

**EFFECTS OF HYDROGEN PEROXIDE BLEACHING ON HUMAN
DENTIN AND ENAMEL MICROSTRUCTURE AND FUNCTION**

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ABSTRACT

EFFECTS OF HYDROGEN PEROXIDE BLEACHING ON HUMAN DENTIN AND ENAMEL MICROSTRUCTURE AND FUNCTION

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In recent years bleaching of vital teeth has become popular among both dentist and patients. Different bleaching agents were used for this purpose. They are either applied professionally at high dose (office bleaching) or by patient at lower dose (home bleaching).

In the present work we studied the effects of a high concentration bleaching agent (35% hydrogen peroxide), and a low concentration bleaching agent (17% carbamide peroxide) on human enamel and dentin using Fourier Transform Infrared (FTIR) Spectroscopic Technique.

The OH stretching band of hydroxyapatite at 3567 cm^{-1} appeared in the spectra of enamel tissue which was absent in dentin. Carbamide peroxide and hydrogen peroxide treatment induced some differences in enamel and dentin

spectra according to control group spectra. While the treatment of hydrogen peroxide led to significant decrease in mineral to matrix ratio, carbamide peroxide treatment did not cause decrease in this level. In addition, organic components of the teeth were changed after the treatment of bleaching agents. The frequency of the Amide A and Amide I bands were significantly changed for enamel tissue after the treatment of high concentration bleaching agent. However, it is observed that these changes result from two different bleaching methods are negligible in dentin tissue.

In conclusion it can be stated that hydrogen peroxide treatment caused dramatic changes in enamel structure according to carbamide peroxide treatment, but two methods did not cause significant changes in dentin tissue.

Key Words: Office bleaching; Home bleaching; Tooth whitening; Hydrogen peroxide; Carbamide peroxide; Enamel; Dentin; FTIR Spectroscopy.

ÖZ

HİDROJEN PEROKSİT DİŞ BEYAZLATMA METODUNUN İNSAN DENTİN VE MİNESİNİN MİKROYAPI VE FONKSİYONU ÜZERİNE ETKİLERİ

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Son yıllarda diş beyazlatma diş hekimleri ve hastalar arasında popüler bir duruma gelmiştir. Diş beyazlatma amacıyla farklı maddeler kullanılmaktadır. Bu maddeler profesyonel olarak yüksek konsantrasyonda (klinik tipi beyazlatma yöntemi), ya da hastalar tarafından bireysel olarak düşük konsantrasyonda (ev tipi beyazlatma yöntemi) uygulanır.

Bu çalışmada yüksek konsantrasyonlu bir diş beyazlatma maddesinin (%35 Hidrojen Peroksit) ve düşük konsantrasyonlu bir diş beyazlatma maddesinin (17% Karbamid Peroksit) insan mine ve dentin dokusu üzerine etkileri Fourier Dönüşüm Kızılötesi Spektroskopi Tekniği kullanılarak araştırıldı.

Mine spektrumlarında hidroksiapatit OH gerilme bandı 3567cm^{-1} de görülürken bu band dentin spektrumlarında gözlenemedi. Karbamid peroksit ve hidrojen peroksit uygulanması mine ve dentin spektrumlarında kontrol grubu spektrumlarına göre çeşitli farklılıklar gösterdi. Mine dokusunda mineral matriks oranı hidrojen peroksit uygulanmasıyla birlikte anlamlı bir biçimde düşerken, karbamid peroksit uygulanması bu seviyede bir düşüşe sebep olmadı. Ayrıca, beyazlatma maddelerinin uygulanmasından sonra dişlerin organik yapısı değişiklik gösterdi. Mine dokusunda Amid A ve Amid I bandlarının frekansları yüksek konsantrasyonlu beyazlatma maddesinin uygulanmasından sonra anlamlı bir değişim gösterdi. Fakat iki farklı beyazlatma metodunun sebep olduğu bu değişimlerin dentinde ihmal edilebilecek kadar düşük seviyede olduğu gözlemlendi.

Sonuç olarak Hidrojen Peroksit uygulanmasının Karbamid Peroksit uygulanmasına göre minede yapısal olarak daha fazla değişime sebep olduğu fakat dentin dokusunda her iki maddenin de anlamlı bir değişime sebep olmadığı söylenebilir.

Anahtar Kelimeler: Klinik tipi beyazlatma; Ev tipi beyazlatma; Diş beyazlatma; Hidrojen peroksit; Karbamid Peroksit; Mine; Dentin; FTIR Spektroskopisi.

To My Parents

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CHAPTER 1

INTRODUCTION

1.1 SCOPE AND THE AIM OF THIS STUDY

Tooth discoloration is a common dental complaint and may be caused by superficial staining from dietary sources such as tea, coffee and red wine, or chemicals such as chlorhexidine found in mouth rinses. However, tooth discoloration may also be caused by dental pathology of developmental origin or environmental factors including tetracycline staining, dental caries and pulp necrosis (Walsh et al., in press). In addition to these factors aging is effective on discoloration of teeth (Goldstein and Garber, 1995). The management of tooth discoloration is as equally varied as its causes, ranging from the removal of superficial staining by polishing with an abrasive paste, microabrasion, bleaching of vital and non-vital teeth, to more destructive forms of treatment such as veneers or crowns (Walsh et al., in press). The concept of this study is limited with two kinds of vital bleaching of teeth: In-office bleaching and home bleaching.

The technique of bleaching or whitening teeth was first described in 1877 by Chapple (Greenwall, 2001). Since then bleaching of teeth has been in use with little change in science or technique during that time (Haywood, 2000). For example in 1937, Ames described a technique for treating mottled enamel

by using a combination of hydrogen peroxide, ether, and heat (Ames, 1937). However current in-office bleaching technique is basically the same as the technique developed between 1880-1916, which uses 35% hydrogen peroxide with rubber dam isolation.

In-office bleaching was never very popular because of the danger to the patient and the dentist, the amount of time it took to do it, and the cost to the patient. The danger to the patient is the potential for tissue burns from the 35% hydrogen peroxide used, which can result from a leaky rubber dam or improper isolation technique. There are also questions about dangers to the pulp related to certain lights used and the heat generated (Haywood, 2000). With the introduction of home bleaching tooth whitening has gained the great popularity and reached the more people in population.

Previous studies on teeth bleaching show controversial results with each other. In one study, treatment of a home bleaching product containing 10% carbamide peroxide resulted in a nonsignificant reduction in microhardness values of human enamel when compared with values of the control nontreated specimens (Araujo et al., 2003). In another research, bleaching enamel with carbamide peroxide could significantly reduce its ultimate tensile strength within a 14 days treatment (Cavalli et al., 2004). Controversial results are also present in safety of high concentration bleaching agents. Using one of the highest concentrations of hydrogen peroxide for tooth bleaching procedures and maximum likely peroxide exposure, there was no evidence of deleterious effects on enamel or dentin (Sulieman et al., 2004). However, another study reported that bleaching of teeth with 30% hydrogen peroxide the mechanical properties of dentin were significantly decreased (Nizam et al., in press). In addition most studies have concentrated on the use of 10% CP, which is equivalent to only 3.35% HP. (Sulieman et al., 2004). In the present work we aimed to study the effect of a high concentration bleaching agent (35% hydrogen peroxide) and a low

concentration bleaching agent (17% carbamide peroxide) on human enamel and dentin composition using Fourier Transform Infrared (FTIR) Spectroscopic Technique. We used FTIR spectroscopy because with this technique biological systems can be observed in molecular level without any damage in structural components (Boyar and Severcan, 1997; Melin et al., 2000; Severcan et al., 2000; Melin et al., 2001). In addition FTIR spectroscopy allows to examine the relative amount of minerals and matrix content and the arrangement of apatite and organic matrix. Therefore, FTIR spectroscopy should become an important tool, because the relative amount of minerals and the arrangement of apatite and organic matrix could be a measure for evaluating bone quality (Takata et al., 2004).

1.2 TOOTH ANATOMY

The teeth develop in sockets along the alveolar borders of the mandibular and maxillary bones. Teeth are unique structures in that two sets form during development. The members of the first set, the primary teeth or deciduous teeth (Figure 1), usually erupt through the gums (gingiva) at regular intervals between the ages of 6 months and 2 years. There are twenty primary teeth-ten in each jaw- and they occur from the midline toward the sides in the following sequence: central incisor, lateral incisor, cuspid (canine), first molar, and second molar (Hole and Koos, 1994).

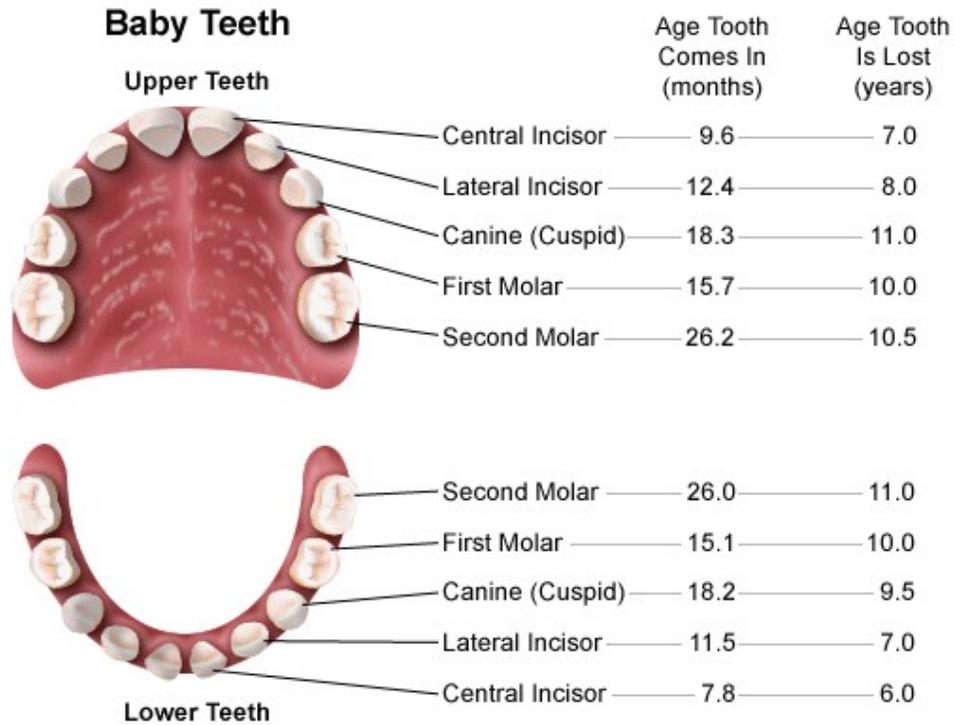


Figure 1 Dental Anatomy of a Baby (MUSC Children’s Hospital, http://www.musckids.com/health_library/dental/teethanat.htm, in visited June 2005).

These teeth will be replaced by the adult secondary dentition or permanent dentition (Figure 2 and 3). The larger adult jaws can accommodate more than 20 permanent teeth, and three additional molars appear on each side of the upper and lower jaws as the individual ages. These teeth extend the length of the tooth rows posteriorly and bring the permanent tooth count to 32 (Martini et al., 2000).

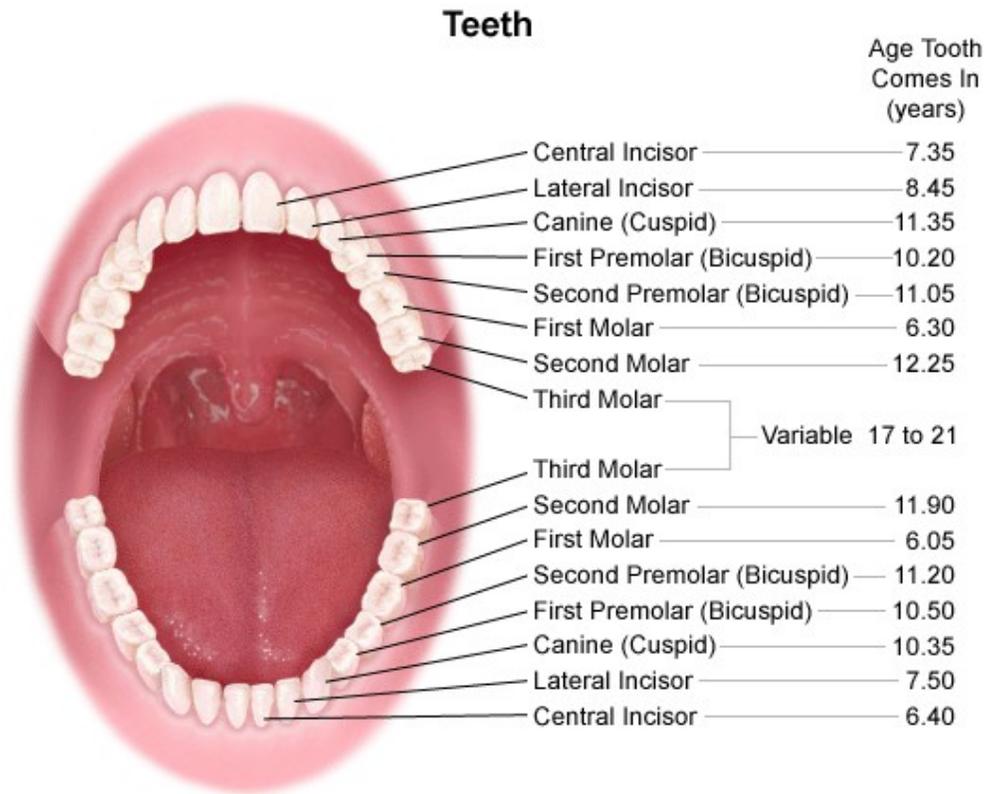


Figure 2 Dental anatomy of an adult (MUSC Children's Hospital, http://www.musckids.com/health_library/dental/teethanat.htm, in visited June 2005).

