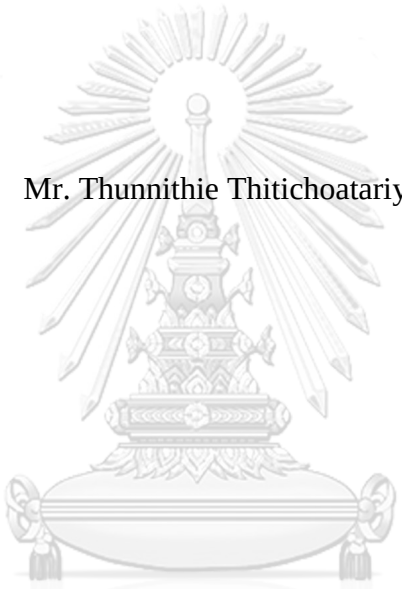
Adding ferrous sulphate to hydrogen peroxide tooth bleaching gels reduced the required bleaching time

Mr. Thunnithie Thitichoatariyakun



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ประสิทธิภาพของสารเฟอรัสซัลเฟตในการลดเวลาการฟอกสีฟันของน้ำยาไฮโดรเจนเปอร์ออกไซด์

นายธัญญ์นิธิ ฐิติโชติอริยกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาทันตกรรมบูรณะเพื่อความสวยงามและทันตกรรมรากเทียม
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Thesis Title	Adding ferrous sulphate to hydrogen peroxide tooth bleaching gels reduced the required bleaching time
By	Mr. Thunnithie Thitichoatariyakun
Field of Study	Esthetic Restorative and Implant Dentistry
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ธัญญ์นิธิ ฐิติโชติอริยกุล : ประสิทธิภาพของสารเฟอรัสซัลเฟตในการลดเวลาการฟอกสีฟันของน้ำยาไฮโดรเจนเปอร์ออกไซด์ (Adding ferrous sulphate to hydrogen peroxide tooth bleaching gels reduced the required bleaching time) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ทญ. ดร.ศิริวิมล ศรีสวัสดิ์, 76 หน้า.

วัตถุประสงค์ เพื่อศึกษาประสิทธิภาพของสารเฟอรัสซัลเฟต ซึ่งถ้าใช้เป็นตัวเร่งปฏิกิริยาในน้ำยาฟอกสีฟันไฮโดรเจนเปอร์ออกไซด์แล้ว สามารถทำให้ฟันขาวขึ้น และ ลดระยะเวลาในการฟอกสีฟัน ได้หรือไม่

วิธีการทดลอง ฟันกรามน้อยมนุษย์บน ซึ่งมีสีฟัน เอ หรือ บี เท่านั้น จำนวน 120 ซี่ ถูกนำมาตัดเป็นชิ้น แล้วลงบล็อกอะคริลิกใส แล้วใช้เครื่องวัดสีฟัน อีซี่เชด วี สเปคโตรโฟโต้มิเตอร์ วัดสีฟันตั้งต้น ตามระบบสีของ ซีไออีแอลเอบี ชิ้นส่วนฟันจะถูกแบ่งเป็น 12 กลุ่ม โดยแบ่งแยกตามผลิตภัณฑ์ฟอกสีฟัน การใส่สารเฟอรัสซัลเฟต และ ระยะเวลาในการฟอกสีฟัน 3 ผลิตภัณฑ์ ที่ใช้ในการศึกษานี้ ได้แก่ โอปาเลสเซนต์ บูสท์ โพล่า ออฟฟิศ และ ซูม ไวท์สปีด โดยในแต่ละกลุ่มผลิตภัณฑ์จะมี 4 กลุ่มการทดลอง กลุ่มที่ 1 คือ คอลโทรล กลุ่มที่ 2 จะมีการใส่สารเฟอรัสซัลเฟต โดยใช้เวลาเท่ากับกลุ่มคอลโทรล กลุ่มที่ 3 จะมีการใส่ สารเฟอรัสซัลเฟต แล้วลดระยะเวลาลงประมาณหนึ่งในสี่ กลุ่มที่ 4 จะมีการใส่ สารเฟอรัสซัลเฟต แล้วลดระยะเวลาลงประมาณครึ่งหนึ่ง แล้ววัดสีฟันทันทีหลังจากฟอกสีฟันเสร็จ ข้อมูลระหว่างกลุ่มการทดลองในผลิตภัณฑ์เดียวกัน จะถูกนำมาวิเคราะห์ทางสถิติด้วย สถิติความแปรปรวนแบบทางเดียว และ เปรียบเทียบด้วยวิธีการของเทอกี หรือ แทมเฮน ที่ระดับนัยสำคัญ 0.05

ผลการทดลอง ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติ ของ เดลต้า แอล เอ บี และ อี ระหว่างกลุ่มที่ 1-3 ในทุกผลิตภัณฑ์ มีแค่ เดลต้าบี ในกลุ่มที่ 4 ของ ซูม ไวท์สปีด ที่แตกต่างอย่างมีนัยสำคัญกับ กลุ่ม คอลโทรล โดยค่าความน่าจะเป็น เท่ากับ 0.001 โดยที่ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติ ระหว่างกลุ่ม 1 และ 4 ในผลิตภัณฑ์ โอปาเลสเซนต์ บูสท์ และ โพล่า ออฟฟิศ

สรุป การใส่สารเฟอรัสซัลเฟตในผลิตภัณฑ์ฟอกสีฟันสามารถลดระยะเวลาในการฟอกสีฟันลงได้ อย่างน้อย 25 เปอร์เซ็นต์ ของทุกๆ ผลิตภัณฑ์ที่ใช้ในการศึกษานี้

สาขาวิชา ทันตกรรมบูรณะเพื่อความสวยงามและทันตกรรมรากเทียม ลายมือชื่อนิสิต .....

ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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5775809932 : MAJOR ESTHETIC RESTORATIVE AND IMPLANT DENTISTRY
KEYWORDS: BLEACHING / CHEMICAL ACTIVATION / FERROUS SULPHATE / HYDROGEN PEROXIDE / TOOTH WHITENING

THUNNITHIE THITICHOATARIYAKUN: Adding ferrous sulphate to hydrogen peroxide tooth bleaching gels reduced the required bleaching time. ADVISOR: ASST. PROF. SIRIVIMOL SRISAWASDI, Ph.D., 76 pp.

Objective To investigate the effect of adding ferrous sulphate ($FeSO_4$) as a chemical activator to hydrogen peroxide tooth bleaching gels on color change and treatment time.

Methods One hundred and twenty extracted human upper premolars, hue A or B, were selected. One enamel-dentin specimen was prepared from each tooth. The specimens were divided into twelve groups (n=10), based on bleaching product, $FeSO_4$ and treatment time. Three in-office bleaching products were used in this study: Opalescence® Boost ™, PolaOffice® and ZOOM!® Whitespeed ™. Each product was used per its specific protocol: 1: untreated control (C), 2: $FeSO_4$ added, using the product's specified treatment time (Fe100%), 3: $FeSO_4$ added, using approximately 75% of the specified time (Fe75%), 4: $FeSO_4$ added, using approximately 50% of the specified time (Fe50%). The color was measured before and immediately after bleaching, using a VITA Easyshade® V spectrophotometer based on the CIE-L*a*b* color system. Data between experimental groups of each product were analyzed using one-way ANOVA and Tukey's or Tamhane's test (p=0.05).

Results Color parameter (ΔL, Δa, Δb and ΔE) evaluation indicated that there were no differences between the C, Fe100% and Fe75% of each product. Compared with the C group, only Δb of the ZOOM!® Whitespeed ™ Fe50% group was significantly different (p=0.001). No differences were found between the Opalescence® Boost ™ and PolaOffice® C and Fe50% groups with each material.

Conclusion Adding $FeSO_4$ was effective in reducing the treatment time of these commercial bleaching products by at least 25%.

Field of Study: Esthetic Restorative and Implant Dentistry Student's Signature .....
Advisor's Signature .....
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Background and Rationale

Nowadays, people are highly demanded for esthetic dentistry. Therefore, tooth whitening has gained more popularity during past decades due to its effectiveness and safety. It is a conservative treatment for discolored teeth, compared to other restorative treatments, such as porcelain veneers, crowns or composite resin restoration.[1]

Tooth whitening can be performed via three methods; the first approach is an at-home technique using low concentrated hydrogen peroxide or carbamide peroxide as an oxidizing agent. The second approach is an in-office technique, which requires high concentrated hydrogen peroxide and this method consumes lesser time than the first one.[1] It should be highlighted that single application of in-office bleaching gel on tooth surface is usually not sufficient to achieve a satisfied result. Repeated treatments maybe needed or followed by an at-home bleaching technique.[2, 3] Due to this reason highly concentrated gel is recommended in an in-office technique, since the level of free radicals such as hydroxyl radical (· OH) in a hydrogen peroxide solution increases in a concentration-dependent manner.[4] The last one is by using over-the-counter products, which normally compose of very low concentrated whitening agent compare to other approaches.

Many researchers have tried to improve quality and properties of bleaching gel.

