color change was detected in the samples that were immersed in distilled water and color changes were found as clinically acceptable at the end of the test period in our study. This finding may be attributed to the fact that distilled water does not contain substances which may cause discoloration in acrylic resins. In addition, the pH of distilled water does not cause surface roughness because of neutrality.

In our study, all acrylic resin groups which immersed in tea and coffee demonstrated marked discoloration at the end of the study and the ΔE values of samples ranged from 4.23 to 5.54, 3.48 to 5.25, respectively. As a result of our study, the ΔE values of all acrylic resin samples immersed in tea and coffee solutions were found above the clinically acceptable threshold value (ΔE>3.3, NBS>3). This finding was associated with yellow colorants which includes different polarities in both tea and coffee. In addition, these could be attributed to the fact that coffee and tea are acidic solutions. Especially, tannic acid in coffee and tea causes discoloration (147).

Similar to the study of Singh *et al.* (145), the difference between the ΔE values of samples immersed in tea and coffee was statistically significant for all acrylic resin groups at the end of the test period in our study. Different results for coffee and tea solutions can be explained by the existence of filler particles, which may have acted as a retentive factor for coloring agents (20).

Similar to the study of Zuo *et al.* (11), red wine produced more apparent discoloration, whereas tea and coffee generally lead to an initial increase in discoloration that subsequently decreased in our study. In addition, coffee caused less discoloration than red wine and the difference between the ΔE values of samples immersed in coffee and red wine was statistically significant ($p<0.000$) for all acrylic resin groups at the end of our study. This finding is consistent with the results of similar studies (135,137). The less discoloration of samples in coffee than red wine is possibly defined by the displacement of the coffee layers from the surface of the samples into the solution as they reach a certain thickness (14).

At the end of other studies (135,137) which evaluates the color stability of acrylic denture base resins have reported that red wine causes the highest discoloration compared with other colorant solutions tested, similar to our study. Red wine includes anthocyanidin pigment that can cause discoloration. Anthocyanidins, especially cyanidin-3-glucoside which is a water-soluble component that gives the color of grapes and berries. In addition, the resin structure may be affected by the acidic pH of red wines. Acidity can soften the polymer matrix of resins and leads to surface roughening and causing discoloration (14).

Tendency of acrylic resin materials to staining is mostly associated with the water absorption (14). Tendency of water absorption can likely to induce absorption of other colorant liquids. Absorbed water molecules soften the resin matrix by playing a role of

plasticizer and cause the expansion of polymer matrix and separation of polymer chains. Thus, it leads to penetration of staining solutions to the acrylic resin and discoloration of the materials (14,148).

CAD/CAM acrylic resins has been known to be possessed of high color stability, less porosity and better mechanical properties than the conventional acrylic resins. Polymerization procedure and composition of a resin matrix may have an effect on the stability of the material's color (149,150). The production of acrylic resins which is conventionally fabricated are dependent on the technician, mixing ratio of the resin components, polymerization type and device and polymerization time (151). According to the results of the study of Dayan *et al.* (135), the least color change was observed in CAD/CAM acrylic resin denture base materials in all solutions. These results are partially consistent with our and Gruber *et al.*'s study (140). However, some researchers (137,142) observed that the color change between heat-polymerized and different CAD/CAM acrylic resin samples were not significantly different. This finding may be explaned by the varability of the acrylic resin brands used in the studies.

Although some results of our study are consistent with the previously mentioned studies, our study may have few limitations. First of all, the condition of experiment cannot actually imitate the real intraoral environment. Some conditions which are effect of the saliva composition, the changes of pH, oral hygiene, diet, masticatory function cannot be imitated completely in *in-vitro* studies. Even if, samples are immersed in different solutions stored in an incubator at a 37°C to mimic the intraoral temperature, variations in intraoral temperature should be taken into attention at mealtimes in clinical situations. Moreover, the prolonged static immersion of the acrylic resins in the colorant solutions does not indicate the natural human eating and drinking process, since nutrition is a process in which nutrients are dynamically retained in the oral cavity.In addition, according to Bagheri *et al.* (144), stains are diluted in oral cavity and their effects are reduced due to saliva and other fluids. Therefore, actual staining in the oral cavity occurs after prolonged and intermittent exposure to beverages which can cause discoloration. All limitations taken into account when evaluating the results of all studies on this subject.

The discoloration of all acrylic resin materials are related with diet and poor oral hygiene. Therefore, patients should be careful about the beverages and foods which cause discoloration, and dentists should educate and motivate the patient about the oral hygiene habits to reduce discoloration with recall sessions.

Within the limitations of this *in-vitro* study, the following conclusions could be listed:

1. For both heat-cured and CAD/CAM acrylic resin groups, the daily consuming drinks (water, tea, coffee, red wine) and the immersion time had a significant effect on the color stability of the acrylic resin materials ($p=0.000$).
2. ΔE values for all acrylic resin groups increased proportionally with increasing immersion time.
3. Among all the staining solutions for each time period, distilled water (control group) caused significantly less discoloration in all acrylic resin denture base groups than tea, coffee, and red wine ($p=0.000$).
4. The color change caused by distilled water was found within clinically acceptable limits at the end of the test period ($\Delta E<3.3$ and $NBS<3$).
5. Red wine was found to be the most colorant solution, followed by tea, coffee and distilled water in all groups at the end of the test period.
6. At the end of immersing for 5 weeks (nearly 3 years consumption of drink), all acrylic resin groups indicated clinically marked color change ($3<NBS<6$) when immersed in tea, coffee, and red wine.
7. At the end of the test period, the lowest ΔE value ($\Delta E=3.48$) was found in the CAD/CAM 2 Merz (CC2.M) group immersed in coffee and the highest ΔE value ($\Delta E=6.28$) was found in the CAD/CAM 1 Dentsply (CC1.D) group immersed in red wine among all CAD/CAM acrylic resin groups except distilled water. In addition, for the heat-cured acrylic resin group, the lowest ΔE value was found in the samples immersed in coffee ($\Delta E=3.64$) and the highest ΔE value was found in the samples immersed in red wine ($\Delta E=5.85$). In summary, the color stability of heat-cured acrylic resin was comparable to that of the CAD/CAM acrylic resin.

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7. CURRICULUM VITAE

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