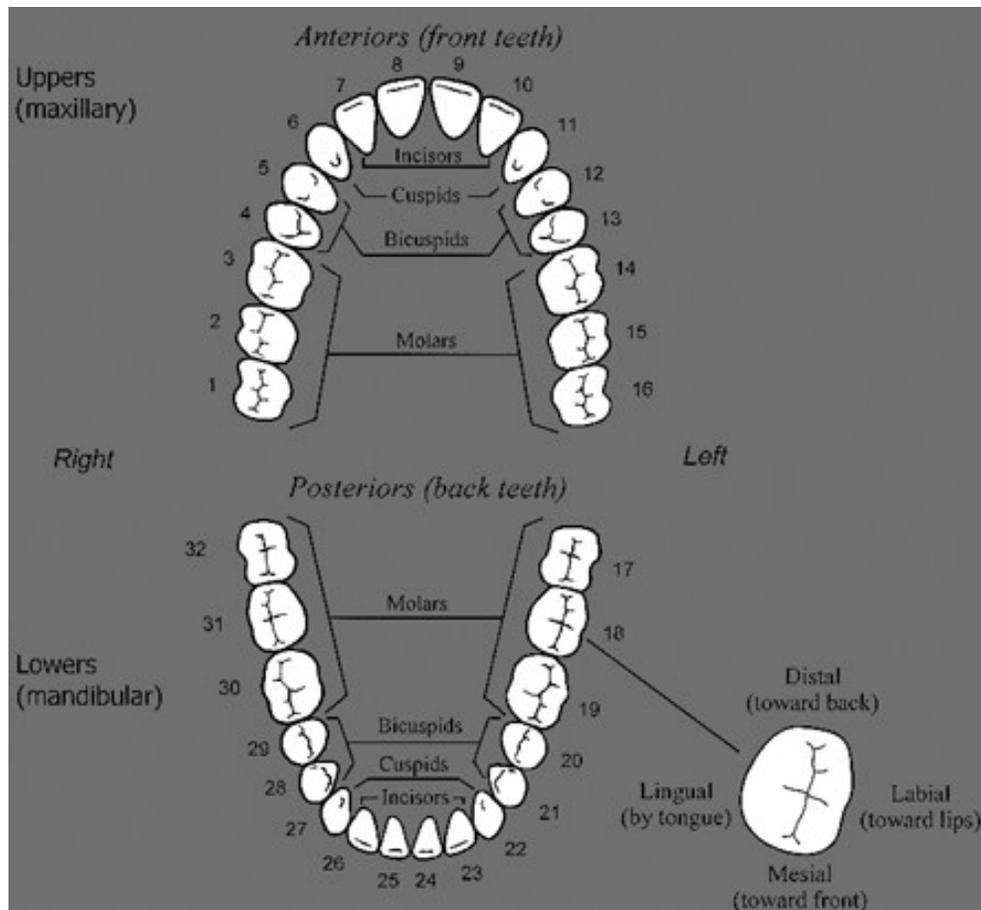


Figure 3 Detailed dental anatomy of humans (MindSpring Internet Service, <http://www.mindspring.com/~adamys/tooth%20anatomy.htm>, in visited June 2005).

Humans and other mammals have heterodont dentition. This means that they have various types of teeth that are adapted to handle food in particular ways (Graaff, 1998). Each of the four types of teeth has a specific function: (1) Incisors, blade-shaped teeth found at the front of the mouth, are useful for clipping or cutting, as when nipping off the tip of a carrot. (2) Cuspids or canines, are conical with sharp ridgeline and pointed tip. They are used for tearing or slashing. (3) Bicuspids or premolars and (4) molars have flattened crowns with prominent ridges. They are used for crushing, mashing, and grinding (Martini and Bartholomew, 2003).

Bleaching agents are applied to incisors, cuspids, or bicuspid(premolars). That is, bleaching process contains the teeth appeared when a person smiles. Therefore we used these types of teeth in our study to examine the effects of bleaching agents on human teeth.

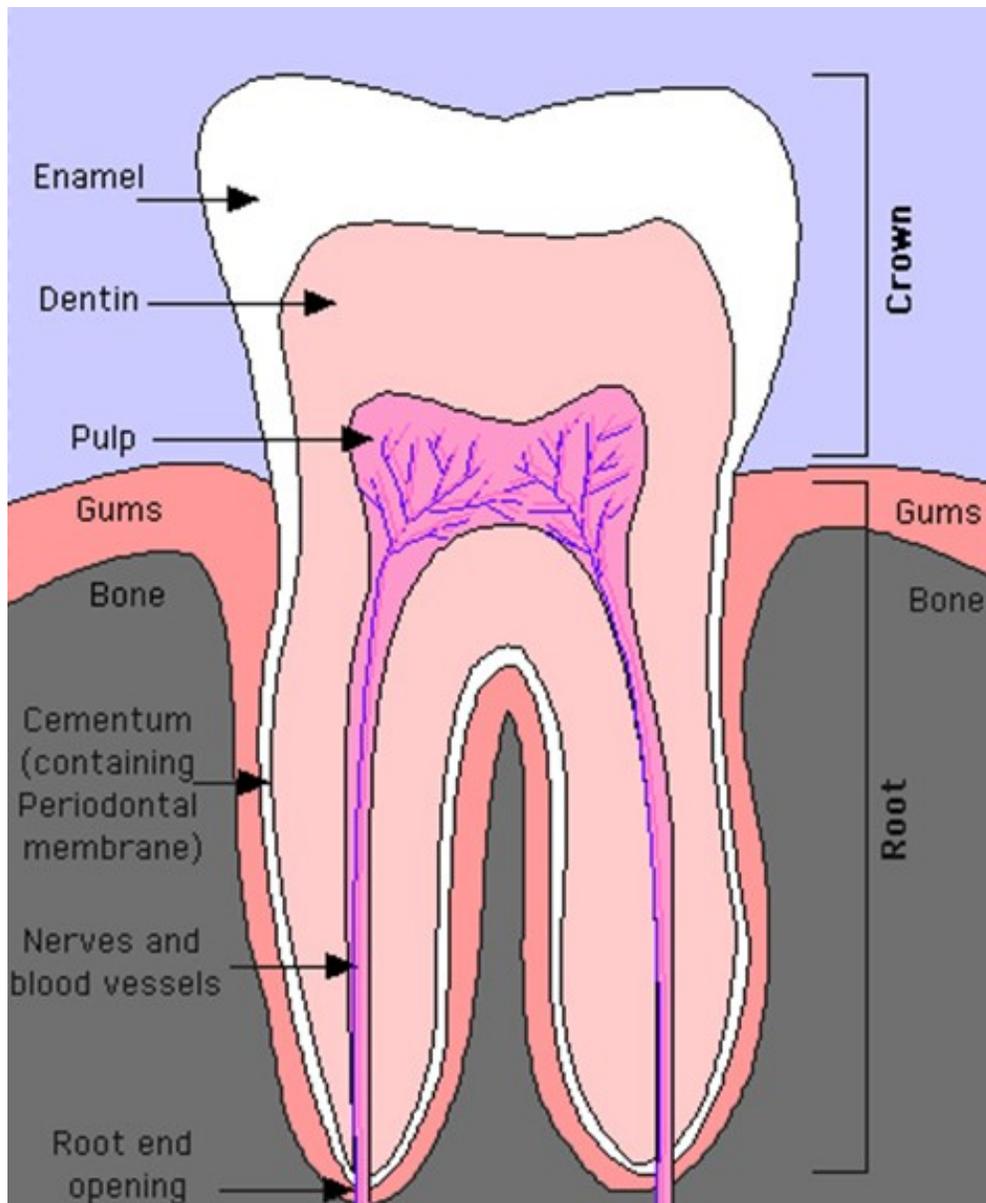


Figure 4 Tooth anatomy (Enchanted Learning, <http://www.enchantedlearning.com/subjects/anatomy/teeth/toothanatomy.shtm>, in visited June 2005).

Figure 4 shows the parts of a tooth. The crown is supported by a neck that is anchored firmly into the jaw by one or more roots (Graaff, 1998). The neck of the tooth marks the boundary between the root and the crown. The crown is covered by a layer of enamel, which contains a crystalline form of calcium phosphate, the hardest biologically manufactured substance. Adequate amounts of calcium, phosphates and vitamin D₃ during childhood are essential if the enamel coating is to be complete and resistant to decay (Martini and Bartholomew, 2003). The bulk of a tooth beneath the enamel is composed of dentin, a substance much like bone, but somewhat harder (Hole and Koos, 1994). Dentin differs from bone also in that it does not contain living cells. Instead, cytoplasmic processes extend into the dentin from cells within the central pulp cavity (Martini and Bartholomew, 2003). The dentin surrounds the tooth's central cavity (pulp cavity) which contains blood vessels, nerves, and connective tissue (pulp). The blood vessels and nerves reach this cavity through tubular root canals that extend upward into the root (Hole and Koos, 1994). The roots of teeth fit into sockets, called dental alveoli, in the alveolar processes of the mandible and maxillae. Each socket is lined with a connective tissue periosteum, specifically called the periodontal membrane. The root of a tooth is covered with a bonelike material called the cementum; fibers in the periodontal membrane insert into the cementum and fasten the tooth in its dental alveolus (Graaff, 1998). Cementum also resembles bone, but it is softer, and remodeling does not occur following its deposition. Where the tooth penetrates the gum surface, epithelial cells form tight attachments to the tooth and prevents bacterial access to the easily eroded cementum of the root (Martini and Bartholomew, 2003).

Of the substructures of the tooth, enamel and dentin were considered for the effects of bleaching agents on these tooth parts in this study.

1.3 TOOTH BLEACHING

1.3.1 Causes of Tooth Discoloration

Teeth can become stained and discolored, sometimes before they even erupt, when the tooth structure itself is altered by a discoloring agent. This happens in a variety of ways.

Administered substances for treatment of some diseases can cause tooth discoloration. The first certain identification was reported in a study of cystic fibrosis patients, for whom tetracycline was (and unfortunately remains) one of the most effective treatments for control of secondary infection of the respiratory system. The severity of the stains and specific color depend on the type of tetracycline administered (more than 2000 variants have been patented), the duration of use, and the stage of tooth formation at the time use. In fact, tetracycline provided dentists much insight into the mechanism by which medications could result in intrinsic stains. Tetracycline is still used, especially for Rocky Mountain spotted fever, chronic bronchitis, and cystic fibrosis, but the Food and Drug Administration has warned for more than 30 years about its use for pregnant women or children when not absolutely necessary (Goldstein and Garber, 1995).

Such studies show that excessive intake of fluoride during enamel formation and calcification results in discoloration and surface defects (Murrin and Barkmeier, 1982; Goldstein, 1987). The type and degree of fluorosis problems depend on genetic vulnerability, the point of development of the enamel at which excessive fluoride intake occurs, and the intensity and length of exposure. The premolar teeth are the most commonly affected, followed by second molars, maxillary incisors, canines and first molars, and mandibular incisors. Where fluoride concentration is very high, primary teeth may also be affected.

Stains can result from systemic conditions. Although there are a number of genetic conditions or childhood illnesses that cause discoloration of the teeth, most are rare and infrequently seen. These conditions include the bluish-green or brown primary teeth that result from postnatal dentin staining by bilirubin in children who suffered severe jaundice as infants; the characteristically brownish teeth caused by destruction of an excessive number of blood-cell erythrocytes in erythroblastosis fetalis, a result of Rh-factor incompatibility between mother and fetus; and the purplish-brown teeth of persons with porphyria, a rare condition that causes an excess production of pigment.

Stain can result from dental conditions or treatments. Dental carries are a primary cause of pigmentation, appearing as either an opaque white halo or gray cast. An even deeper brown to black discoloration can result from bacterial degradation of food debris in areas of tooth decay. Tooth-colored restorations such as acrylics, glass ionomers, or composites can cause teeth to look grayer and discolored as the restoration ages and degrades. Metal restorations, even silver amalgams and gold inlays, can reflect discoloration through the enamel, a problem that may become more evident with the thinning and translucency of enamel that occurs with aging. A more difficult discoloration occurs when oils, iodines, nitrates, root-canal sealers, pins, and other materials used in dental restoration have penetrated the dentinal tubules.

Tooth color changes due to aging. Unfortunately, all of the numerous genetic, environmental, medical, and dental causes described above move teeth further away from that ideal, and aging intensifies all of their effects. Food and drink have a cumulative staining effect, and these and other stains become even more visible in the older patient because of the inevitable cracking and other changes on the enamel surface of the tooth, within its crystalline structure, and in the underlying dentin. Furthermore, amalgams

and other restorations placed years ago inevitably degrade over time, causing further staining (Goldstein and Garber, 1995).

The management of tooth discoloration is as equally varied as its causes, ranging from the removal of superficial staining by polishing with an abrasive paste, microabrasion, bleaching of vital and non-vital teeth, to more destructive forms of treatment such as veneers or crowns (Walsh et al., in press). The concept of this study is limited with the vital bleaching of teeth.

1.3.2 History of Tooth Bleaching

The technique of bleaching or whitening teeth was first described in 1877 (Greenwall, 2001). Since then bleaching of teeth has been in use with little change in science or technique during that time (Haywood, 2000). For example in 1937, Ames described a technique for treating mottled enamel by using a combination of hydrogen peroxide, ether, and heat (Ames, 1937). However current in-office bleaching technique is basically the same as the technique developed between 1880-1916, which uses 35% hydrogen peroxide with rubber dam isolation (Haywood, 2000).

The history of “modern day” tooth bleaching, however, began in 1989. In 1989 Haywood and Heyman introduced the nightguard vital bleaching method (Haywood and Heyman 1989). However, the technique actually originated over 20 years earlier. The nightguard vital bleaching technique was discovered accidentally like many famous discoveries. Dr Bill Klusmier, an orthodontist in Fort Smith, Arkansas, was completing treatment of a patient who sustained trauma to the mouth. The patient was in the retention phase of the orthodontic treatment and periodically was wearing an orthodontic positioner (similar to a custom-fitted nightguard). In an effort to facilitate tissue healing, Dr Klusmier instructed his patient to place an over-the-counter oral antiseptic, Gly-oxide (Marion Merrell Dow), containing 10% carbamide

peroxide into the orthodontic positioner at night. Dr Klusmier noted a significant improvement in tissue health, but more importantly, discovered that the patient also returned with lighter teeth after an extended period of time. This event led to researches and development of nightguard bleaching technique. In addition to Dr Klusmier, Dr Jerry Wagner, Dr Tom Austin, Dr David Freshwater, contributed to development of nightguard vital bleaching technique, till Haywood and Heyman introduced the nightguard vital bleaching method with the publication of the first article on this technique (Goldstein and Garber, 1995). When home 'nightguard' bleaching using carbamide peroxide was introduced in 1989, it appeared that the in-office approach would become less popular. However, at the present time there has been a recent resurgence in-office bleaching, primarily due to aggressive marketing of various 'high tech' light sources such as lasers and plasma arc lights, coupled with claims of reducing bleaching time, even to a single office visit (Haywood, 2000).

1.3.3 Mechanism of Tooth Bleaching

Although process of in-office bleaching and home bleaching are different, they are based on hydrogen peroxide as an active agent for bleaching. However, concentrations of hydrogen peroxide in home bleaching and in-office bleaching are different. While a 10% carbamide peroxide bleaching product contains 7% urea and 3% hydrogen peroxide, an in-office bleaching product contains 35% hydrogen peroxide (Haywood, 2000). The mechanism of the action of bleaching agents is thought to be due to the ability of hydrogen peroxide to form oxygen free radicals that interact with adsorbed colored organic molecules and oxidize these macromolecules and pigment stains, producing dental discoloration into smaller and lighter molecules (Cavalli et al., 2004).