The newly developed products and techniques have been launched into the market and made an in-office bleaching approach more practical and predictable.[1] There are many methods to accelerate a dissociation of hydrogen peroxide and formation of free radicals, for instance, physical activation method by using electric heating device or light sources such as the blue light produced by quartz-tungsten-halogen lamps, plasma arc lamps, light emitting diodes, or LASER.[5] However, it is still inconclusive about effectiveness and safety of these methods.[2] The other method is by using chemical activator, for instance, some enzymes and salt of transition metals such as Fe, Cu, Cr, or Mn can act as a catalyst. Some studies show that the dissociation of metal salts with the bleaching gel is able to improve the dental bleaching effect.[6, 7]

Review of literature

1. Composition of commercial bleaching agents

In general, bleaching agents contain both active and inactive ingredients. The active ingredients include hydrogen peroxide or carbamide peroxide compounds. Carbamide peroxide is a complex of urea and molecular hydrogen peroxide. On the other hand, hydrogen peroxide can only exist by itself in aqueous form and is generally unstable except at relatively low pH.

The major inactive ingredients may include thickening agents, carrier, surfactant, pigment dispersant, preservative and flavoring.[5]

1.1 Thickening agents: Carbopol (carboxypolymethylene) is the most

commonly used thickening agent in bleaching materials. Its concentration is usually between 0.5% and 1.5%. This high-molecular-weight polyacrylic acid polymer is needed for two main purposes. Firstly, it increases the viscosity of the bleaching material to gain better retention of the bleaching gel in the dental bleaching tray or on the tooth surface. Secondly, it can increase the active oxygen-releasing time of the bleaching material by up to 4 times.[8]

The ability to obtain a stable gel is particularly difficult where it is desired to use a polymeric tackifying agent such as carboxypolymethylene. Carboxypolymethylene is unable to maintain its desired stickiness and gelling capabilities over time when mixed with either concentrated acidic aqueous hydrogen peroxide solutions or strong basic solutions used to neutralize the acidic hydrogen peroxide solution. Where it is desired to mass produce highly concentrated yet stable hydrogen peroxide bleaching compositions, it has been necessary to entirely replace carboxypolymethylene with non-polymeric thickeners such as fumed silica in order to obtain stable gels.[9, 10]

1.2 Carrier: Glycerin and propylene glycol are the most commonly used carriers, needed to maintain moisture and help to dissolve other ingredients. Other carriers that can be used are sorbitol, polypropylene glycol and polyethylene glycol.

1.3 Surfactant and pigment dispersant: Gels with surfactant or pigment dispersant may be more effective than those without them.[5] The surfactant acts as a surface-wetting agent, which allows the active bleaching ingredient to diffuse. Whereas, the pigment dispersant keeps pigments in suspension.

1.4 Preservative: Methyl, propylparaben and sodium benzoate are commonly used as preservative substances. They have the ability to prevent the growth of bacteria in bleaching material. In addition, these agents can accelerate the breakdown of hydrogen peroxide by releasing transitional metals such as iron, copper, and magnesium.

1.5 Flavoring: Flavoring is a substance used to improve the taste and the consumer acceptance of bleaching products, for example, peppermint, spearmint, winter-green, sassafras, anise, and a sweetener such as saccharin.

1.6 Light energy absorbing agents (optional): Carotene, etc.

Hydrogen peroxide is more stable in acid solutions than in basic solutions. When hydrogen peroxide is to be stored, weak acid is added to the solution to prevent it from decomposing.[9]

Some companies sell tooth bleaching agents that comprises of two parts. A first part includes a stable concentrated dental bleaching agent at low pH and the second part includes a base dispersed within a stable gel that, when mixed with the first part, raises the pH of the mixed composition to an acceptable level and, optionally, in order to destabilize the dental bleaching agent and trigger the release of oxygen radicals to accelerate bleaching activity. The main purpose of the two-part system is to keep bleaching potency. This is because storing active ingredient at very low pH in the first part can reduce destabilization of hydrogen peroxide and to maintain gel form having a desired range of stickiness and/or viscosity during transportation and subsequent storage. Upon mixing the two parts together, the bleaching gel composition having a

desired bleaching potency can be formed. This allows the two-part composition to be manufactured and then shipped and stored as desired prior to mixing the parts together without any significant loss in bleaching potency and the break down of the gel that includes the strong base.[10]

2. Mechanism of tooth bleaching

The mechanism of tooth bleaching by hydrogen peroxide is not well understood. In-office and home bleaching gel contains hydrogen peroxide or its precursor, carbamide peroxide. Bleaching with carbamide peroxide differs from hydrogen peroxide. First, carbamide peroxide breaks down into urea and hydrogen peroxide. Ten percent carbamide peroxide breaks down into two products: 6.6% urea + 3.4% hydrogen peroxide. The urea further breaks down into carbon dioxide and ammonia. The hydrogen peroxide breaks down into H_2O + O_2 through an intermediary perhydroxyl free radical (HOO ·). Other conditions can give rise to free radical formation, for example, by homolytic cleavage of either an O–H bond or the O–O bond in hydrogen peroxide to give H· + · OOH (perhydroxyl radical) and 2 · OH (hydroxyl radical), respectively. Under photochemical reactions initiated by light or lasers, showing that the formation of hydroxyl radicals from hydrogen peroxide has increased.[11] Hydrogen peroxide is an oxidizing agent that, as it diffuses into the tooth, dissociates to produce unstable free radicals, which are hydroxyl radical (HO ·), perhydroxyl radical (HOO ·), perhydroxyl anion (HOO–), and superoxide anion (OO · –), which will attack organic pigmented molecules in the spaces between the inorganic salts in tooth enamel by attacking double bonds of chromophore molecules within tooth tissues.[12-15] The change in double-bond conjugation results in smaller, less heavily

pigmented constituents, and there will be a shift in the absorption spectrum of chromophore molecules; thus, bleaching of tooth tissues occur. The simpler molecules formed by the bleaching process reflect more light, changing the tooth's appearance to a lighter shade. Another theory for the mechanism of action of a peroxide is that it opens the carbon-ring of pigment molecules, converting them to chain that are lighter in color. Yellow double-bond color compounds are converted into almost colorless hydroxyl compounds.[4, 15]

It has been suggested that H_2O_2 and · OH do not influence the inorganic tissue of dentin but attack the organic component of dentin. These facts suggest that · OH has the main role in tooth bleaching with H_2O_2.[4]

In one SEM study found that higher concentration of H_2O_2 and longer application time increased the damage to intertubular dentin and peritubular dentin. This showed that destruction of dentin occurred as a result of H_2O_2 application. As the X–ray-diffraction revealed that the structure of HAP was not changed by H_2O_2 treatment. These results showed that · OH and H_2O_2 did not damage the inorganic tissue of the tooth, but did damage other substances such as organic tissue.[4]

3. Types of tooth whitening

There are three fundamental approaches for tooh bleaching.[16]

3.1 In-office or power bleaching

In-office-bleaching procedures seem to be an appropriate alternative to home-

bleaching applications with trays, foils or gels, especially in the case of very severe discolorations, discolorations of single teeth, lack of patient compliance or if a rapid treatment is desired. In-office-bleaching could also be applied as a kind of boost therapy, thereby initiating the bleaching process, which might be continued afterwards by home-bleaching procedures.[1]

High concentration of hydrogen peroxide, normally around 17–50%, is used.[16, 17] An operator has complete control and the ability to stop a treatment when a desired result is achieved or when any problem happens throughout the procedure; for instance, a patient cannot tolerate a treatment due to tooth sensitivity.

In this procedure, the bleaching gel is applied to the teeth after protection of soft tissues. The peroxide will further be activated (or not) by heat or light for around one hour in a dental office.[18, 19] Different types of curing lights including; halogen curing lights, Plasma arc lamp, Xe–halogen light (Luma Arch), Diode lasers (both 830 and 980 nm wavelength diode lasers), or Metal halide (Zoom) light can be used to activate the bleaching gel or accelerate the whitening effect. It should be noted that a single application of an in-office treatment is usually not sufficient to achieve an optimal bleaching result, repeated the treatment procedure during an appointment or multiple appointments may be needed. [3, 17, 20, 21]

Many commercial in-office bleaching products claimed that their products do not need light activated such as Opalescence® Boost ™ PF 40% (Ultradent), Niveous® (Shofu), PolaOffice® (SDI Limited), etc. For Niveous® and PolaOffice®, light activation is optional. Opalescence® Boost ™ PF 40% contains active ingredients, which are 40% hydrogen peroxide and PF (potassium nitrate and fluoride). The

company claims that potassium nitrate helps reducing sensitivity and fluoride helps reducing caries and strengthening enamel. Opalescence Boost uses a unique syringe-to-syringe mixing method to ensure fresh, effective product for every application and no light is needed. After mixing the product, the pH will be 7.

Higher concentration bleaching agent can produce more peroxide radicals, resulting in a faster whitening process. However, this rapid process of bleaching may increase side effects such as tooth sensitivity, gingival irritation, throat irritation, and nausea.[17]

When assessing the color of bleached teeth, dentists should also keep in mind that teeth might be mildly dehydrated during the bleaching therapy with bleaching gel. The dehydration is probably greater when rubber dam isolation is used, which might in turn increase the brightening effect temporarily. This positive effect on tooth lightening is reversed due to the rehydration of the teeth in the following period. This means that both dentists and patients should not be over enthusiastic with the result of an in-office-bleaching therapy immediately after completion of the treatment.[3]

3.2 At home or dentist supervised night guard bleaching

Low concentration of whitening agent (10–20% carbamide peroxide, which equals 3.5–6.5% hydrogen peroxide) is used. Haywood and Heymann recommended the use of 10% carbamide peroxide (equivalent to 3.6% hydrogen peroxide) gel applied with a thin plastic individualized tray for each patient and its use for several hours a day at home for a period of 1-2 weeks.[22, 23] In general, it is recommended that the 10% carbamide peroxide is used for 8 h per day, and the 15–20% carbamide peroxide

is used for 3–4 h per day. The patients carry out this treatment by themselves but a dentist should supervise it during recall visits. The bleaching gel is applied to teeth through a custom-fabricated mouth guard worn at night for at least 2 weeks. This technique has been used for many decades and is probably the most widely used.[20]

The at home technique offers many advantages such as self-administration by the patient, less chair-side time, high degree of safety, fewer adverse effects, and low cost. However, patient compliance is mandatory.[24, 25] In addition, color change is dependent on how often the patients wear the bleaching trays. However, the results are sometimes less than ideal because some patients do not remember to wear them every day. In contrast, excessive use by overzealous patients reported to be as high as 67% is also possible, which frequently causes thermal sensitivity.[22]

Some authors suggested that the use of an in-office bleaching gel, which is followed, by an at-home gel with tray are more effective in color changing than without following the at-home tray bleaching process.[3]

3.3 Bleaching with over-the-counter (OTC) products

These products are composed of low concentrated whitening agent, which is normally in the range of 3–6% hydrogen peroxide, and are self-applied to tooth surfaces via gum shields, strips, or paint-on product formats.[26] They are also available as whitening dentifrices, pre-fabricated trays, whitening strips, and toothpastes.[26] It should be applied twice per day for up to 2 weeks. However, these products maybe questioned about their safety since some products are not accepted by the Food and Drug Administration.

4. Means to increase bleaching efficiency

4.1 Physical activation method

4.1.1) Electric heating device

Heat accelerates the permeation of hydrogen peroxide into enamel and dentine.[7, 27, 28] In addition, heat may be hazardous to pulp cells because it might aggravate pulp damage from hydrogen peroxide.[29] It also can increase an occurrence of post-operative sensitivity and the risk of irreversible pulpal damage.[2] Although increasing in penetration can improve the bleaching result of dentine, the diffusion of peroxide into the pulp also leads to an oxidative stress that can negatively affect the pulpal cell metabolism.[2, 30] Moreover, after a thermo-catalytic bleaching procedure in non-vital tooth bleaching, the probability of cervical reabsorption was increased.[8]

4.1.2) Light sources such as blue light produced by quartz-tungsten-halogen lamps, plasma arc lamps, light emitting diodes, or LASER.[2]

The potential of increased efficacy of bleaching gels by light has been questioned, and some studies show that the benefit of its use is limited or in no significant level.[2] Furthermore, light application may cause a temperature rise.[12]

If light is projected onto a bleaching product, such as a bleaching gel, a small fraction is absorbed and the energy is converted into heat. Most likely, this is the main mechanism of action of all light-activated bleaching procedures. In order to increase

light absorption and, as a result, heat conversion, some bleaching products are mixed with specific colorants, e.g. carotene. The orange-red color of carotene increases the absorption of blue light. In order to increase the absorption of red and infrared light, small silica particles in the nm- or lower μm- scale may be added, which gives these products a bluish appearance.[10]

The degree of tooth brightening achieved with activated bleaching methods depends on the mode and type of activation, but also on the composition of the applied bleaching substance. There is one study which tested that using the bleaching gel Opalescence Xtra (35% H_2O_2) with an application of halogen light resulted in the highest color difference when compared to the non-activated application mode. However, when using the bleaching gel QuickWhite (35% H_2O_2) with infrared (and not the halogen) light was the most effective. It should also be noted that the infrared light caused the highest tooth temperature increase in this study. Due to this finding, selection of a specific combination of bleaching agent and light that demonstrates good color change and little temperature elevation for in-office-bleaching is recommended.[31]

Neither the application of light, nor the application of heat increased the decomposition rate of H_2O_2 . Although, the temperature of a bleaching gel with reddish appearance containing carotene increased considerably, the temperature increase was not high enough to accelerate peroxide decomposition significantly.[3] Past existing literature pointed that acceleration of disassociation of hydrogen peroxide by heat, light or LASER may have a deleterious effect on pulpal tissue due to an increase of intra-pulpal temperature exceeding the critical value of 5.5 $^{\circ}$C.[2] An intra-pulpal

temperature increase of 5.5 °C is nowadays regarded as the threshold value, which should not be exceeded to avoid irreversible pulp damage.[32]

4.2 The use of chemical activators

Some enzymes and salts of transition metals, such as Fe, Cu, Cr, or Mn can act as a catalyst, promoting the dissociation of hydrogen peroxide molecule and formation of free radicals. Many studies show that the association of metal salts with bleaching gel is able to improve dental bleaching result.[6, 32-38] Other studies also report the use of enzyme, such as catalase, peroxidase, dopamine and mulberry root extract as a chemical activator.[33, 37] One of the advantages of using chemical activator is the lack of pulpal heating during procedure, thus reducing harmful potential of the in-office bleaching agent. In addition, it can have some influence on H_2O_2 penetration since the reaction is expected to happen between it and the chemical activator.[6, 38] There is an increase in the formation of free radicals on the tooth surface, resulting in a specific and fast reaction with the local chromophores, improving the bleaching effectiveness and reducing the quantity of unreacted H_2O_2 available for diffusion into the dental structure.[39]

Chen et al. (1993) found that iron (III) chloride can accelerate the release of hydrogen but its brown color stained the solution, making it unacceptable for dental bleaching.[32] Gaffar et al. (1997) reported that The ΔE values of teeth bleached with peroxygen compounds either hydrogen peroxide, urea peroxide or sodium percarbonate mixed with the activator manganese gluconate substantially increased the tooth whitening efficacy about 1.5 to 8 times.[36] Zhao et al. (2003) suggested the use of

mulberry root extract 0.1% to 1% as a whitening catalyst for enhancing effectiveness, efficiency and stability of tooth whitening process of the whitening compound.[37] Mulberry root extract contains phenyl flavonoid, which is effective as human skin whitener. In contrast to Torres et al. (2010), they concluded that mulberry root extract as a chemical activator is not effective. They also found that the addition of ferrous sulphate or manganese gluconate in 35% hydrogen peroxide bleaching gel produced a statistically significant reduction of hydrogen peroxide penetration into the pulp chamber and higher mean of ΔE compare with the control group without chemical activator. And just small amount of ferrous sulphate was used, only 0.004% w/w, which was the lowest amount compared to other chemical activators.[6] This study determined that adding more than 0.004% w/w $FeSO_4$ to a bleaching gel resulted in excessive bubble formation that caused the gel to breakdown, thus, the concentration of 0.004% w/w was used in the present study.[6] Travassos et al. (2010) also showed better ΔE results by adding ferrous sulphate in 35% hydrogen peroxide bleaching gel.[38]

These metal salts used as the chemical activators are not toxic when used in the right concentration, since they are used as nutritional supplement in human diet.[6] Though, there is no scientific proof about safeness of these metal salts when they are mixed with hydrogen peroxide used in tooth whitnening, except ferrous sulphate. Duque et al. (2014) evaluated that the effect of ferrous sulphate on the transenamel and transdentinal cytotoxicity of the bleaching gel to two culture lineages obtained from dental pulp in an *in vitro* study. The viability of odontoblast-like cells (MDPC-23) from tooth bleached with 35% hydrogen peroxide gel mixed with ferrous sulphate was 15% higher than the viability observed for those bleached with 35% hydrogen peroxide gel alone. For Human dental pulp cells (HDPCs), no significant difference in cell viability

was observed between both groups. However, for both cell lineages, a significant reduction in cell viability was observed when either the group bleached with 35% hydrogen peroxide mixed with ferrous sulphate or 35% hydrogen peroxide alone when compared to no treatment group. They also found a significant increase in the value of ΔE around 30% in hydrogen peroxide mixed with ferrous sulphate group.[39] However, most studies of chemical activators did not use commercial tooth whitening product in their studies.[6, 32-37]

Ferrous sulfate is a salt with the formula $FeSO_4$. It is used medically to treat iron deficiency, and also for industrial applications. It can be used in the form of Fenton's reagent, which is a solution of hydrogen peroxide and an iron catalyst that is used to oxidize contaminants or waste water. Fenton's reagent can be used to destroy organic compounds such as trichloroethylene (TCE) and polychloroethylene (PCE). It was developed in the 1890s by Henry John Horstman Fenton as an analytical reagent.[40]