1.3.3.1 In-Office Bleaching (Hydrogen Peroxide Bleaching)

Hydrogen peroxide is an oxidizing agent and has the ability to produce free radicals, $\text{HO}_2\cdot + \text{O}\cdot$, which are very reactive ($\text{HO}_2\cdot$ is the stronger free radical). In pure aqueous form, hydrogen peroxide is weakly acidic (to reduce breakdown and extend shelf life) and ionizes as shown in Fig 5.

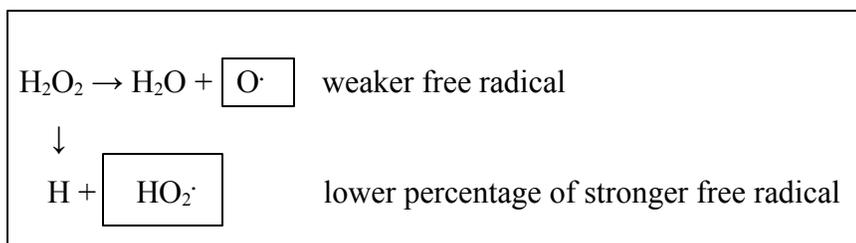


Figure 5 Ionization of hydrogen peroxide at acidic pH.

The result is that a larger proportion of the weaker free radical $\text{O}\cdot$ is produced. The perhydroxyl $\text{HO}_2\cdot$ is the more potent free radical. In order to promote the formation of $\text{HO}_2\cdot$ ions the H_2O_2 needs to be made alkaline. The optimum pH for this to occur is at pH of 9.5 to 10.8 (Goldstein and Garber, 1995)

In the ionization of buffered hydrogen peroxide in this pH range, a greater amount of perhydroxyl $\text{HO}_2\cdot$ free radicals are produced, which results in a greater bleaching effect in the same time as at other pH levels (Frysh et al., 1993; Zaragoza, 1984). Thus, hydrogen peroxide is most effective between pH 9.5 and pH 10.8.

In the presence of some decomposition catalysts and enzymes, the hydrogen peroxide can not produce free radicals thus hydrogen peroxide becomes ineffective as a bleaching agent. These enzymes, some of which are present in the mouth, are an important part of the body's defense against oxygen toxicity. It is thus important to have teeth dry and free of debris when applying a bleaching agent (Goldstein and Garber, 1995).

1.3.3.2 Home Bleaching (Carbamide Peroxide Bleaching)

In dental bleaching, carbamide peroxide is usually used at a concentration of 10% to 15% (Goldstein and Garber, 1995). The original at-home bleaching products used a 10% solution of carbamide peroxide as the bleaching agent, which are basically 3% hydrogen peroxide and 7% urea. The urea in carbamide peroxide primarily acts as a stabilizer to give these products a longer shelf life, slower release of the hydrogen peroxide, and it has additional cariostatic benefits. Hydrogen peroxide penetrates the tooth more quickly than carbamide peroxide. The basic mechanism of action is the same, but the formulation affects shelf life and time required for penetration of the tooth (Haywood, 2003). Carbamide peroxide breaks down as shown in Fig 6.

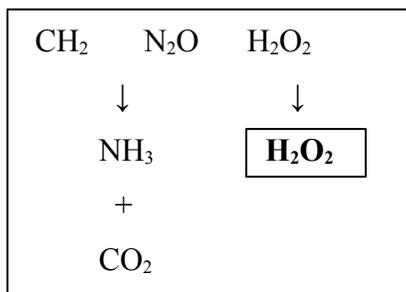


Figure 6 Chemical breakdown of carbamide peroxide (Goldstein and Garber, 1995).

Carbamide peroxide dissociates into hydrogen peroxide and urea when in contact with soft-tissues or saliva at oral temperatures (Cavalli et al., 2004). The resulting hydrogen peroxide then ionizes as shown in Fig 5 (Goldstein and Garber, 1995). On the other hand, urea degrades into ammonia and carbon dioxide (Haywood and Heymann, 1991).

1.4 BASIC PRINCIPLES OF OPTICAL SPECTROSCOPY

Light, in its wave aspect, consists of mutually perpendicular electric and magnetic fields, which oscillate sinusoidally as they are propagated through space (Figure 7).

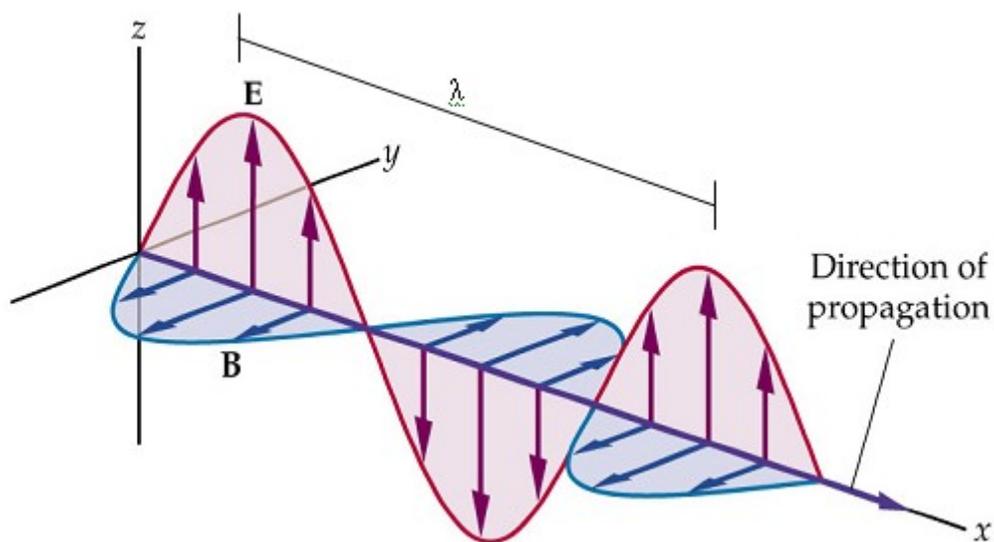


Figure 7 Propagation of an electromagnetic wave through space. The E and B vectors are mutually perpendicular at all times.

The energy E of the wave is

$$E = h \frac{c}{\lambda} = h\nu$$

In which h is Planck's constant, c is the velocity of light, λ is the wavelength, and ν is the frequency. When such a wave encounters a molecule, it can be either scattered (i.e., its direction of propagation changes) or absorbed (i.e., its energy is transferred to the molecule). A molecule can have various energy levels including vibrational energy levels.

The absorption of energy is most probable only if the amount absorbed corresponds to the difference between energy levels. This can be expressed by stating that light of wavelength λ can be absorbed only if

$$\lambda = hc / E_2 - E_1$$

in which E_1 is the energy level of the molecule before absorption and E_2 is an energy level reached by absorption.

A change between energy levels is called a transition. A plot of the probability of absorption versus wavelength is called an absorption spectrum and absorption spectroscopy refers to the gathering and analysis of absorption data.

For most molecules, the wavelengths corresponding to transitions between the ground state and any vibrational level of the first excited state fall in the range of ultraviolet and visible light. Low-energy transitions are also possible between vibrational levels within a single electronic level. These transitions produce radiation in the infrared range (Freifelder, 1982). Figure 8 shows the part of the electromagnetic spectrum producing radiation in different frequency ranges.

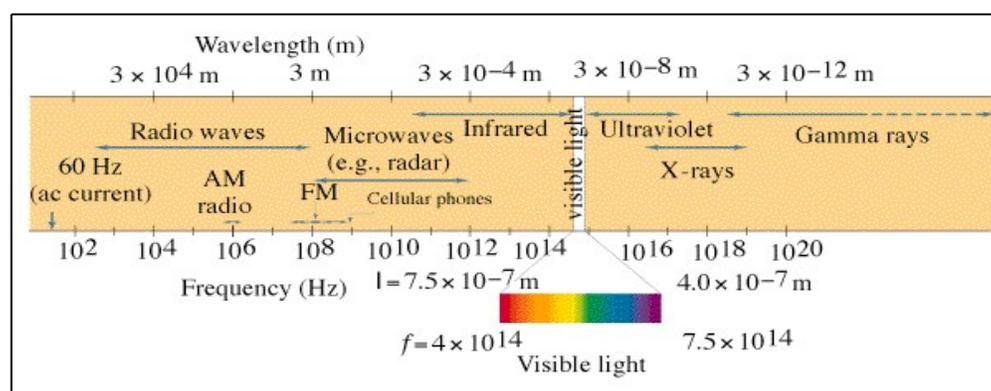


Figure 8 The part of the electromagnetic spectrum.

1.4.1 Infrared Spectroscopy

1.4.1.1 Basis of Infrared Spectroscopy

Transitions between vibrational levels of the ground state of a molecule result from the absorption of light in the infrared (IR) region: from 10^3 nm to 10^5 nm (Figure 8). These vibrational levels and hence, infrared spectra are generated by the characteristic motions (bond stretching, bond bending, and more complex motions) of various functional groups (e.g., methyl, carbonyl, amide, etc.). The value of infrared spectral analysis comes from the fact that the modes of vibration of each group are very sensitive to changes in chemical structure, conformation, and environment (Freifelder, 1982). There are several types of vibrations, the two most basic vibrational modes are stretching and bending. Figure 9 shows the major vibrational modes of water (Stuart, 1997).

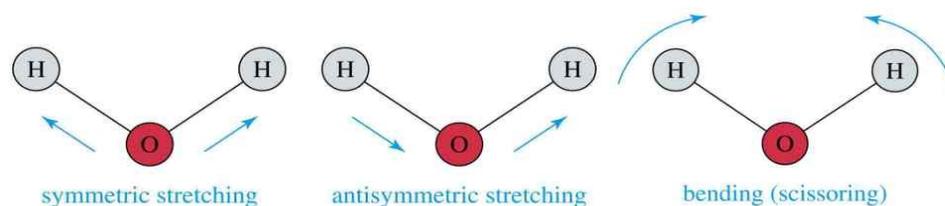


Figure 9 Major vibrational modes of H₂O

(<http://faculty.ccri.edu/wsuits/MolecularStructureandOrganicChemistry.ppt>)

In simple terms, IR spectra are obtained by detecting changes in transmittance (or absorption) intensity as a function of frequency. Most commercial instruments separate and measure IR radiation using dispersive spectrometers or Fourier transform spectrometers.

Infrared radiation spans a section of the electromagnetic spectrum having wavenumbers from roughly 13,000 to 10 cm^{-1} , or wavelengths from 0.78 to

1000 μm . It is bound by the red end of the visible region at high frequencies and the microwave region at low frequencies.

IR absorption positions are generally presented as wavenumbers (ν). Wavenumber defines the number of waves per unit length. Thus, wavenumbers are directly proportional to frequency, as well as the energy of the IR absorption. The wavenumber unit (cm^{-1} , reciprocal centimeter) is more commonly used in modern IR instruments that are linear in the cm^{-1} scale. Wavenumbers and wavelengths can be interconverted using the following equation:

$$\nu \text{ (in } \text{cm}^{-1}\text{)} = 1 / \lambda \text{ (in } \text{cm}^{-1}\text{)} \times 10^4$$

IR absorption information is generally presented in the form of a spectrum with wavenumber as the x-axis and absorption intensity or percent transmittance as the y-axis. Transmittance, T, is the ratio of radiant power transmitted by the sample (I) to the radiant power incident on the sample (I_0). Absorbance (A) is the logarithm to the base 10 of the reciprocal of the transmittance (T).

$$A = \log_{10} (1/T) = -\log_{10} T = -\log_{10} I/I_0$$

The IR region is commonly divided into three smaller areas: near IR, mid IR, and far IR.

	Near IR	Mid IR	Far IR
Wavenumber	13,000–4,000 cm^{-1}	4,000–200 cm^{-1}	200–10 cm^{-1}
Wavelength	0.78–2.5 μm	2.5–50 μm	50–1,000 μm

Most frequently used region is mid IR region, between 4000 and 400 cm^{-1} (2.5 to 25 μm).

Most commercial instruments separate and measure IR radiation using dispersive spectrometers or Fourier transform spectrometers. However, Fourier transform spectrometers have recently replaced dispersive instruments due to their distinct advantages over dispersive spectrometers:

- Better speed and sensitivity (Fellgett advantage). A complete spectrum can be obtained during a single scan of the moving mirror, while the detector observes all frequencies simultaneously.
- Increased optical throughput (Jacquinot advantage). Energy-wasting slits are not required in the interferometer because dispersion or filtering is not needed. Instead, a circular optical aperture is commonly used in FTIR systems. The beam area of an FT instrument is usually 75 to 100 times larger than the slit width of a dispersive spectrometer. Thus, more radiation energy is made available. This constitutes a major advantage for many samples or sampling techniques that are energy-limited.
- Internal laser reference (Connes advantage). The use of a helium neon laser as the internal reference in many FTIR systems provides an automatic calibration in an accuracy of better than 0.01 cm^{-1} . This eliminates the need for external calibrations.
- Simpler mechanical design. There is only one moving part, the moving mirror, resulting in less wear and better reliability.
- Elimination of stray light and emission contributions. The interferometer in FTIR modulates all the frequencies. The unmodulated stray light and sample emissions (if any) are not detected.
- Powerful data station. Modern FTIR spectrometers are usually equipped with a powerful, computerized data system. It can perform a wide variety of

data processing tasks such as Fourier transformation, interactive spectral subtraction, baseline correction, smoothing, integration, and library searching. Although the spectra of many samples can be satisfactorily run on either FTIR or dispersive instruments, FTIR spectrometers are the preferred choice for samples that are energy-limited or when increased sensitivity is desired. A wide range of sampling accessories is available to take advantage of the capabilities of FTIR instruments (Settle, 1997).

1.4.1.2 Fourier Transform Infrared Spectroscopy

In recent years, Fourier-transform infrared (FT-IR) spectroscopy has found increasing favour in laboratories. This more recent method is based on the old idea of the interference of radiation between two beams to yield an interferogram. An interferogram is a signal produced as a function of the change of pathlength between the two beams. The two domains of distance and frequency are interconvertible by the mathematical method of Fourier transformation. Although the basic optical components of FT-IR instruments, namely the Michelson interferometer, has been known for almost a century, it was not until recent advances in computing that the technique could be successfully applied (Stuart, 1997).

There are three basic spectrometer components in an FT system: radiation source, interferometer, and detector. A simplified optical layout of a typical FTIR spectrometer is illustrated in Fig 10.