Iron can react with hydrogen peroxide and accelerate the production of free radicals, to obtain a faster, more efficient bleaching result.[6, 40] Iron (II) is oxidized by hydrogen peroxide to iron (III), forming a hydroxyl radical and a hydroxide ion in the process. Iron (III) is then reduced back to iron (II) by another molecule of hydrogen peroxide, forming a perhydroxyl radical and a proton. The net effect is a disproportionation of hydrogen peroxide to create two different oxygen-radical species, with water ($H^+ + OH^-$) as a byproduct.

$$(1)\ Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO\bullet + OH^-$$

$$(2)\ Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HOO\bullet + H^+$$

The free radicals generated by this process then engage in secondary reactions. For example, the hydroxyl is a powerful, non-selective oxidant. Oxidation of an organic compound by Fenton's reagent is rapid and exothermic and results in the oxidation of contaminants to primarily carbon dioxide and water.[39, 40]

Reaction (1) was also suggested by Haber and Weiss in the 1930s as part of what would become the Haber–Weiss reaction. Iron (II) sulfate is typically used as the iron catalyst. The Haber–Weiss's reaction generates hydroxyl radicals from superoxide and hydrogen peroxidase shown in the following equations: $\bullet O_2^- + H_2O_2 \rightarrow \bullet OH + HO^- + O_2$. The reaction is very slow, but can also be catalyzed by iron.[40]

5. Color Measurement

Visual color matching is subjective and influenced by variety of factors. Observer-related variables such as sex, color blindness, experience and eye fatigue could influence the final results.[41, 42] A more standardized assessment of tooth color may be possible with the use of special devices such as colorimeters, spectrophotometers, digital cameras, spectroradiometers, or combinations of these.[43-45] Colorimeters measure tristimulus color values from light reflected by an object after it has passed through green, red, and blue filters.[46, 47] They are useful in quantifying color differences among specimens. Various factors such as curvature of the tooth surface, translucency, nonuniform color properties of teeth, edge-loss effects, aging of filters, and metamerism may affect the performance of these instruments.[48, 49] Studies comparing visual shade evaluation with colorimetric assessment have reported wide variation.[45, 50-52]

Spectrophotometers measure the spectral reflectance or transmittance curve of an object. They have a longer working life than colorimeters and are useful in the measurement of surface color.[46] More accurate results were reported with the use of dental spectrophotometer compared with visual methods.[53-57] Intraoral spectrophotometer can be used for measuring and matching tooth color both *in vivo* and *in vitro.* [12, 58] Studies have demonstrated that the performance of the VITA Easyshade® spectrophotometer (Vita Zahnfabrik, Bad Säckingen, Germany) was more accurate than that of a naked eye.[53, 59, 60] VITA Easyshade® V spectrophotometer (Vita Zahnfabrik, Bad Säckingen, Germany) provides accurate shade determination for natural dentition and a variety of restorations. The instrument covers the broad spectrum of the VITA SYSTEM 3D-MASTER® shades, including the BLEACHED SHADE GUIDE shades as well as the VITA classical A1-D4® shades. It also translates data into CIE-L*a*b* system. This device measures in small spot area approximately 5 mm diameter of optical tip. The advantage of Vita Easyshade® compact is portable, small, battery operated, and contact type.[43]

Research Questions

1. Does ferrous sulphate improve efficacy of hydrogen peroxide commercial tooth bleaching gel?

2. Does ferrous sulphate help reducing operating time of in-office commercial tooth whitening?

Research objectives

The aim of this study was to investigate effects of adding ferrous sulphate to hydrogen peroxide commercial tooth bleaching products on color change and bleaching time required.

Statement of hypothesis

Null hypothesis

1. There was no significant difference in tooth color between teeth bleached with hydrogen peroxide gel containing $FeSO_4$ and teeth bleached with hydrogen peroxide gel alone

2. There was no significant difference in tooth color between teeth bleached with hydrogen peroxide gel containing $FeSO_4$ using reduced treatment time and teeth bleached with hydrogen peroxide gel alone at the manufacturer's recommended time.

Alternative hypothesis

1. There was a significant difference in tooth color between teeth bleached with hydrogen peroxide gel containing $FeSO_4$ and teeth bleached with hydrogen peroxide gel alone

2. There was a significant difference in tooth color between teeth bleached with hydrogen peroxide gel containing $FeSO_4$ using reduced treatment time and teeth bleached with hydrogen peroxide gel alone at the manufacturer's recommended time.

Conceptual framework

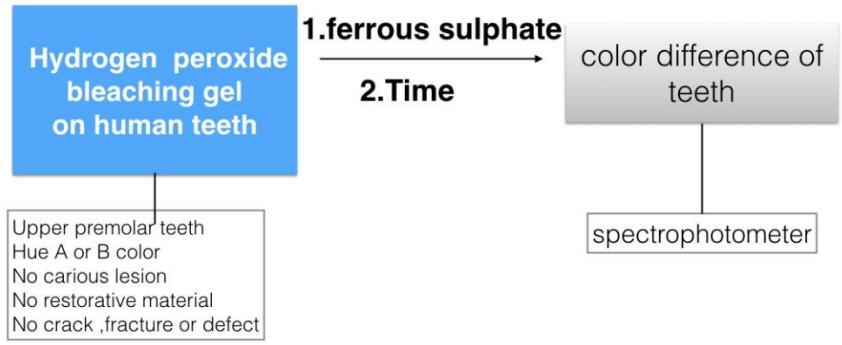


Figure 1

Conceptual framework.

Type of study

Experimental study

Research methodology

Materials and methods

Materials

1. Opalescence® Boost ™ PF 40% (40% hydrogen peroxide) - 4 syringes (Ultradent, South Jordan, UT, USA)

2. ZOOM!® Whitespeed ™ (25% hydrogen peroxide) - 4 boxes (Discus Dental/Philips Oral Healthcare, Stamford, CT, USA)

3. PolaOffice® (35% hydrogen peroxide) - 4 boxes (SDI Limited, Victoria, Australia)

4. Clear Epoxy Resin (Super Silicone & Resin Art, Bangkok, Thailand)

5. Ferrous sulphate (Merck KGaA, Darmstadt, Germany)

6. Fluoride-free pumice (3M ESPE, St. Paul, MN, USA)

7. 0.1% Thymol solution (Merck KGaA, Darmstadt, Germany)

8. Absorbent gauze sponge (Union Science, Chiangmai, Thailand)

9. 600,800,1,000 and 1200-grit silicon carbide paper (3M ESPE, St. Paul, MN, USA)

10.Acrylic sheet, 1 mm. thick (3A MEDES, Goyang-si, Gyeonggi-do, Republic of Korea)

11. Acrylic sheet, 2 mm. thick (3A MEDES, Goyang-si, Gyeonggi-do, Republic of Korea)

12.Ligature elastic: Patented design 33898 (Dent-mate, Bangkok, Thailand)

Equipments

1. Vita Easyshade V spectrophotometer (Vita Zahnfabrik, Bad Säckingen, Germany)

2. Stereomicroscope: ML 9300 (Meiji, Saitama, Japan)

3. Polishing cup (3M ESPE, St. Paul, MN, USA)

4. NTI sintered diamond disc: D354-220 (Kerr, Orange, CA, USA)

5. Digital vernier caliper (Mitutoyo digital caliper, Mitutoyo corp., Kanogawa, Japan)

6. Carbide round bur (2 mm. in diameter): 400276-JK5 (Kerr, Orange, CA, USA)

7. Magnetic stirrer: Stuart Scientific (Bibby Scientific, Burlington, NJ, USA)

8. Magnetic stirring bar: 3 mm. in diameter and 5 mm. in length (ProSciTech Pty Ltd, Queenland, Australia)

9. Glass beaker: 5 ml. (Union Science, Chiangmai, Thailand)

10.Vita classical A1-D4 shade guide (Vita Zahnfabrik, Bad Säckingen, Germany)

11.Trephine drill: 7 mm. in diameter (Hu-friedy, Chicago, IL, USA)

12.Trephine drill: 5 mm. in diameter (Hu-friedy, Chicago, IL, USA)

Methods

1. Sample description

Sample size recommendation, according to ISO 28399: Dentistry-Products for external tooth bleaching[61], is six to ten specimens per group.

The sample size per group was ten in this study. For twelve groups, one hundred and twenty freshly extracted human upper premolar teeth were collected after informed consent has been obtained under a protocol approved by the Ethics Committee of the Faculty of Dentistry, Chulalongkorn University (Study Code: HREC-DCU 2016-045). Teeth were cleaned thoroughly and stored in 0.1% thymol (Merck KGaA, Darmstadt, Germany) at 4°C for up to 3 months after extraction.

2. Preparation of samples

Freshly extracted intact non-carious and non-restored human upper premolars were stored in 0.1% thymol solution (Merck KGaA, Darmstadt, Germany) at room temperature. All of them were examined under Stereomicroscope (ML 9300, Meiji, Saitama, Japan) at the magnification of 20x to detect enamel crack or fracture, carious, stain, and other defects. The teeth in this study were of only hue A or B, as compared with the Vita classical A1-D4® shade guide (Vita Zahnfabrik, Bad Säckingen, Germany). Teeth with cracks, imperfections or not hue A or B were discarded, and

replaced with teeth meeting these specifications. A NTI® sintered diamond disc (D352-220; Kerr, Orange, CA, USA) was used to obtain 5 mm x 5 mm enamel/dentin specimens from the labial surface of each tooth. The dentin side of the specimen was ground flat with 600, 800, 1,000, 1,200-grit silicon carbide sand paper (3M ESPE, St. Paul, MN, USA), under a constant water flow until the remaining dentin layer was 1 mm thick.

Twelve acrylic sheets, 6 cm x 12 cm and 2 mm thick (3A MEDES, Goyang-si, Gyeonggi-do, Republic of Korea), were punched with a 7 mm diameter trephine drill (Hu-friedy, Chicago, IL, USA) to create ten 7 mm internal diameter holes in the middle area of the acrylic sheets. (Figure 2)

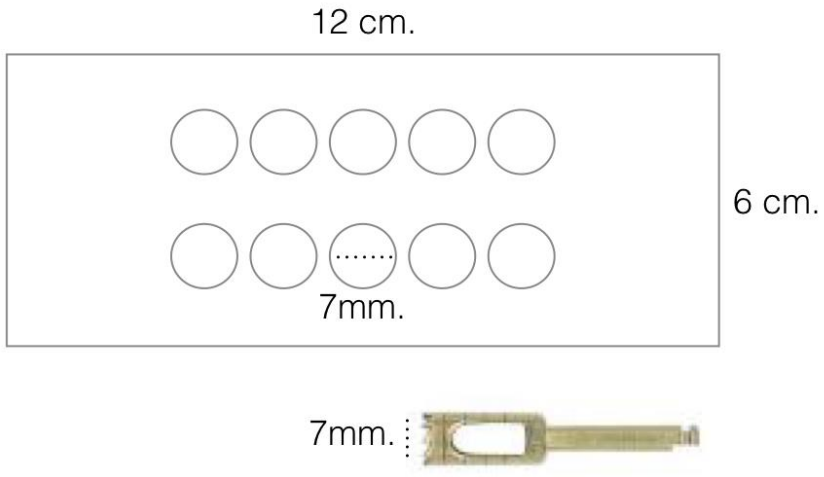


Figure 2

Ten 7 mm internal diameter holes were drilled in the middle area of the acrylic sheet.

Ten enamel/dentin specimens per sheet were then randomly positioned in the center of the holes, dentin side down, and embedded in clear epoxy resin (Super Silicone & Resin Art, Bangkok, Thailand). (Figure 3) The enamel side of each enamel/dentin block was ground flat using 600, 800, 1,000 and 1,200-grit sand paper (3M ESPE, St. Paul, MN, USA), under a constant water flow, until the enamel/dentin block was 2 mm thick. (Figure 4) in accordance with ISO 28399 (Dentistry – Products for external tooth bleaching).[61] The thickness of each block was measured using a digital vernier caliper (Mitituyo digital caliper, Mitutoyo Corp., Kanogawa Japan).

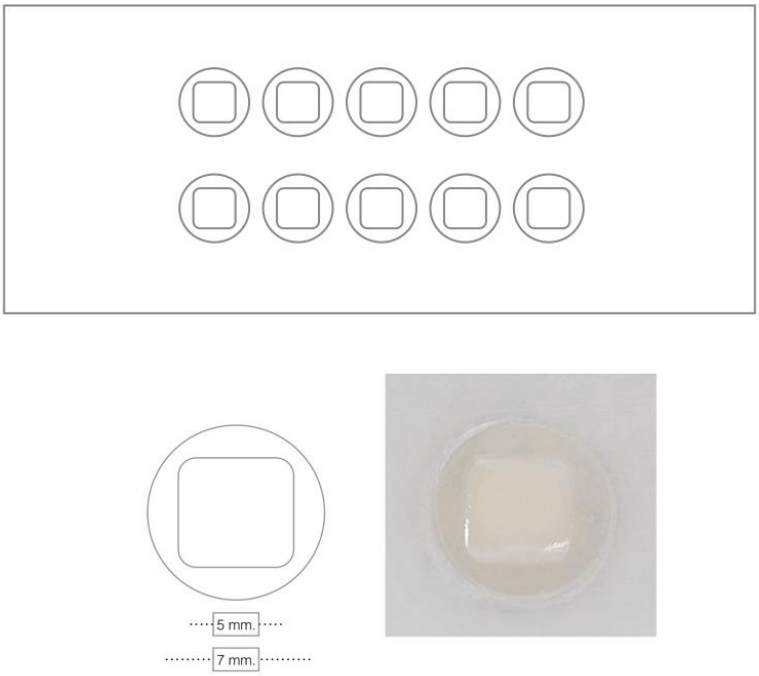


Figure 3

Ten enamel/dentin specimens per sheet were then randomly positioned in the center of the holes and embedded in clear epoxy resin.

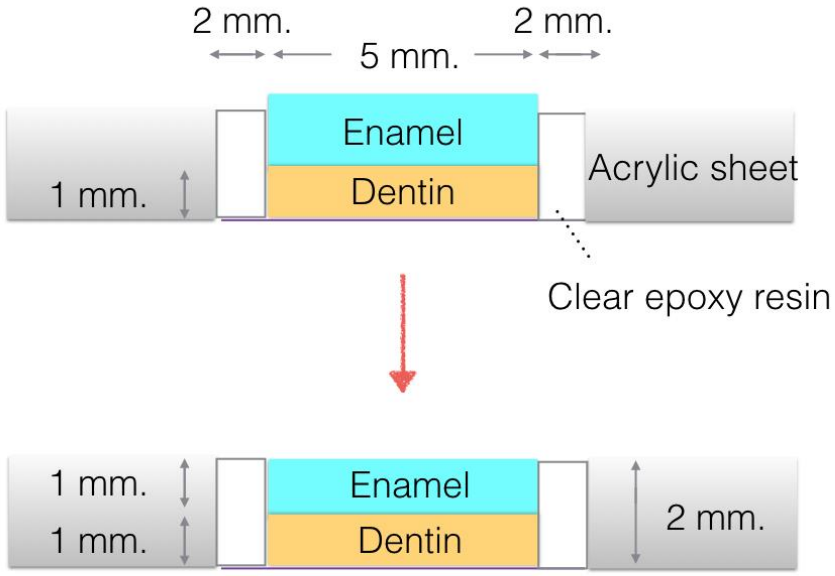


Figure 4

The enamel side of each enamel/dentin block was ground flat, until the thickness of enamel/dentin block was 2 mm thick.

A 2 mm diameter carbide round bur (Kerr, Orange, CA, USA) was used to drill a hole 1 mm away from each specimen. The holes served as registration marks to assure that repeated color readings were performed at the same position. (Figure 5)

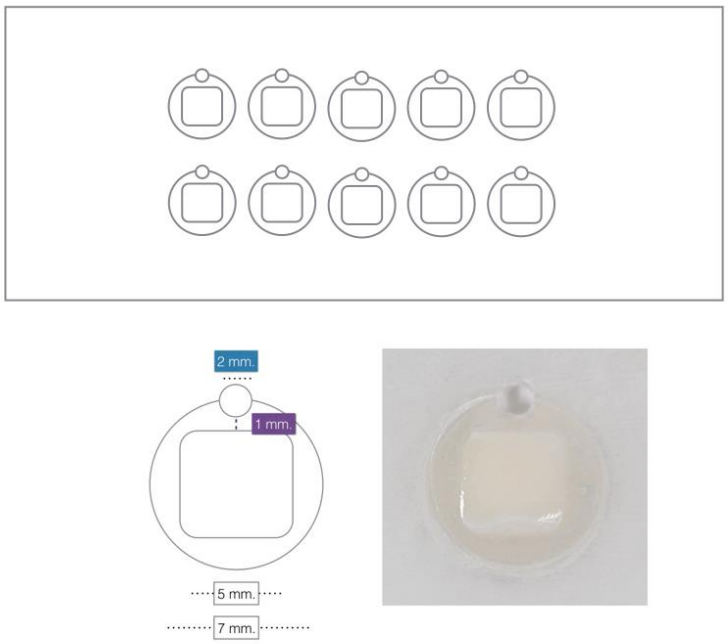


Figure 5

A 2mm. diameter hole was drilled 1 mm away from each specimen.

3. Allocation technique

The twelve acrylic sheets were randomly allocated into twelve groups, according to three commercial bleaching products: Opalescence® Boost ™ (OB) (Ultradent, South Jordan, UT, USA), PolaOffice® (PO) (SDI Limited, Victoria, Australia) and ZOOM!® Whitespeed ™ (ZW) (Discus Dental/Philips Oral Healthcare, Stamford, CT, USA), the addition of $FeSO_4$ (Merck KGaA, Darmstadt, Germany) as a chemical activator to the gel, and the amount of time the gel was applied (Table 1) Each product had 4 experimental groups: 1: untreated control (C), 2: $FeSO_4$ added, using the

product's specified treatment time (Fe100%), 3: $FeSO_4$ added, using approximately 75% of the specified time (Fe75%), 4: $FeSO_4$ added, using approximately 50% of the specified time (Fe50%).