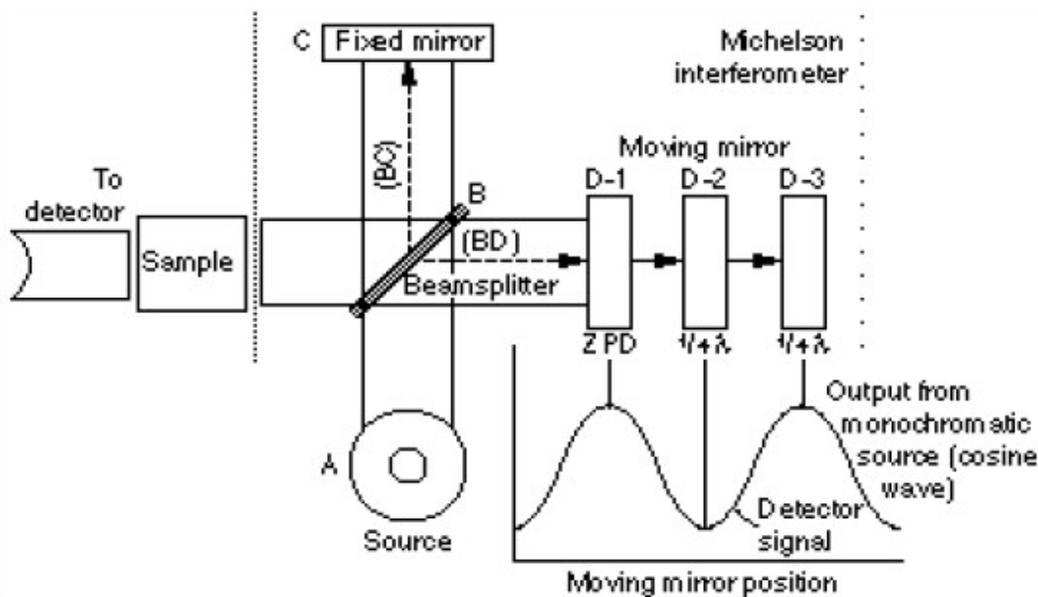


Figure 10 Simplified optical layout of a typical FTIR spectrometer.

The monochromator is replaced by an interferometer, which divides radiant beams, generates an optical path difference between the beams, then recombines them in order to produce repetitive interference signals measured as a function of optical path difference by a detector. As its name implies, the interferometer produces interference signals, which contain infrared spectral information generated after passing through a sample. The most commonly used interferometer is a Michelson interferometer. It consists of three active components: a moving mirror, a fixed mirror, and a beamsplitter (Fig. 10). The two mirrors are perpendicular to each other. The beamsplitter is a semireflecting device and is often made by depositing a thin film of germanium onto a flat KBr substrate. Radiation from the broadband IR source is collimated and directed into the interferometer, and impinges on the beamsplitter. At the beamsplitter, half the IR beam is transmitted to the fixed mirror and the remaining half is reflected to the moving mirror. After the divided beams are reflected from the two mirrors, they are recombined at the beamsplitter. Due to changes in the relative position of the moving mirror to

the fixed mirror, an interference pattern is generated. The resulting beam then passes through the sample and is eventually focused on the detector.

For an easier explanation, the detector response for a single-frequency component from the IR source is first considered. This simulates an idealized situation where the source is monochromatic, such as a laser source. As previously described, differences in the optical paths between the two split beams are created by varying the relative position of moving mirror to the fixed mirror. If the two arms of the interferometer are of equal length, the two split beams travel through the exact same path length. The two beams are totally in phase with each other; thus, they interfere constructively and lead to a maximum in the detector response. This position of the moving mirror is called the point of zero path difference (ZPD). When the moving mirror travels in either direction by the distance $\lambda/4$, the optical path (beamsplitter–mirror–beamsplitter) is changed by $2(\lambda/4)$, or $\lambda/2$. The two beams are 180° out of phase with each other, and thus interfere destructively. As the moving mirror travels another $\lambda/4$, the optical path difference is now $2(\lambda/2)$, or λ . The two beams are again in phase with each other and result in another constructive interference.

When the mirror is moved at a constant velocity, the intensity of radiation reaching the detector varies in a sinusoidal manner to produce the interferogram output shown in Fig. 10. The interferogram is the record of the interference signal. It is actually a time domain spectrum and records the detector response changes versus time within the mirror scan. If the sample happens to absorb at this frequency, the amplitude of the sinusoidal wave is reduced by an amount proportional to the amount of sample in the beam.

The interferogram contains information over the entire IR region to which the detector is responsive. A mathematical operation known as Fourier transformation converts the interferogram (a time domain spectrum

displaying intensity versus time within the mirror scan) to the final IR spectrum, which is the familiar frequency domain spectrum showing intensity versus frequency. This also explains how the term Fourier transform infrared spectrometry is created (Settle, 1997).

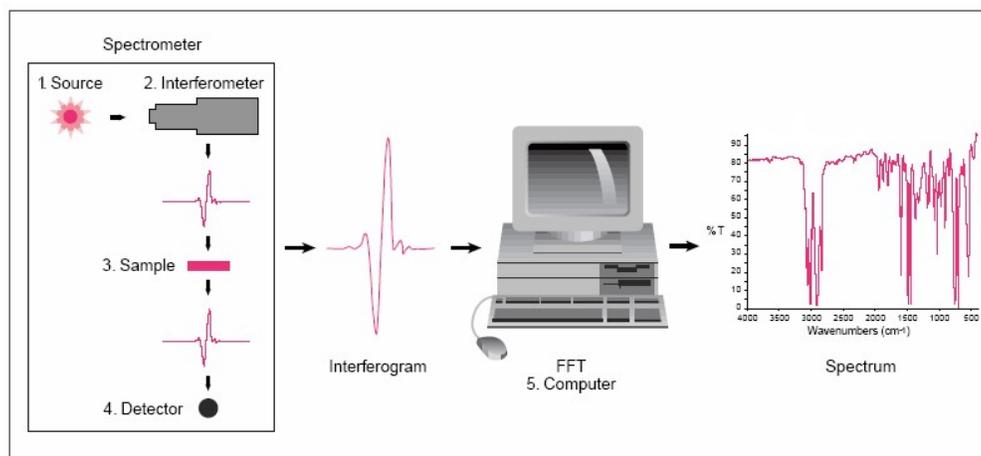


Figure 11 Schematic representation of obtaining an FTIR spectrum.
(http://mmrc.caltech.edu/mmrc_html/FTIR/FTIRintro.pdf)

CHAPTER 2

MATERIALS AND METHODS

2.1 CHEMICALS

2.1.1 Acrylic

This chemical was used to obtain sections from teeth samples. Teeth samples were embedded in acrylic and under appropriate pressure (~200 bars) for nearly 5 minutes acrylic transformed into rigid structure which we could take sections from.

2.1.2 Liquid Nitrogen

Liquid nitrogen at -196 °C was used to grind enamel and dentin samples in Retsch MM200 Grinder Device.

2.1.3 Potassium Bromide (KBr)

This chemical was used to obtain pellets from powdered enamel and dentin samples. Then these pellets were put in Perkin-Elmer spectrometer and their spectra were obtained. KBr were used for this process because this chemical

under appropriate pressure (~200 bar for our samples) for certain times (~6minutes for our samples) transforms pellet and it has nearly no absorbance in infrared region. In addition, light can pass through the KBr pellet.

2.2 DEVICES

2.2.1 Isomet 4000 Linear Precision Saw

The teeth were sectioned in 0.5 mm thick pieces from the buccal surfaces up to pulp of teeth with Isomet 4000 Linear Precision Saw (Figures 12 and 13). We worked with the device blade speed with 3900rpm and feed rate with 10,1mm/min.

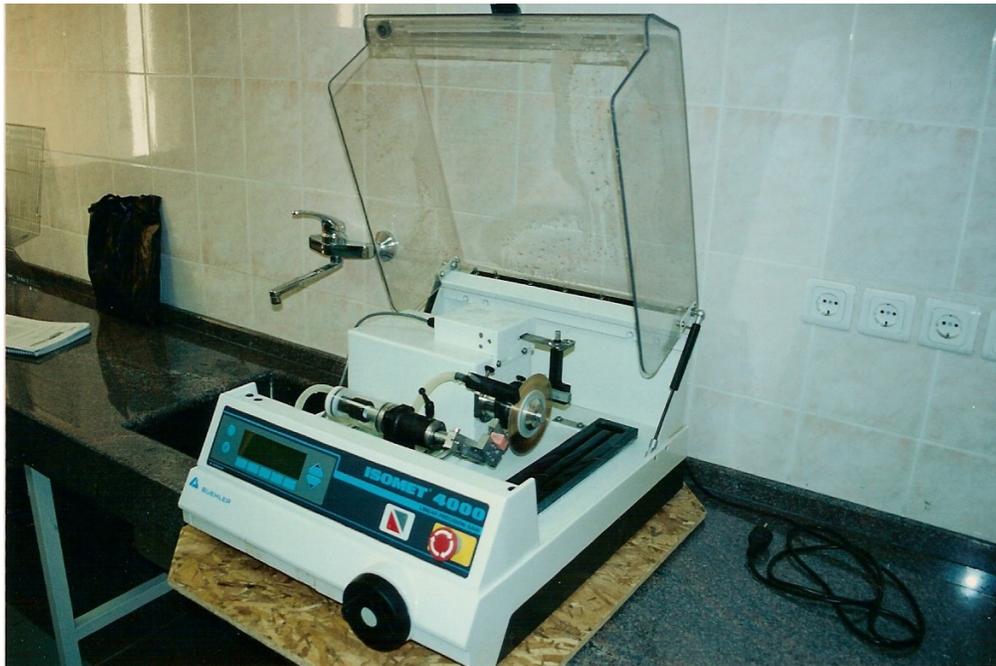


Figure 12 View of the Buehler Isomet 4000 Linear Precision Saw.



Figure 13 View of the tooth which is in acrylic during the sections were being taken from it.

2.2.2 Retsch MM200 Grinder

This device was worked in frequency value of 17 for 4 minutes to grind enamel and dentin samples.

2.2.3 Manfredi Hydraulic Press

This device was used to transform KBr and powdered tissue samples into pellets. We worked with Manfredi Hydraulic Press in 200 bars for 6 minutes to obtain KBr pellets.

2.2.4 Perkin-Elmer Spectrometer

FT-IR spectra were obtained with Perkin-Elmer Spectrometer (Figure 14). Spectra were obtained between 4000cm^{-1} and 450cm^{-1} . Resolution value was 4cm^{-1} .



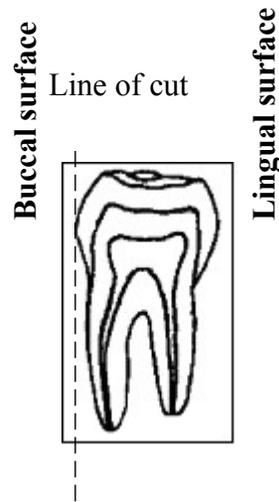
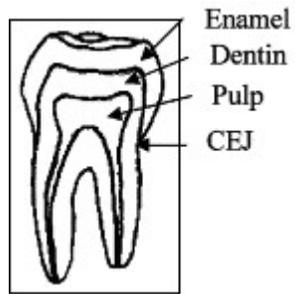
Figure 14 View of the Perkin Elmer Spectrometer.

2.3 SPECIMENS

Human teeth newly extracted for periodontal reasons were used. The teeth were collected from Hacettepe University Dentistry Department. They were randomly divided into control, hydrogen peroxide, and carbamide peroxide groups of ten specimens.

2.3.1 Control

Ten human teeth newly extracted for periodontal reasons were used. They were stored in distilled water for 7 days after their extraction. Then teeth were embedded in acrylic to obtain sections from buccal surfaces of them. The specimens were sectioned with a high-speed diamond rotary instrument (Buehler Isomet 4000 Linear Precision Saw) using water and air spray (Figure 13). Sectioning process from buccal surfaces of the teeth is illustrated from upper view in Figure 15. Enamel and dentin specimens were ground in liquid nitrogen and then, were investigated in the form of KBr pellets by using FTIR spectroscopic technique. A Perkin-Elmer spectrometer (Figure 14) was used with 4 cm^{-1} resolution for this purpose. After obtaining their spectra nine enamel tissue spectra and nine dentin tissue spectra were used for analysis.



A) Tooth embedded in acrylic

B) Reference plane

Line of cut

Line of cut



C) First slicing plane

D) Second slicing plane

Line of cut

Line of cut



E) Third slicing plane

F) Final slicing plane

Figure 15 Schematic diagrams showing sectioning process. A) Tooth embedded in acrylic, B) Reference plane, C) First slicing plane, D) Second slicing plane, E) Third slicing plane, F) Final slicing plane.

2.3.2 In-Office Bleaching:

Ten human teeth newly extracted for periodontal reasons were used. They were stored in distilled water for 7 days after their extraction. The roots of each tooth were sealed with nail varnish to prevent the penetration of bleaching agent (Figure 16). The specimens were exposed to 35% hydrogen peroxide (Pola Office Southern Dental Industries Cologne, Germany) as follows: The specimens were applied on the buccal surface then waited for three minutes (Figure 17), then Hilux Expert Curing Light was applied, and then the specimens were washed and dried. This procedure was repeated for four times. Teeth were embedded in acrylic to obtain sections from buccal surfaces of them. The specimens were sectioned with a high-speed diamond rotary instrument (Buehler Isomet 4000 Linear Precision Saw) using water and air spray (Figure 13). Sectioning process from buccal surfaces of the teeth is illustrated from upper view in Figure 15. Enamel and dentin specimens were ground in liquid nitrogen and then, were investigated in the form of KBr pellets by using FTIR spectroscopic technique. A Perkin-Elmer spectrometer (Figure 14) was used with 4 cm^{-1} resolution for this purpose. After obtaining their spectra ten enamel tissue spectra and seven dentin tissue spectra were used for analysis.



Figure 16 View of the teeth after the root of each tooth were sealed with nail varnish.



Figure 17 View of the office bleaching agent used in this study and the teeth after application of this agent.

2.3.3 Home Bleaching:

Ten human teeth newly extracted for periodontal reasons were used. The roots of each tooth were sealed with nail varnish to prevent the penetration of bleaching agent (Figure 16). The specimens were exposed to 17% carbamide peroxide bleaching agent (Perfect Bleach, Voco GmbH P.O.B. 767 274457 Cuxhaven/Germany) for 4 hours a day (Figures 18 and 19) and they were stored in distilled water remaining time of a day during 14 days. Teeth were embedded in acrylic to obtain sections from buccal surfaces of them. The specimens were sectioned with a high-speed diamond rotary instrument (Buehler Isomet 4000 Linear Precision Saw) using water and air spray (Figure 13). Sectioning process from buccal surfaces of the teeth is illustrated from upper view in Figure 15. Enamel and dentin specimens were ground in liquid nitrogen and then, were investigated in the form of KBr pellets by using FTIR spectroscopic technique. A Perkin-Elmer spectrometer (Figure 14) was used with 4 cm^{-1} resolution for this purpose. After obtaining their spectra nine enamel tissue spectra and nine dentin tissue spectra were used for analysis.



Figure 18 View of the home bleaching agent used in this study.



Figure 19 View of the teeth after application of home bleaching agent.

Teeth



Enamel or Dentine Samples



24 Hours Freze Dry



0,001g Sample + 0,1g KBr (Dry)



With 2000kg/cm Pressure & Air Vacuum



Semitransparent KBr Disk (Pellet)



FTIR

Fig 20 Summary of experimental procedure.