Table 1 *The three bleaching products and the experimental groups evaluated in this study*

Bleaching Product	Group	$FeSO_4$	Treatment time (minutes x cycles)
Opalescence® Boost ™ (OB)	C	No	20x2= 40 min
	Fe100%	Yes	20x2 = 40 min
(40% Hydrogen peroxide/	Fe75%	Yes	15x2 = 30 min
Lot.BC25B)	Fe50%	Yes	10x2 = 20 min
PolaOffice® (PO)	C	No	8x4 = 32 min
	Fe100%	Yes	8x4 = 32 min
(35% Hydrogen peroxide/	Fe75%	Yes	6x4 = 24 min
Lot.1082345)	Fe50%	Yes	4x4 = 16 min
ZOOM!® Whitespeed ™ (ZW)	C	No	15x3 = 45 min
	Fe100%	Yes	15x3 = 45 min
(25% Hydrogen peroxide/	Fe75%	Yes	10x3 = 30 min
Lot.22-3764)	Fe50%	Yes	5x3 = 15 min

4. Initial color measurement

Prior to treatment, baseline color of each specimen was assessed under standard conditions based on the CIE-L*a*b* system using a VITA Easyshade® V spectrophotometer (Vita Zahnfabrik, Bad Säckingen, Germany). (Figure 6)

Figure 6

VITA Easyshade® V spectrophotometer.

An elastic ligature (Patented design 33898; Dent-mate, Bangkok, Thailand) was prepared as shown in Figure 7. This was then placed on the tip of the VITA Easyshade® V spectrophotometer (Figure 8)

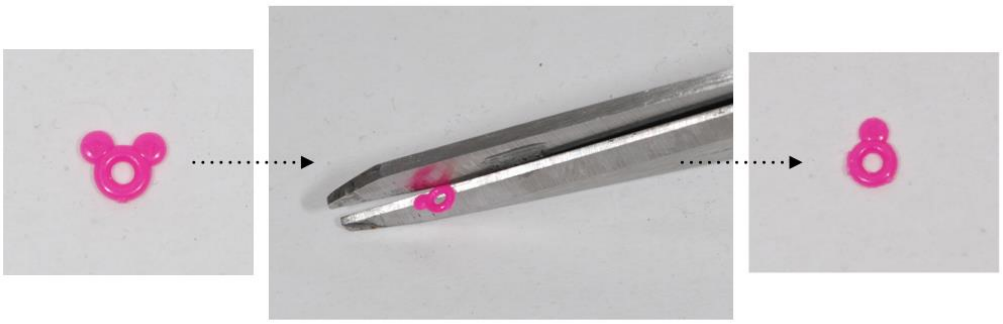


Figure 7

Elastic ligature was prepared.

Figure 8

Elastic ligature was then placed on the tip of the VITA Easyshade® V spectrophotometer.

Each sample was dried with an absorbent gauze sponge (Union Science, Chiangmai, Thailand) for 3 seconds, and the color was immediately measured. A neutral gray cloth was used as a background. During the measurements, the instrument tip was placed perpendicular to, and in complete contact with enamel surface of the enamel/dentin block. The 2 mm hole near each enamel/dentin specimen was used to register the sample position for color reading (Fig. 2g), by placing the 2 mm circle of the elastic ligature on top of the 2 mm hole of the acrylic sheet that was next to each

enamel/dentin specimen. (Figure 9)

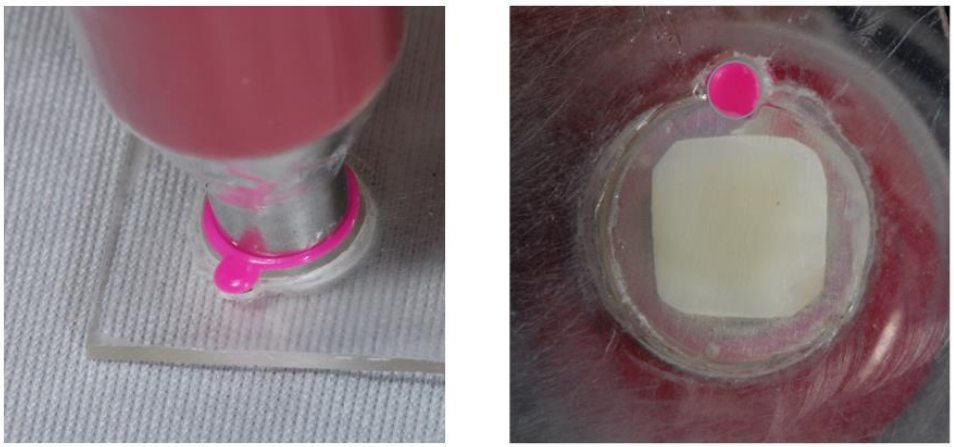


Figure 9

The 2 mm hole near each enamel/dentin specimen was used to register the sample position for color reading, by placing the 2 mm circle of the elastic ligature on top of the 2 mm hole of the acrylic sheet that was next to each enamel/dentin specimen.

The color of each sample was measured 5 times and averaged. The L*, a*, b* measurements served as the baseline color of each sample. All measurements were made by single investigator. Before any measurement, the device was calibrated by

placing a probe tip on the instrument's calibration port aperture, following the manufacturer's instruction.

5. Bleaching procedure

Before bleaching procedure, the enamel/dentine blocks were cleaned using a prophylaxis paste (fluoride-free pumice; 3M ESPE, St. Paul, MN, USA), with a polishing cup (3M ESPE, St. Paul, MN, USA) using a slow speed handpiece. The paste was removed using a water spray. Bleaching gels were then mixed according to manufacturer's recommendation, and transferred to a 5 ml glass beaker (Union Science, Chiangmai, Thailand). In the appropriate groups, 0.004% (w/w) $FeSO_4$ (Merck KGaA, Darmstadt, Germany) was added to the gel and mixed on a magnetic stirrer (Bibby Scientific, Burlington, NJ, USA) using a magnetic stirring bar, 3 mm in diameter and 5 mm in length (ProSciTech Pty Ltd, Queenland, Australia), for 1 minute. Only ZW used light activation (light machine obtained from the gel manufacturer) when the gel was placed on the samples. The distance from light source to the samples was 4.4 cm.

A 6 cm x 12 cm, 1 mm thick acrylic sheet (3A MEDES, Goyang-si, Gyeonggi-do, Republic of Korea) was drilled with 5 mm diameter trephine drill (Hu-friedy, Chicago, IL, USA) to create ten 5 mm internal diameter holes in the middle area of the acrylic sheet. (Figure 10)

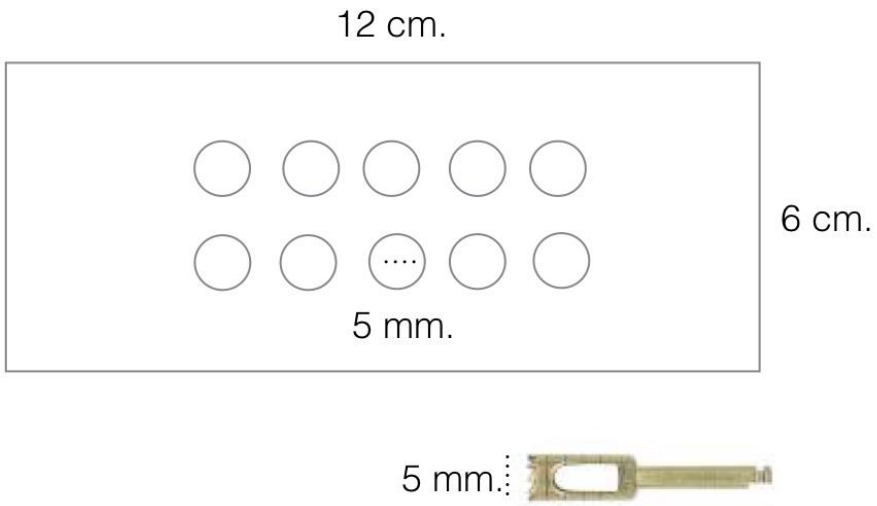


Figure 10

Ten 5 mm internal diameter holes were drilled in the middle area of the acrylic sheet.

This acrylic sheet was placed on top of the enamel/dentin specimen acrylic sheet. Therefore, a 1 mm layer of bleaching gel was applied on the enamel surface of each specimen. (Figure 11) An aspiration cannula was used to remove the gel between each application, simulating a clinical bleaching treatment. The specimens were then washed with a water spray. And then, the enamel/dentin specimen acrylic sheet was stored in distilled water.