CHAPTER 3

RESULTS

3.1 Infrared Spectrum of a Tooth

FT-IR technique gives information about biological systems at molecular level without any damage in structural components (Boyar and Severcan, 1997; Melin et al., 2000; Severcan et al., 2000; Melin et al., 2001). Therefore examining the FT-IR bands give us information about changes in molecular structures of teeth.

Figure 21 illustrates the typical FT-IR spectrum of an untreated (control) tooth in the 4000-450 cm^{-1} region. The main bands were labeled in this figure and detailed spectral band assignments based on the literature were given in Table1.

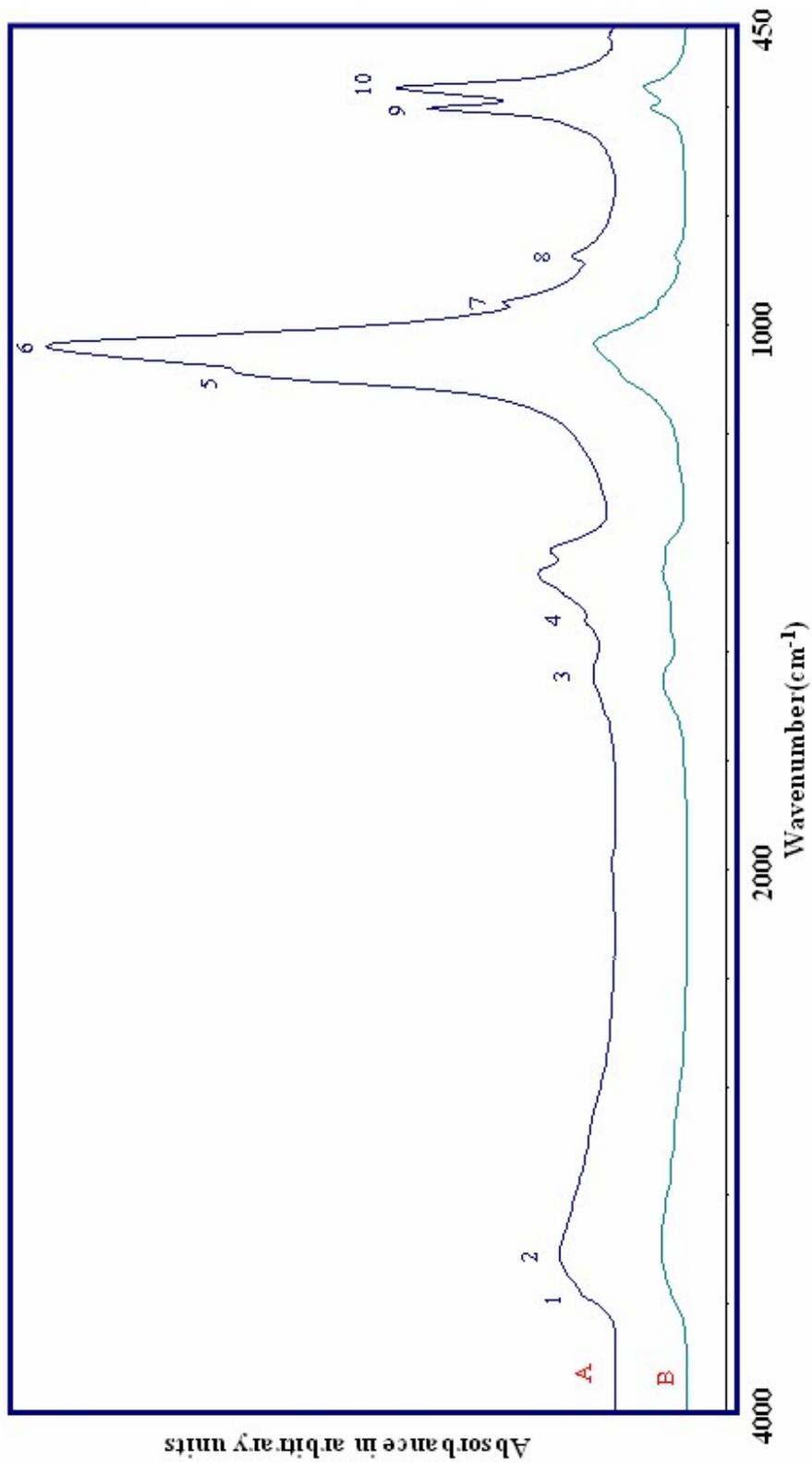


Fig. 21 A typical FTIR spectra of enamel(A) and dentine(B) tissues in the 4000 – 450 cm⁻¹ region.

Table 1. Major absorptions in IR spectra of control enamel and dentin cited in literature (Bachmann et al., 2003; Courrol et al., 2004; Magne et al., 2001; Morris and Finney, 2004; Sasaki et al., 2002).

Band #	Frequency (cm ⁻¹)		Definitions of the spectral assignment
	Enamel	Dentin	
1	3567	–	OH stretching
2	3369	3363	O-H and N-H group stretching vibration: polysaccharides, protein
3	1637	1652	Amide I (protein C=O stretch)
4	1544	1547	Amide II (protein N-H bend, C-N stretch), $\nu_3 \text{CO}_3^{-2}$ (mineral)
5-7	1200-900	1200-900	$\nu_1 \nu_3 \text{PO}_4^{-3}$ stretching (mineral)
8	890-850	890-850	$\nu_2 \text{CO}_3^{-2}$ (mineral) type B
9-10	700-450	700-450	$\nu_4 \text{PO}_4^{-3}$ bending (mineral)

3.2 Comparison of the Spectra of Control, Carbamide Peroxide and Hydrogen Peroxide Treated Teeth

3.2.1 Control, Carbamide Peroxide and Hydrogen Peroxide Spectra

FT-IR spectra were collected in the frequency range of 4000-450 cm^{-1} . The spectra were analyzed in two frequency ranges. These ranges are between 4000-2000 cm^{-1} and 2000-450 cm^{-1} . Figures 22 and 23 show the normalized spectra of enamel and dentin tissues respectively for control, carbamide peroxide, and hydrogen peroxide treatments in the 4000-2000 cm^{-1} region. Figures 24 and 25 illustrate the normalized spectra of enamel and dentin tissues respectively for control, carbamide peroxide treatment, and hydrogen peroxide treatment groups in the 2000-450 cm^{-1} region.

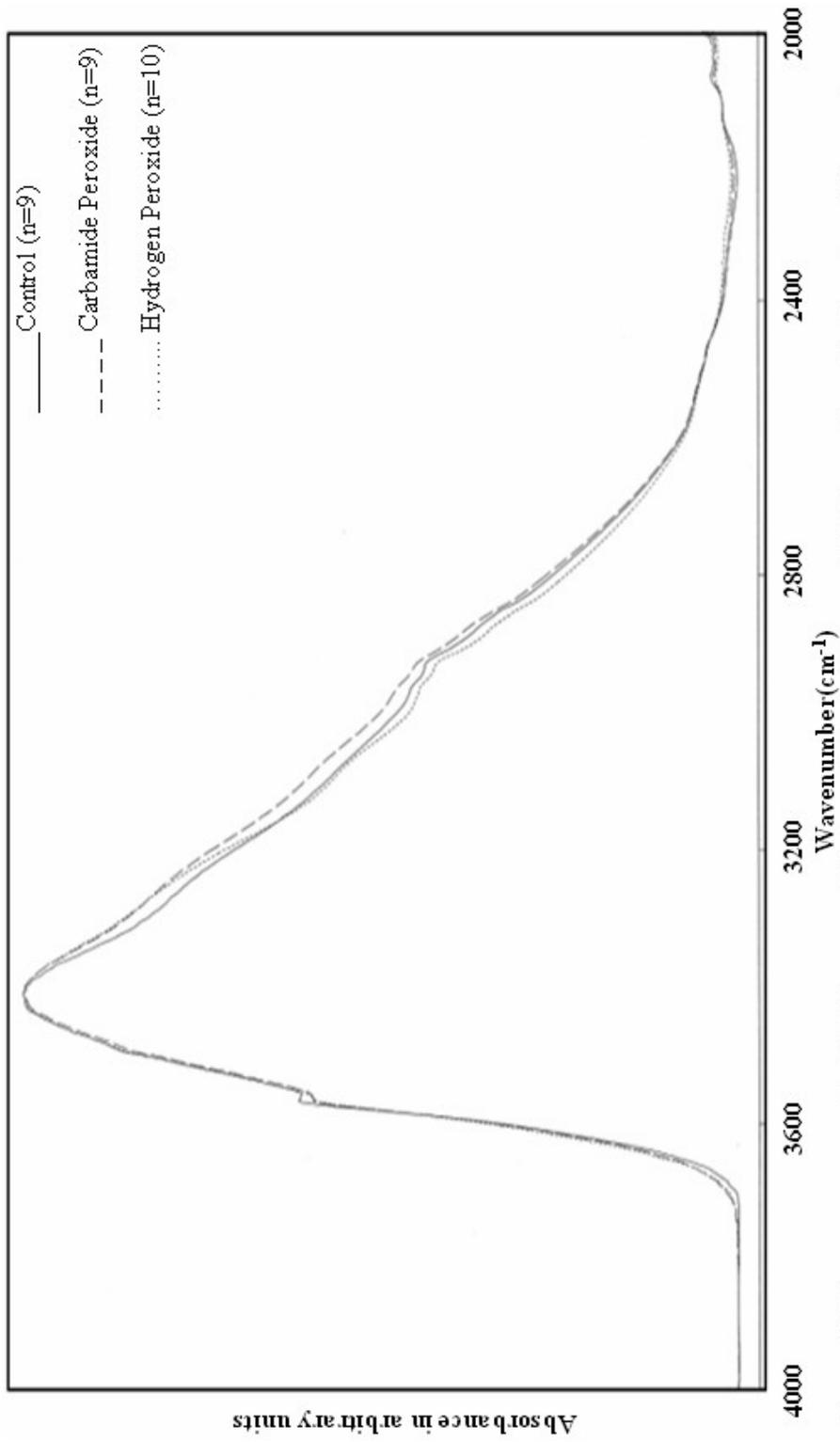


Figure 22 The average normalized FTIR spectra of Carbamide Peroxide Treated, Hydrogen Peroxide Treated and Untreated enamel groups in the 4000-2000 cm⁻¹.

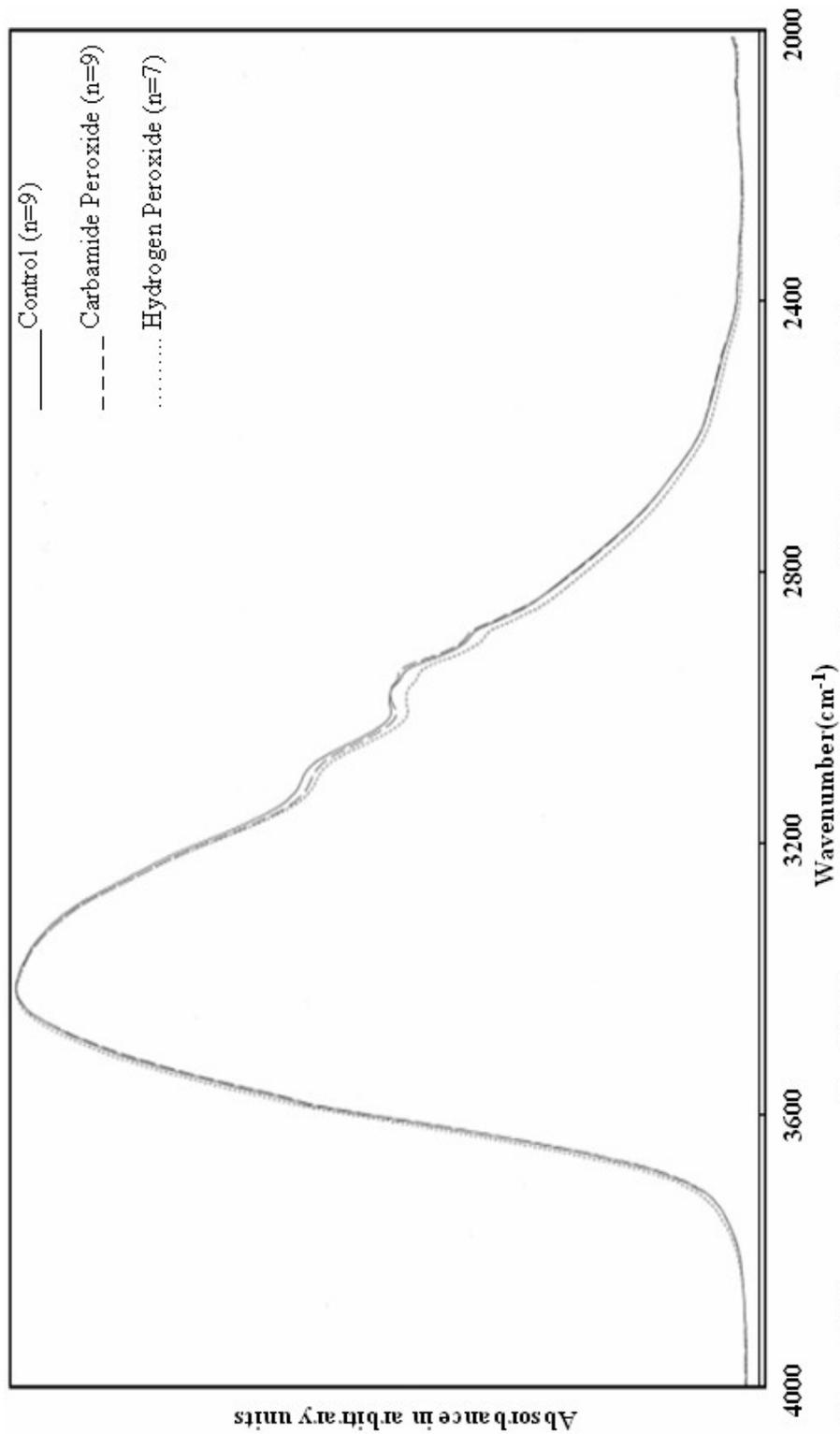


Figure 23 The average normalized FTIR spectra of Carbamide Peroxide Treated, Hydrogen Peroxide Treated and Untreated dentin groups in the 4000-2000 cm⁻¹.

3.2.2 Comparison of Control and Treated Teeth Spectra in the 4000-2000cm⁻¹ Region

As can be seen from table 1, although the OH stretching band (hydroxyapatite band) located at nearly 3567cm⁻¹ and the band originated from O-H and N-H group stretching (3369 cm⁻¹) are present for enamel tissue. For dentin tissue only the band located at 3363 cm⁻¹ (the O-H and N-H group stretching) is present.

In an infrared spectrum for biological samples the 3000-2800cm⁻¹ region generally contains lipid bands. Teeth do not contain lipid at high amount, and this can be clearly seen in Figures 22 and 23. Bachmann and coworkers (2003) could not observe lipid bands in this region for enamel spectra. However, we observed shoulders between 3000-2800 cm⁻¹ region and these shoulders were very small especially for enamel tissue. Therefore, the lipid region was ignored in the present study. Instead, Amide A band and hydroxyapatite bands were analyzed to show the differences between control and treatment groups' spectra in the 4000-2000cm⁻¹ region.

The frequency of Amide A band shifted to lower values in the carbamide peroxide treated enamel from 3390.21±2.20 cm⁻¹ to 3383.43±2.11 cm⁻¹. Similarly the frequency of Amide A band shifted to lower values in the hydrogen peroxide treated enamel from 3390.21±2.20 cm⁻¹ to 3371.45±2.04 cm⁻¹ (p<0.05). The frequency of Amide A band shifted to lower values in the carbamide peroxide treated dentin from 3362.30±2.42 cm⁻¹ to 3360.81±1.80 cm⁻¹. Similarly the frequency of Amide A band shifted to lower values in the hydrogen peroxide treated dentin from 3362.30±2.42 cm⁻¹ to 3360.79±2.08 cm⁻¹. However, for dentin tissue these reductions were not found as significant.

The frequency of OH stretching band observed only in enamel tissue spectra shifted to lower values with the treatment of carbamide peroxide and hydrogen peroxide. While the frequency of this band changed from $3568.09 \pm 1.13 \text{ cm}^{-1}$ to $3566.71 \pm 1.94 \text{ cm}^{-1}$ after the treatment of carbamide peroxide, the treatment of hydrogen peroxide caused the change of frequency of this band from $3568.09 \pm 1.13 \text{ cm}^{-1}$ to $3566.50 \pm 1.43 \text{ cm}^{-1}$. However, these reductions were not found as significant.

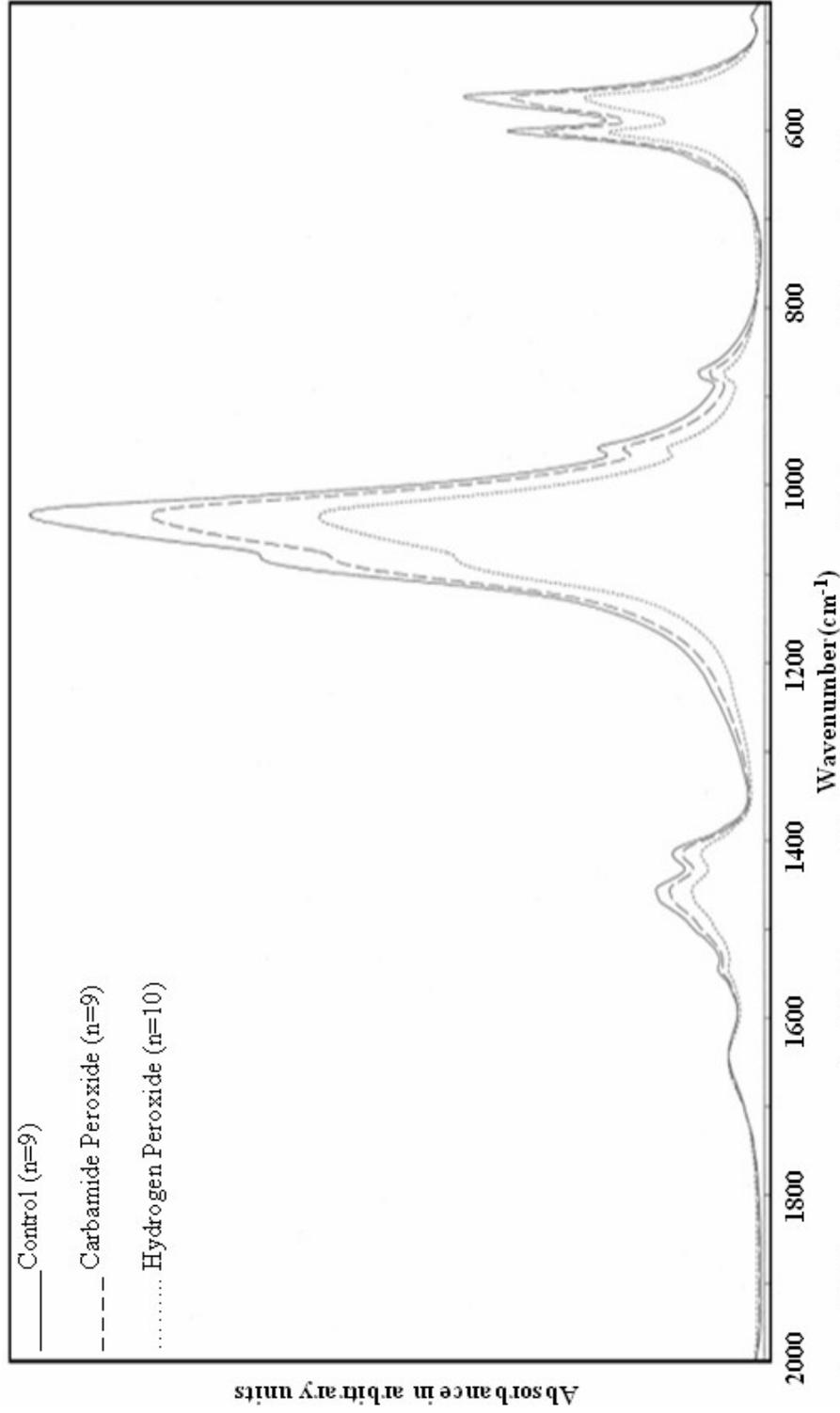


Figure 24 The average normalized FTIR spectra of Carbamide Peroxide Treated, Hydrogen Peroxide Treated and Untreated enamel groups in the 2000-450cm⁻¹.

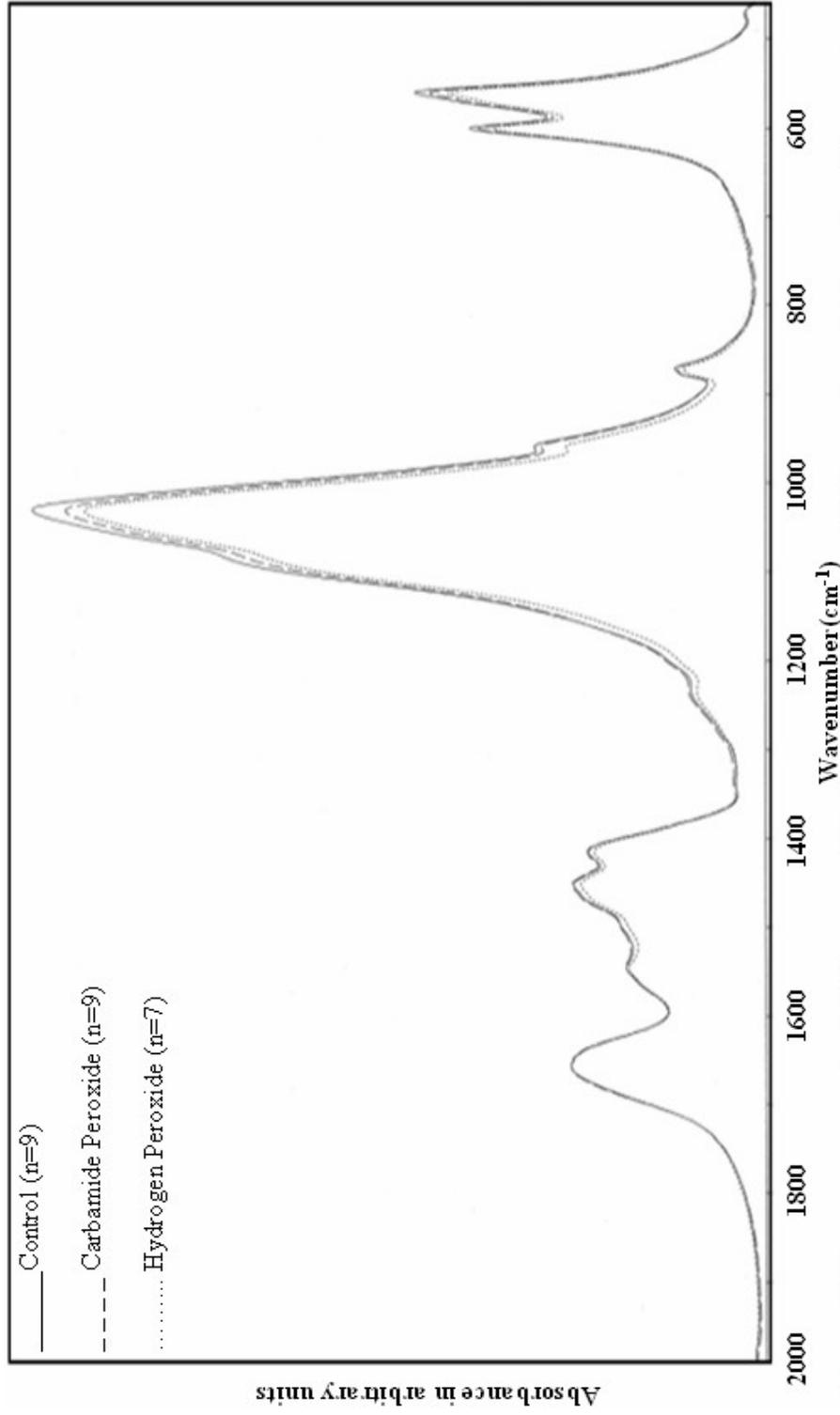


Figure 25 The average normalized FTIR spectra of Carbamide Peroxide Treated, Hydrogen Peroxide Treated and Untreated dentin groups in the 2000-450cm⁻¹.

3.2.3 Comparison of Control and Treated Spectra in the 2000-450 cm⁻¹ Region

In the 2000-450 cm⁻¹ region Amide I, Amide II, Phosphate (PO₄), and Carbonate (CO₃²⁻), bands are present. Amide I band gives information about proteins (C=O stretch). Amide II band gives information about protein N-H bend and C-N stretch. However, carbonate bands overlaps with the amide II bands at around 1545 cm⁻¹ (Bachmann et al., 2003; Di Renzo et al. 2001; Sasaki et al., 2002). Therefore, in the present study the amide II band of the protein matrix were not taken into consideration. The band between 1200 and 900cm⁻¹ gives information about $\nu_1\nu_3$ stretching modes of PO₄. The band between 890 and 850cm⁻¹ gives information about ν_2 mode of CO₃²⁻. And the band between 700 and 450cm⁻¹ gives information about ν_4 bending mode of PO₄ (Table 1).

The ratios of the intensity (absorbance) of some bands in this region were calculated and given in Table 2. As can be seen from Table 2 the ratios of the mineral to matrix intensity (ν_1, ν_3 PO₄³⁻ / AmideI) decreased for both enamel and dentin, after the treatment of both carbamide peroxide and hydrogen peroxide. The mineral to matrix ratio showed significant decrease for hydrogen peroxide treated enamel samples (p<0.01).

While the treatment of carbamide peroxide and hydrogen peroxide caused decreases in mineral to matrix ratio for enamel and dentin, the treatment of carbamide peroxide and hydrogen peroxide caused increases in carbonate to phosphate ratio (CO₃²⁻ / ν_1, ν_3 PO₄³⁻), but these changes were not found as significant for enamel and dentin.

Table 2. The ratios calculated for the control, carbamide and hydrogen peroxide groups.

	Mineral/Matrix ($v_1, v_3 \text{ PO}_4^{3-} / \text{AmideI}$)		Carbonate/Phosphate ($\text{CO}_3^{2-} / v_1, v_3 \text{ PO}_4^{3-}$)	
	enamel	dentin	enamel	dentin
Control	53.561±2.972	6.121±0.352	0.037±0.003	0.064±0.005
Carbamide peroxide	43.410±2.334	5.564±0.191	0.039±0.006	0.074±0.006
Hydrogen peroxide	27.343±1.691 **	5.542±0.282	0.038±0.003	0.068±0.005

Values are given by average \pm stdev. p value significant at $p < 0.01$ illustrated by **.

3.2.4 Numerical Comparison of the Bands of Control, Carbamide Peroxide and Hydrogen Peroxide FTIR Spectra

Numerical comparison of the control and treatment groups was carried out to show the spectral changes after treatment of bleaching agents. The numerical values were listed in table 3 and 4. Carbonate bands overlaps with the amide II bands at around 1545 cm^{-1} (Bachmann et al., 2003; Di Renzo et al. 2001; Sasaki et al., 2002). Therefore, amide II band (4th band) of the protein matrix was not taken into consideration in these tables. As can be seen from the tables there are significant changes in some of the band frequency and intensity values. For example, the frequency of the Amide A and Amide I bands changed significantly after the treatment of hydrogen peroxide. In addition to the frequency values, the intensity values of these bands changed significantly after the application of hydrogen peroxide.

Table 3 Band frequency and band intensity values for enamel tissue. *p value less than or equal to 0.05 was accepted as significant).