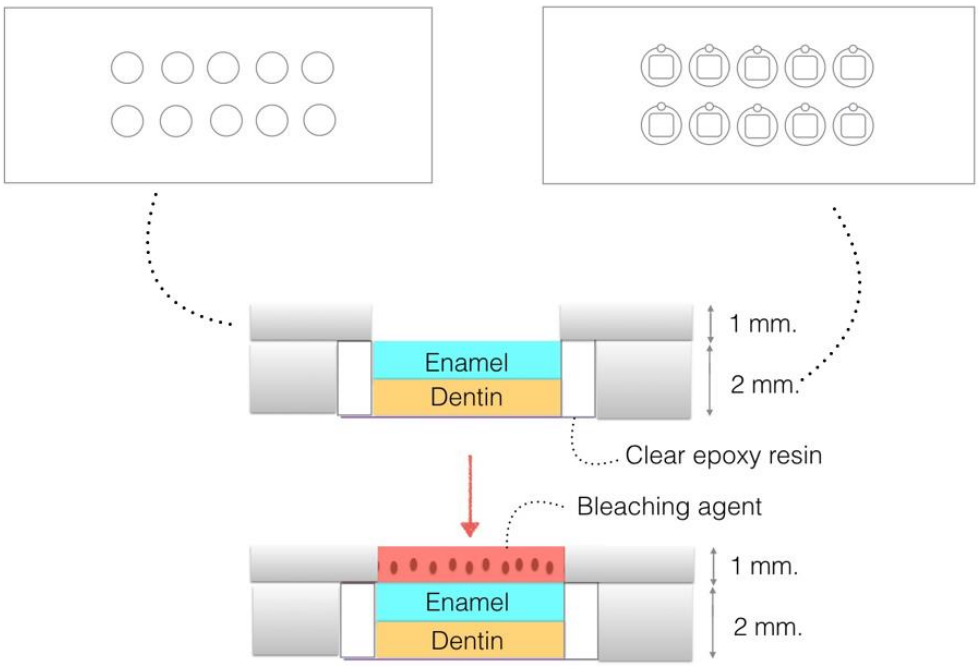


Figure 11

The 1 mm thick acrylic sheet was used to control amount of bleaching agent placed on the labial surface of each enamel/dentin specimen.

The company recommends 2 cycles for Opalescence® Boost ™. Minimizing contacting time differences of bleaching gel and samples, the first cycle, we started from sample 1 toward 10. The second cycle, we started from sample 10 toward 1.

For PolaOffice®, the company's recommendation is 4 cycles. We started the first cycle from sample 1 toward 10. The second cycle, we started from sample 10 toward 1. The third cycle, was done as following sequence, from sample 6, then 5, 7, 4, 8, 3, 9, 2, 10, 1. And the last cycle, was done as following sequence, from sample 1,

then 10, 2, 9, 3, 8, 4, 7, 5, 6.

And the company's recommendation for ZOOM!® Whitespeed ™ is 3 cycles. We did the same as the PolaOffice®'s group, from the first to the third cycle. (Figure 12)

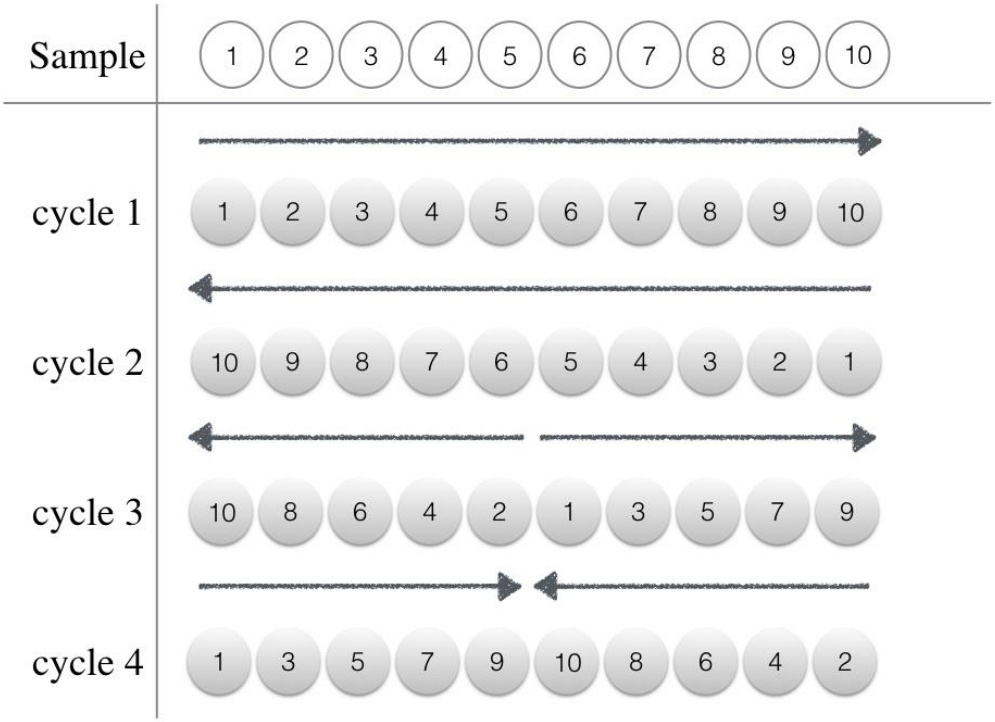


Figure 12

Bleaching sequence in each cycle

6. After bleaching color measurement

After bleaching, the same method as for the initial color measurement was used. Color of each sample was measured 5 times and averaged. The averaged L*, a*, b* measurements served as the after bleaching color of each sample. Because we wanted to observe the immediate effect of $FeSO_4$ as a chemical activator, bleaching was performed on one experimental group at a time, and the color of the samples in each group was measured within 10 minutes after bleaching.

7. Outcome Measurement

The results of color measurement were quantified in three coordinate values (L*, a*, b*), established by the Commission Internationale de l'Eclariage (CIE), which locates the color of an object in three-dimensional color space. The L* axis represents the degree of lightness within a sample, ranging from 0 (black) to 100 (white). The a* plane represents the degree of green/red, and the b* plane represents the degree of blue/yellow in the sample. Comparing tooth color before and after treatment, color changes (ΔE) and differences (ΔL, Δa, Δb) were calculated with the following color definition of respective positive (+) and negative (-) values: ΔL = (+) white, (-) black; Δa = (+) red, (-) green; Δb = (+) yellow, (-) blue.

8. Data Collection

Determination of the color change of each specimen after the bleaching

procedure was made by calculating the variation of L* (ΔL), a* (Δa), and b* (Δb). The total color change (ΔE) were calculated according to the following formula:

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

where; $\Delta L = L_{Final} - L_{Initial}$; $\Delta a = a_{Final} - a_{Initial}$ and $\Delta b = b_{Final} - b_{Initial}$.

9. Data Analysis

The data were determined to have a normal distribution, and then statistically analyzed using SPSS 17.0 for Windows (Chicago, IL, USA). The color measurement data (ΔE, ΔL, Δa and Δb) were analyzed using one-way ANOVA to compare the differences between experimental groups of each commercial product, followed by the Tukey's homogeneous test. If Levene's test showed that homogeneity of variance was violated, the Tamhane's test was used instead of Tukey's homogeneous test. The level of significance was determined as $p = 0.05$.

Ethical consideration

All teeth were collected following ethical approval from the Ethics Committee, the Faculty of Dentistry, Chulalongkorn University (Study Code: HREC-DCU 2016-045).

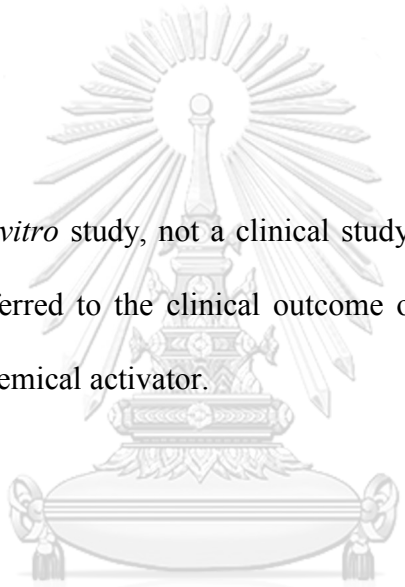
Expected of benefit of study

1. To study effects of ferrous sulphate in hydrogen peroxide tooth bleaching gel.

2. To reduce contacting time of the in-office bleaching gel.

Limitation

This was an *in vitro* study, not a clinical study. Therefore, the results of this study might not be inferred to the clinical outcome of these bleaching agents with ferrous sulphate as a chemical activator.



Results

1. Results of ΔL, Δa, Δb and ΔE of Opalescence® Boost ™ groups

The Shapiro-Wilk test showed a normal distribution of ΔL, Δa, Δb and ΔE in all groups ($p > 0.05$). The one-way ANOVA showed no significant difference in ΔL, Δa, Δb and ΔE between the experimental groups ($p > 0.05$), as shown in table 2.