Band #	Band Frequency (cm ⁻¹)			Band Intensity (A)		
	Control	Carbamide Peroxide	Hydrogen Peroxide	Control	Carbamide Peroxide	Hydrogen Peroxide
1	3568.09± 1.13	3566.71± 1.94	3566.50± 1.43	0.04± 0.00	0.03± 0.00	0.02± 0.00
2	3390.21± 2.20	3383.43± 2.11	3371.45± 2.04 *	0.13± 0.03	0.14± 0.02	0.16± 0.03 *
3	1635.37± 1.49	1641.55± 2.11	1646.42± 1.82 *	0.02± 0.01	0.03± 0.01	0.05± 0.02 *
5-7	1046.10± 1.36	1046.53± 1.62	1046.27± 0.85	1.14± 0.04	1.22± 0.05	1.21± 0.02
8	874.08± 0.68	874.34± 0.75	874.33± 0.28	0.04± 0.01	0.05± 0.01	0.05± 0.01
9-10	567.58± 0.75	568.23± 0.82	567.86± 0.35	0.47± 0.04	0.50± 0.04	0.49± 0.03

Table 4 Band frequency and band intensity values for dentin tissue

Band #	Band Frequency (cm^{-1})				Band Intensity (A)			
	Control	Carbamide Peroxide	Hydrogen Peroxide	Control	Control	Carbamide Peroxide	Hydrogen Peroxide	Control
1	-	-	-	-	-	-	-	-
2	3362.30± 2.42	3360.81± 1.80	3360.79± 2.08	0.23± 0.02	0.21± 0.01	0.20± 0.02	0.20± 0.02	0.20± 0.02
3	1656.55± 2.23	1657.29± 1.06	1657.00± 1.90	0.12± 0.01	0.12± 0.02	0.12± 0.02	0.12± 0.02	0.12± 0.02
5-7	1046.03± 1.71	1046.56± 1.71	1046.05± 2.07	0.74± 0.03	0.71± 0.04	0.68± 0.04	0.68± 0.04	0.68± 0.04
8	873.56± 0.56	873.54± 0.40	873.47± 0.60	0.04± 0.01	0.05± 0.01	0.04± 0.01	0.04± 0.01	0.04± 0.01
9-10	567.60± 0.82	567.39± 0.48	567.50± 0.48	0.36± 0.02	0.35± 0.03	0.31± 0.01	0.31± 0.01	0.31± 0.01

CHAPTER 4

DISCUSSION

The tooth mainly composed of enamel, dentin, dentin-enamel junction cementum and pulp. Enamel is the hardest tissue found in the human body (Balooch et al., 1998). The enamel of mammalian teeth is the hardest mineralized tissue known. Both bones and dentin are relatively spongy assemblage of the apatite crystals interwoven with type I collagen and other proteins, producing a very tough material. Tooth enamel is much more heavily mineralized than bone, making it much harder, and enamel does not contain collagen, though it does, when mature, contain small amounts of specialized matrix proteins (Wilt, 2005). Mature enamel is highly mineralized (Balooch et al., 1998). It contains 96% inorganic material, 1% organic material and 3% water by weight. The inorganic component is mainly calcium phosphate in the form of hydroxyapatite crystals. Other elements present are small amounts of carbonate, magnesium, potassium, sodium and fluoride. Dentin is softer than enamel. The composition of dentin is approximately 70% inorganic material, 20% organic material and 10% water by weight. The main inorganic component is hydroxyapatite, and the main organic component is Type I collagen (Nizam et al., in press). Our findings are consistent with the literature information mentioned above. We obtained the sharper peaks and lower amide peaks for enamel spectra according to dentin spectra (See the Appendix). The sharpness of the peaks in the FTIR

profile indicates the degree of crystallinity of the material, and the proportion of the peaks is related to the amount of the tissue components (LeGeros, 1991). Therefore the sharper peaks and lower amide peaks obtained for enamel tissue, compared to dentin tissue, indicating the higher mineral (more crystalline structure) and lower organic contents of enamel, as in previous studies.

In addition to quantitative difference there are changes in the chemical composition between enamel and dentin organic matrixes. The dentin organic matrix is composed basically of collagen and other components in lower quantity such as citric acids, lipids, mucopolysaccharide, and insoluble proteins, peptides, proteins, keratin and little fraction of collagen compose the enamel (Provenza, 1964).

The absorption spectra of the tissues are composed of the sum of the spectra of their primary components. Therefore the dental hard tissues are constituted by a sum of the mineral matrix composed of phosphate, carbonate and hydroxyl bands,(Rey et al., 1990; Rey et al., 1991) organic matrix which is composed of collagen or non-collagen proteins with bands originated from chemical compounds such as C-H, C=O, C-N, N-H, NCO⁻, NCN²⁻ (Dowker and Elliot, 1983; Lazarev et al., 1978; Payne and Veis, 1988). In addition the tissue contains water (LeGeros et al., 1978; Bayly et al., 1963). Water absorbs the light in infrared region and in the present of water other molecular bands may not be observed. Therefore in this study we dried the samples to eliminate this situation, and therefore the unbound water bands were not observed in our FTIR spectra. In the present case, effects of bleaching agents on teeth can be examined in terms of the effects on mineral matrix and effects on organic matrix.

MINERAL MATRIX

The mineral matrix is composed in its majority of crystals of carbonate hydroxyapatite (Enanes, 1979). The absorbed components of it in the infrared region are the hydroxyl (OH⁻), carbonate (CO₂⁻³) and phosphate radical (PO₄⁻³).

One of the infrared mode from the hydroxyl radical was observed between 3560 and 3570 cm⁻¹ associated to the stretching mode (Dahm, 1999). This mode was observed only in the enamel tissue, which has a high degree of crystallinity, while in the dentin tissue, which has a small crystallinity, these bands could not be observed (Figures 22 and 23) (Bachmann et al., 2003). There are some differences in OH⁻ stretching band frequency and intensity values belongs to carbamide peroxide treated, hydrogen peroxide treated, and control enamel groups. For example after the treatment of carbamide peroxide the frequency of this band changed from 3568.09±1.13 cm⁻¹ to 3566.71±1.94 cm⁻¹. On the other hand the treatment of hydrogen peroxide caused the change of frequency of this band from 3568.09±1.13 cm⁻¹ to 3566.50± 1.43 cm⁻¹. In addition band intensity values shifted to lower values from 0.04± 0.00 to 0.03± 0.00 after the treatment of carbamide peroxide and from 0.04± 0,00 to 0.02±0.00 after the treatment of hydrogen peroxide. Therefore it can be concluded that both of the bleaching agents decreased the crystallinity of enamel tissue, and the deleterious effect of hydrogen peroxide treatment is more dramatic than that of carbamide peroxide treatment. This result is logical when it is taken into consideration that with the hydrogen peroxide treatment, teeth are exposed to more concentrated hydrogen peroxide than that of carbamide peroxide treatment. However, the reductions in enamel crystallinity were not found as significant.

The phosphate radical shows four vibrational modes in the infrared region (4000–400 cm^{-1}) (Bachmann et al., 2003). The phosphate moiety present in hydroxyapatite gives rise to the peaks in two different spectral regions: the ν_4 asymmetric P–O bending vibration as a sharp band in the 500–650 cm^{-1} region and the P–O symmetric and asymmetric stretching vibrations (ν_1 and ν_3) which occur in the 900–1200 cm^{-1} spectral region as a broad band (Pleshko et al., 1991). Parallel to these information we observed ν_1 , ν_3 and ν_4 vibrational modes of phosphate radical in enamel and dentin spectra (Figure 21 and Table 1). The bands which give information about phosphate content of enamel and dentin can be seen from Figures 24 and 25. Changes in these bands after the treatment of carbamide peroxide and hydrogen peroxide can also be seen from these figures. With the treatment of two bleaching agents, intensity of the ν_1 , ν_3 PO_4 stretching and ν_4 PO_4 bending bands decreased for enamel (Figure 24). The treatment of the high concentration bleaching agent caused more dramatic decrease in phosphate bands intensities. Decreasing in phosphate band intensities shows the loss of phosphate ion from enamel tissue, and this lost is more dramatic in hydrogen peroxide treatment. However in dentin tissue, changes in phosphate bands intensities after the treatment of high concentration and low concentration bleaching agents are negligible (Figure 25). Therefore, it can be concluded that deleterious effects of bleaching agents are more dramatic in terms of phosphate loss in enamel tissue and hydrogen peroxide treatment caused more dramatic changes according to carbamide peroxide treatment. Evaluation of changes in the ν_1 and ν_3 phosphate absorption region is appropriate for monitoring changes in the relative mineral content of bone tissue (Pleshko et al., 1991). Therefore, investigations of mineral crystallinity was focused essentially on the $\nu_1\nu_3$ phosphate band between 1200-900 cm^{-1} during calculation of relative carbonate to phosphate ratio.(Magne et al., 2001; Ikemura et al., 2003).

Among the four vibration modes of the free carbonate ion, only two are of importance for infrared investigations in calcium phosphates. These are the ν_2

and ν_3 bands. The ν_3 mode (1400–1600 cm^{-1}), corresponding to the strongest infrared bands of the ion, is obscured by several absorption bands of proteins (CH, Amide II, COO^-) or glycosaminoglycans (NH) (Termine et al., 1973). The ν_2 vibrational domain of the carbonate ion is free of absorption by organic substituents (Boyar et al., 2004). Therefore in this study the ν_2 carbonate band (between 850 and 890 cm^{-1}) was taken into consideration when we were investigating the effects of bleaching agents on carbonate content of the enamel and dentin tissue. The Amide II band of the protein matrix and ν_3 carbonate band of mineral were not taken into consideration due to overlap of these bands. Effects of two bleaching agents on the ν_2 carbonate bands of enamel and dentin tissues are illustrated in Figures 24 and 25. Carbonate plays an important role in affecting crystallinity and solubility of the mineral matrix. As seen from Figure 24 the intensity of carbonate band between 850-890 cm^{-1} was seen to decrease after the treatment of both high concentration and low concentration bleaching agents. However in the case of high concentration bleaching agent (hydrogen peroxide) treatment, decreasing in intensity of this band was more dramatic. This situation implies that there is a decrease in carbonate content in treated enamel samples, and loss of carbonate is more dramatic after the treatment of hydrogen peroxide (Magne et al., 2001; Ikemura et al., 2003). This decrease in CO_3^{2-} content may disturb or disorganize the apatite lattice. However, this decrease is not great for dentin tissues.

Relying on the hydroxyl, carbonate and phosphate absorption bands, the spectra of the carbamide peroxide treated samples indicate that less mineral has been removed from teeth samples, compared to hydrogen peroxide treated samples.

ORGANIC MATRIX

Starting from greater wavenumbers in the enamel and dentin spectra, the first band groups between 3370 and 2800 cm^{-1} can be observed. In this region, the bands are assigned to Amide A, and CH stretching mode (Lazarev et al., 1978). A second region where absorption bands are assigned to the organic matrix in the dentin spectra, starting at 1660 cm^{-1} and finishing at 1500 cm^{-1} . In this region some overlapping happens with the bands arises from other chemical components, such as water and carbonate. Theoretically near the water band predicted at 1645 cm^{-1} another band at 1655 cm^{-1} assigned to amide I (Payne and Veis, 1988) was observed as a common band positioned at $\sim 1660 \text{ cm}^{-1}$ (Bachmann et al., 2003). However, since we dried our samples we evaluated these bands at $\sim 1635 \text{ cm}^{-1}$ for enamel and at $\sim 1655 \text{ cm}^{-1}$ for dentin as Amide I bands. Concerning smaller wavenumbers, carbonate bands are overlapped with Amide II band (Tenmine et al., 1973). Therefore instead of Amide II band, Amide I band was taken into consideration during the evaluation of changes in organic matrix after the treatment of two different bleaching agents.

The broad bands at $\sim 3370 \text{ cm}^{-1}$ for enamel and $\sim 3360 \text{ cm}^{-1}$ for dentin correspond to NH stretching vibrations of Amide A and intermolecular OH bonding as seen from Figures 22 and 23. The frequency of this band shifted to lower values in carbamide peroxide treated enamel from $3390.21 \pm 2.20 \text{ cm}^{-1}$ to $3383.43 \pm 2.11 \text{ cm}^{-1}$ and in hydrogen peroxide treated enamel from $3390.21 \pm 2.20 \text{ cm}^{-1}$ to $3371.45 \pm 2.04 \text{ cm}^{-1}$ ($p < 0.05$) (Table 3). These changes in Amide A frequency after the treatment of bleaching agents indicate that NH groups were involved in a new set of hydrogen bonds of weaker strength (Magne et al., 2001; Ikemura et al., 2003). Changes in Amide A band frequency was also observed in bleaching agent treated dentin samples but these changes were not significant. The frequency of this band shifted to lower values in carbamide peroxide treated dentin from $3362.30 \pm 2.42 \text{ cm}^{-1}$ to

3360.81±1.80 cm⁻¹ and in hydrogen peroxide treated dentin from 3362.30±2.42 cm⁻¹ to 3360.79±2.08 cm⁻¹ (Table 4). By comparing the changes in these frequency values it can be concluded that change in the strength of hydrogen bonds in which NH groups were involved is more dramatic in enamel tissue and in the case of hydrogen peroxide treatment.

The bands at ~1635 cm⁻¹ for enamel and ~1650 cm⁻¹ for dentin are attributed to the Amide I vibration of structural proteins (Courrol et al., 2004; Haris and Severcan, 1999). The Amide I band corresponds to the stretching C=O and bending C-N vibrational modes of the polypeptide and protein backbone. The Amide I region is useful for determination of protein secondary structure because the frequency of vibration is very sensitive to changes in the nature of hydrogen bonds in the different types of protein secondary structures (Haris and Severcan, 1999; Lyman et al., 1998). It is known that α helix locates around 1650 cm⁻¹, β -sheet locates around 1630 cm⁻¹ and random coil locates around 1640 cm⁻¹ (Sackett and Shai, 2005; Jorgensen et al., 2003). These values indicate that the proteins of enamel are mainly in β -sheet structure and proteins of dentin are in α helical structure. As can be seen easily from Table 3 the frequency of this band shifted to higher values in the carbamide peroxide treated enamel from 1635.37±1.49 cm⁻¹ to 1641.55±2.11 cm⁻¹ and in the hydrogen peroxide treated enamel from 1635.37±1.49 cm⁻¹ to 1646.42±1.82 cm⁻¹ (p<0.05). This situation shows that protein structure changed from β -sheet to random coil structure and protein denaturation took place in case of hydrogen peroxide treatment (Ellepola et al., in press). On the other hand the increase in frequency value of this band after the application of bleaching agents was not so great in dentin tissue. The frequency of this band shifted to higher values in the carbamide peroxide treated dentin from 1656.55±2.23 cm⁻¹ to 1657.29±1.06cm⁻¹ and in hydrogen peroxide treated dentin from 1656.55±2.23 cm⁻¹ to 1657.00± 1.90 cm⁻¹ (Table 4). Based on these statistical values it can be concluded that application of bleaching agents caused changes in protein secondary structures of enamel and dentin

tissue and the changes in protein secondary structures are more pronounced in enamel tissue in the case of hydrogen peroxide treatment.

In addition to evaluation of the mineral component and organic component distinctly, the ratios of the absorbance of some bands were calculated to illustrate the changes in relative ratios of some structural components of teeth after the application of high and low concentration bleaching agents.

MINERAL TO MATRIX AND CARBONATE TO PHOSPHATE RATIOS

The ratios of the intensity (absorbance) of the ν_1 , ν_3 phosphate stretching and the amide I regions were calculated to determine the relative ratio of mineral and protein matrix phases. The mineral to matrix ratio was shown as directly related to the ash weight of a tissue. Therefore, it is indicative of the relative quantity of mineral present in calcified tissues. In addition to mineral to matrix ratio the relative carbonate to phosphate ratio, which is indicative of the quantity of carbonate present in the apatite phase was calculated by taking the ratio the intensity (absorbance) of ν_2 carbonate and ν_1 , ν_3 phosphate stretching bands (Boyar et al., 2004). These ratios were given in Table 2.

With the application of two bleaching agents the mineral to matrix ratio decreased to lower values in both enamel and dentin tissue. However, this reduction was greater in enamel tissue and in this tissue mineral to matrix ratio decreased significantly after the treatment of hydrogen peroxide ($p < 0.01$). Both of the bleaching agents did not cause any statistically significant reduction in mineral to matrix ratio in dentin tissue. Theoretically, the decrease in this ratio may indicate five possible phenomena: 1) a decrease in the amount of mineral components and no change in the amount of organic components; 2) no changes in the amount of mineral components and an increase in the amount of organic components; 3) an increase in the amount

of both organic and inorganic components, but a larger increase of the organic ones; 4) a decrease in the amount of both organic and inorganic components, but a larger decrease of the mineral ones; 5) a decrease and an increase of the amount of the mineral and organic components respectively. The further formation of organic and inorganic components is unlikely, because an additional source of protein and phosphate ions would be necessary (Sasaki et al., 2002). Therefore, the most likely event during bleaching process is that the loss of mineral components of the dental tissues occurs in larger proportions than does the loss of proteins. This event can be explained with the interaction of free radicals with proteins. The generated free radicals during bleaching process can react rapidly with a range of targets including the side chains of amino acids (Farkas et al., 2003). The repertoire of side chain groups in proteins is embellished with some substances including minerals (Mathews et al., 2000). Interaction of oxygen free radicals formed during bleaching process (Goldstein and Garber, 1995) with mineralized side chains of proteins can clarify the decrease in mineral to matrix ratio after the treatment of bleaching agents.

While the treatment of carbamide peroxide and hydrogen peroxide caused decrease in the mineral to matrix ratio for enamel and dentin, the treatment of carbamide peroxide and hydrogen peroxide caused increases in carbonate to phosphate ratio, these changes were not found as significant for enamel and dentin. Based on Sasaki and coworkers' approach (2002) mentioned above, we can suggest five possible phenomena from increasing in carbonate to phosphate ratio: 1) a decrease in the amount of phosphate components and no change in the amount of carbonate components; 2) no changes in the amount of phosphate components and an increase in the amount of carbonate components; 3) an increase in the amount of both carbonate and phosphate components, but a larger increase of the carbonate ones; 4) a decrease in the amount of both carbonate and phosphate components, but a larger decrease of

the phosphate ones; 5) a decrease and an increase of the amount of the phosphate and carbonate components respectively. During bleaching process the increase in the concentration of the carbonate and phosphate is unlikely because for that an additional source of carbonate and phosphate ions would be necessary. As seen from Figure 24 after the two types of bleaching process both carbonate and phosphate band intensities decreased in terms of control spectrum for enamel. Therefore, the most likely event during bleaching process is that the loss of the phosphate component of the dental tissues occurs in larger proportions than does the loss of carbonate component. However the increasing in carbonate to phosphate ratio was not statistically significant for enamel and dentin.

The reason of changes in mineral components, organic components and the ratios of the absorbance of some bands of the teeth mentioned above can be understood easily when the mechanism of dental bleaching is taken into consideration. The mechanism of the action of bleaching agents is thought to be due to the ability of hydrogen peroxide to form oxygen free radicals that interact with adsorbed colored organic molecules and oxidize these macromolecules and pigment stains, producing dental discoloration into smaller and lighter molecules (Cavalli et al., 2004).

In dental bleaching hydrogen peroxide diffuses through the organic matrix of the enamel and dentin. Because, the radicals have unpaired electrons, they are extremely electrophilic and unstable and will attack most of the other organic molecules to achieve stability, generating other radicals. These radicals can react with most unsaturated bonds, resulting in disruption of electron conjugation and a change in the absorption energy of the organic molecules in tooth enamel. And then simpler molecules that reflect less light are formed, creating a successful whitening action. This process occurs when the oxidizing agent (hydrogen peroxide) reacts with organic material in the spaces between the inorganic salts in tooth enamel. A simple example of this

type of reaction is the oxidation of beta carotene, which is deep red. When oxidized, this molecule is split in half to produce two molecules of vitamin A, which are colorless. However not all bleaching reactions are that simple. Albers (1991) has described a more extensive bleaching process: ‘the extent of bleaching determines the amount of whitening compared to the amount of material loss. During the initial bleaching process highly pigmented carbon-ring compounds are opened and converted into chains which are lighter in color. Existing carbon double-bond compounds, usually pigmented yellow, are converted into hydroxy groups (alcohol like), which are usually colorless. As these processes continue the bleached material continually lightens.’ (Goldstein and Garber, 1995).

CHAPTER 5

CONCLUSION

In the present study it was determined that bleaching treatment led to some structural changes in enamel and dentin tissue by using FTIR spectroscopic method. Loss of mineral components observed from carbonate and phosphate bands. This situation indicates that demineralization took place in enamel and dentin tissue. However, the loss of mineral components was more dramatic in enamel tissue in the case of hydrogen peroxide treatment. The loss of these components from dentin tissue after the treatment of high and low concentration bleaching agents was negligible. The loss of phosphate content led to decrease in hardness of enamel samples (Nizam et al., in press). Furthermore, the decrease in total carbonate content was associated with lower mineral crystallinity (Magne et al., 2001; Ikemura et al., 2003).

In addition to mineral components, organic components of the teeth were changed after the treatment of bleaching agents. The frequency of the Amide A and Amide I bands were significantly changed for enamel tissue after the treatment of high concentration bleaching agent. Change in Amide A band shows change in strength of hydrogen bonds in which NH groups were involved. Change in Amide I band shows that change in protein secondary structure took place after the treatment of bleaching agents. The changes in organic components were more dramatic in enamel tissue in the case of

hydrogen peroxide treatment as in the changes in mineral components. However neither of the bleaching agents caused statistically significant changes in Amide A and Amide I frequencies in dentin tissue.

In conclusion, it can be stated that hydrogen peroxide treatment caused dramatic changes in enamel structure according to carbamide peroxide treatment, but two methods did not cause significant changes in dentin tissue.

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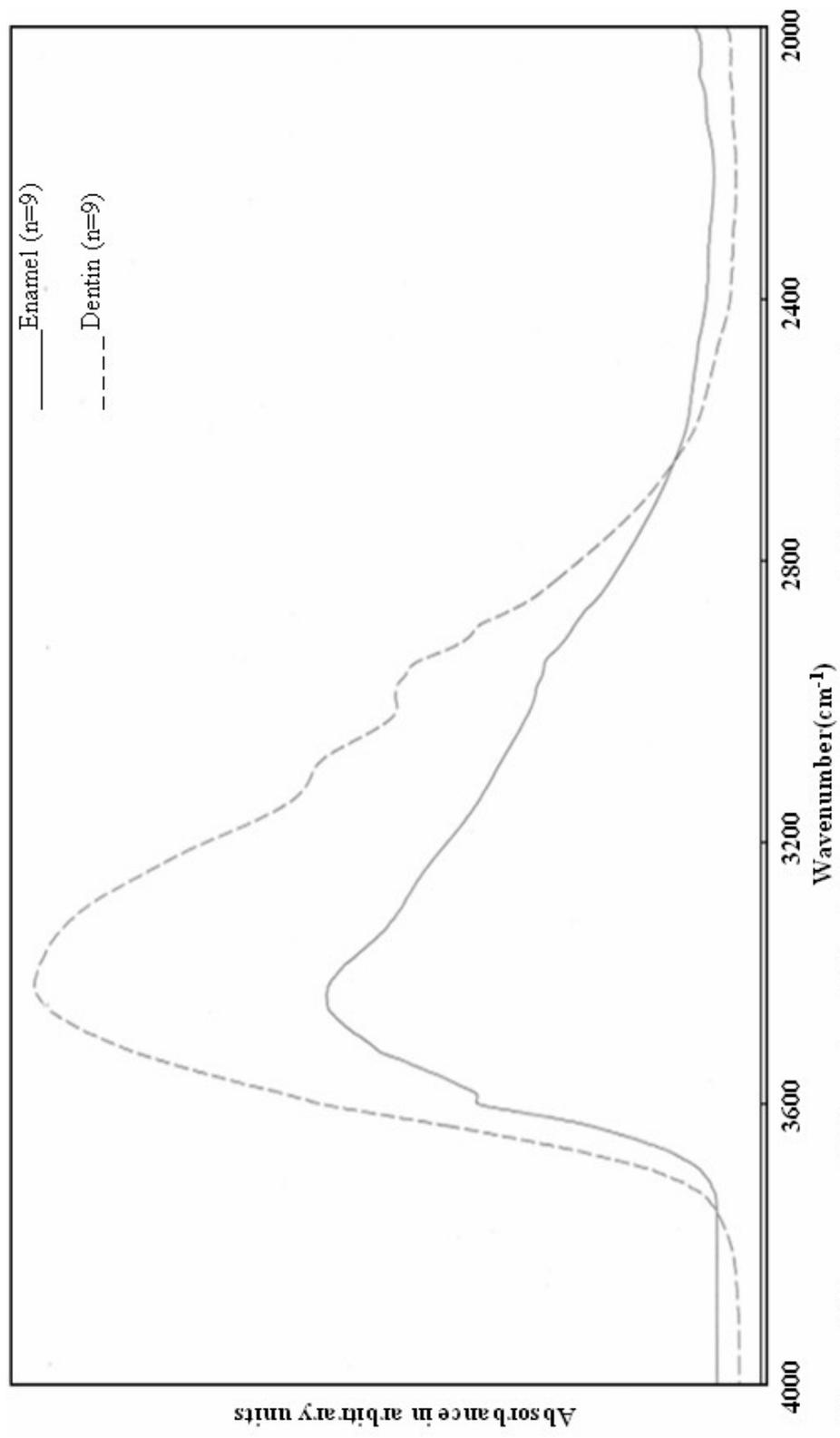


Figure 26 The average FTIR spectra of Untreated Enamel and Dentin groups in the 4000-2000cm⁻¹.

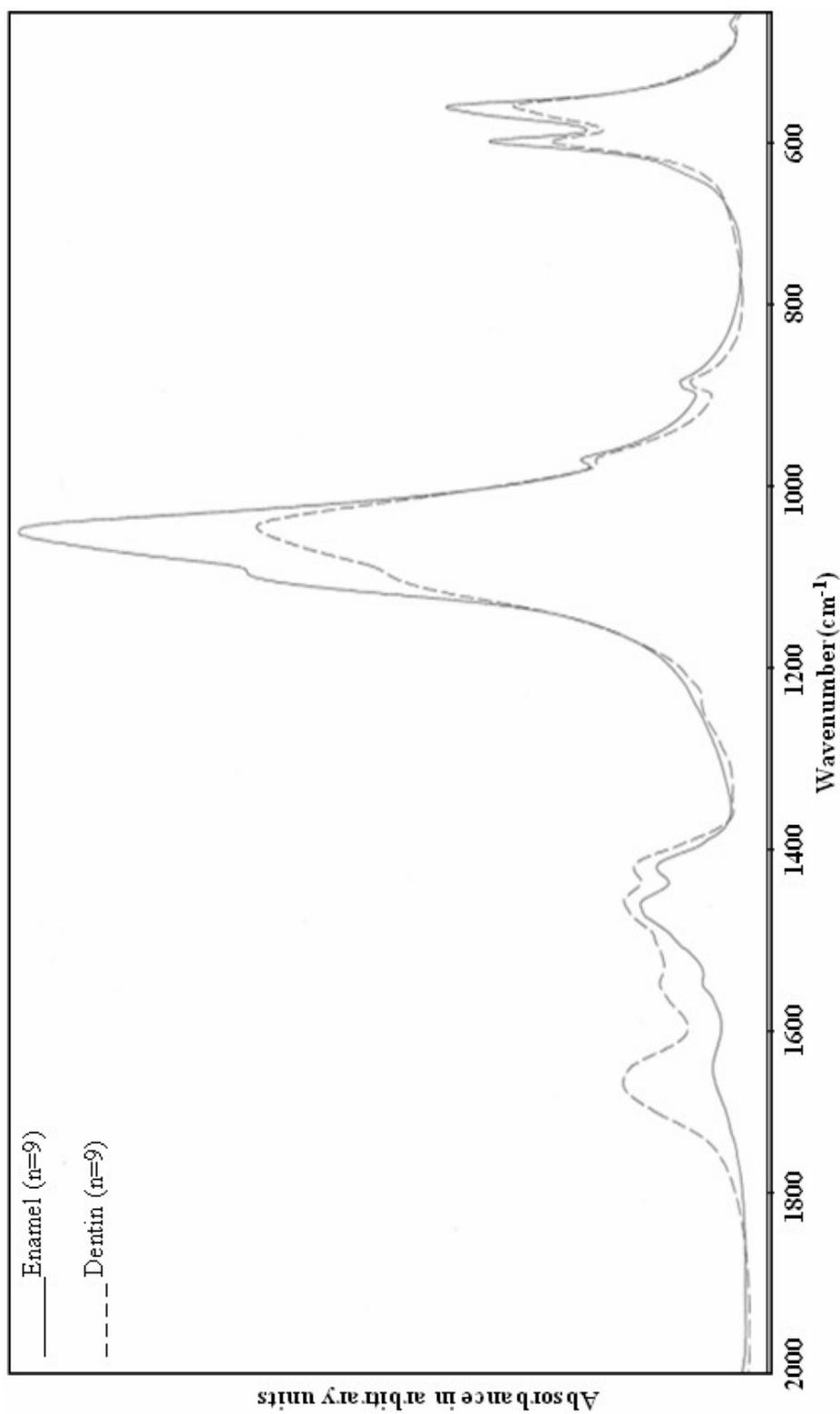


Figure 27 The average FTIR spectra of Untreated Enamel and Dentin groups in the 2000-450cm⁻¹.

Appendix C: PUBLICATIONS RELATED WITH THIS STUDY

Article:

Ayca Dogan, Kurtulus Gokduman, Sukran Bolay, Feride Severcan, *Evaluation of In-Office Bleaching on Enamel and Dentine: An FTIR Study*, WSEAS Transactions on Biology and Biomedicine, Issue 4, Volume 2, October 2005, pp: 365-369.

Talk:

Kurtuluş Gökdoğan, Ayça Doğan, Şükran Bolay, Saadet Gökçalp, Feride Severcan, *Klinik ve Ev Tipi Diş Beyazlatma Metodlarının Mine ve Dentin Üzerine Etkileri*, **XVII. National Biophysics Congress, Ege University, Izmir-Turkey, September 2005.**

Poster:

-Ayça Doğan, Kurtuluş Gökdoğan, Şukran Bolay, Saadet Gökçalp, and Feride Severcan, *FTIR Spectroscopic Characterization of Bleaching Agents on Human Teeth* Poster at Biophysical Society 49th Annual Meeting, Long Beach, CA, February 2005.

-Kurtuluş Gökdoğan, Ayça Doğan, Şükran Bolay, Saadet Gökçalp, Feride Severcan, *Klinik ve Ev Tipi Diş Beyazlatma Metodlarının Mine ve Dentin Üzerine Etkileri*, Poster at IX. National Spectroscopy Congress, Bilkent University, Ankara-Turkey, June 2005.

-Kurtulus Gokduman, Ayca Dogan, Sukran Bolay, Feride Severcan, *Evaluation of Office and Home Bleaching on Enamel and Dentine: An FTIR Study*, Poster at International University Bremen Summer School(Biosensing with channels: faster, smaller, smarter), Bremen-Germany, 30 July-5 August 2005.