Table 2 *Results of one-way ANOVA of Opalescence® Boost™ groups*

		Sum of Squares	df	Mean Square	F	Sig.*
ΔL	Between Groups	16.277	3	5.426	1.014	.398
	Within Groups	192.719	36	5.353		
	Total	208.996	39			
Δa	Between Groups	.027	3	.009	.045	.987
	Within Groups	7.123	36	.198		
	Total	7.150	39			
Δb	Between Groups	18.842	3	6.281	1.385	.263
	Within Groups	163.290	36	4.536		
	Total	182.132	39			
ΔE	Between Groups	.478	3	.159	.105	.956
	Within Groups	54.441	36	1.512		
	Total	54.919	39			

* Significant differences ($p < 0.05$).

Where; Δ L = L Final – L Initial; Δ a = a Final - a Initial; Δ b = b Final – b Initial and Δ E = E Final – E Initial.

Means and standard deviations of ΔL, Δa, Δb and ΔE from Opalescence® Boost ™ groups are shown in table 3. The C got the highest ΔL (2.40±1.55), followed by Fe75% (1.93±2.50), Fe50% (1.30±2.40) and Fe100% (0.69±2.65) groups, respectively. The highest decrease in a* values were found within the C (-0.59±0.52), followed by Fe75% (-0.54±0.47), Fe50% (-0.54±0.41) and Fe100% (-0.51±0.36) groups,. The

highest decrease in b* values were found within the Fe75% (-2.24±0.97), followed by C (-2.21±1.21), Fe100% (-1.31±2.81) and Fe50% (-0.59±2.80) groups. And ΔE was the highest within the Fe100% (3.89±0.98), followed by Fe75% (3.78±1.23), C (3.66±1.21) and Fe50% (3.61±1.45) groups, respectively.

Table 3 *Means and Standard deviation values of ΔL, Δa, Δb and ΔE from Opalescence® Boost™ groups*

Group	Mean of ΔL (SD)	Mean of Δa (SD)	Mean of Δb (SD)	Mean of ΔE (SD)
C	2.40 (1.55)	-0.59 (0.52)	-2.21 (1.21)	3.66 (1.21)
Fe100%	0.69 (2.65)	-0.51 (0.36)	-1.31 (2.81)	3.89 (0.98)
Fe75%	1.93 (2.50)	-0.54 (0.47)	-2.24 (0.97)	3.78 (1.23)
Fe50%	1.30 (2.40)	-0.54 (0.41)	-0.59 (2.80)	3.61 (1.45)

Where; Δ L = L Final – L Initial; Δ a = a Final - a Initial; Δ b = b Final – b Initial and Δ E = E Final – E Initial.

2. Results of ΔL, Δa, Δb and ΔE of PolaOffice® groups

The Shapiro-Wilk test showed a normal distribution of ΔL, Δa, Δb and ΔE in all groups ($p > 0.05$). The one-way ANOVA showed a significant difference in ΔL ($p = 0.016$) but no significant difference was found in Δa, Δb and ΔE between the experimental groups ($p > 0.05$), as shown in table 4.

Table 4 *Results of one-way ANOVA of PolaOffice® groups*

		Sum of Squares	df	Mean Square	F	Sig.*
ΔL	Between Groups	30.861	3	10.287	3.951	.016*
	Within Groups	93.730	36	2.604		
	Total	124.591	39			
Δa	Between Groups	.654	3	.218	1.177	.332
	Within Groups	6.666	36	.185		
	Total	7.320	39			
Δb	Between Groups	3.119	3	1.040	.545	.654
	Within Groups	68.609	36	1.906		
	Total	71.728	39			
ΔE	Between Groups	4.903	3	1.634	2.077	.120
	Within Groups	28.331	36	.787		
	Total	33.234	39			

* Significant differences ($p < 0.05$).

Where; Δ L = L Final – L Initial; Δ a = a Final - a Initial; Δ b = b Final – b Initial and Δ E = E Final – E Initial.

Means and standard deviations of ΔL, Δa, Δb and ΔE from PolaOffice® groups are shown in table 5. ΔL was the highest within the Fe100% (1.98±1.43), followed by C (1.88±1.34), Fe75% (0.41±2.07) and Fe50% (-0.01±1.51) groups, respectively. The highest decrease in a* values were found within the Fe75% (-0.13±0.44), followed by C (-0.09±0.26) group. In contrast to Fe100% (0.02±0.44) and Fe50% (0.20±0.54) groups, the data showed slightly increase in a* values. The highest decrease in b* values were found within the Fe75% (-0.60±1.62), followed by Fe50% (-0.14±0.98) and C (-0.11±0.36) groups. The FE100% (0.18±1.47) group showed slightly increase in b* values. ΔE was highest within the FE100% (2.66±0.98), followed by C (2.51±0.82), Fe75% (2.41±1.13) and Fe50% (1.74±0.49) groups, respectively.

Table 5 *Means and Standard deviation values of ΔL, Δa, Δb and ΔE from PolaOffice® groups*

Group	Mean of ΔL (SD)	Mean of Δa (SD)	Mean of Δb (SD)	Mean of ΔE (SD)
C	1.88 (1.34)	-0.09 (0.26)	-0.11 (1.36)	2.51 (0.82)
Fe100%	1.98 (1.43)	0.02 (0.44)	0.18 (1.47)	2.66 (0.98)
Fe75%	0.41 (2.07)	-0.13 (0.44)	-0.60 (1.62)	2.41 (1.13)
Fe50%	-0.01 (1.51)	0.20 (0.54)	-0.14 (0.98)	1.74 (0.49)

Where; Δ L = L Final – L Initial; Δ a = a Final - a Initial; Δ b = b Final – b Initial and Δ E = E Final – E Initial.

Table 6 is given the results of Tukey's homogeneous test for ΔL of PolaOffice® group. The data analysis revealed that only ΔL of the Fe100% and Fe50% groups were significant difference from each other.

Table 6 *Results of Tukey's homogeneous test of ΔL of PolaOffice® groups.*

Group	Mean of ΔL (SD)	Tukey's homogeneous groups
Fe100%	1.98 (1.43)	A
C	1.88 (1.34)	A B
Fe75%	0.41 (2.07)	A B
Fe50%	-0.01 (1.51)	B

The groups accompanied by the same letters do not present significant differences.
Where; Δ L = L Final – L Initial.

3. Results of ΔL, Δa, Δb and ΔE of ZOOM!® Whitespeed ™ groups

The Shapiro-Wilk test showed a normal distribution of ΔL, Δa, Δb and ΔE in all

groups ($p > 0.05$). The one-way ANOVA showed a significant difference in Δb ($p = 0.001$), but no significant difference was found in ΔL, Δa and ΔE between the experimental groups ($p > 0.05$), as shown in table 7.

Table 7 *Results of one-way ANOVA of ZOOM!® Whitespeed™ groups*

		Sum of Squares	df	Mean Square	F	Sig.*
ΔL	Between Groups	54.255	3	18.085	.858	.472
	Within Groups	758.616	36	21.073		
	Total	812.872	39			
Δa	Between Groups	4.933	3	1.644	2.816	.053
	Within Groups	21.017	36	.584		
	Total	25.950	39			
Δb	Between Groups	258.903	3	86.301	6.386	.001*
	Within Groups	486.485	36	13.513		
	Total	745.388	39			
ΔE	Between Groups	43.093	3	14.364	2.228	.102
	Within Groups	232.064	36	6.446		
	Total	275.157	39			

* Significant differences ($p < 0.05$).

Where; Δ L = L Final – L Initial; Δ a = a Final - a Initial; Δ b = b Final – b Initial and Δ E = E Final – E Initial.

Means and standard deviations of ΔL, Δa, Δb and ΔE from ZOOM!®

Whitespeed ™ groups are shown in table 8. The data showed a slightly increase in ΔL within the C group (0.43±2.88), but a decrease in ΔL within Fe50% (-2.74±4.40), Fe100% (-1.25±6.16) and Fe75% (-0.46±4.32) groups, respectively. The highest decrease in a* values were found within the Fe75% (-0.39±0.87), followed by C (-0.30±0.70) group. In contrast to the Fe100% (0.24±0.92) and Fe50% (0.44±0.50) groups, the data showed slightly increase in a* values. The highest decrease in b* values were found within the C (-6.68±2.78), followed by Fe100% (-5.07±1.70), Fe75% (-2.34±5.60) and Fe50% (-0.04±3.47) groups, respectively. ΔE was the highest within the Fe100% (7.89±1.94), followed by C (7.41±2.33), Fe75% (6.81±2.40) and Fe50% (5.14±3.29) groups, respectively.

Table 8 *Means and Standard deviation values of ΔL, Δa, Δb and ΔE from ZOOM!® Whitespeed ™ groups*

Group	Mean of ΔL (SD)	Mean of Δa (SD)	Mean of Δb (SD)	Mean of ΔE (SD)
C	0.43 (2.88)	-0.30 (0.70)	-6.68 (2.78)	7.41 (2.33)
Fe100%	-1.25 (6.16)	0.24 (0.92)	-5.07 (1.70)	7.89 (1.94)
Fe75%	-0.46 (4.32)	-0.39 (0.87)	-2.34 (5.60)	6.81 (2.40)
Fe50%	-2.74 (4.40)	0.44 (0.50)	-0.04 (3.47)	5.14 (3.29)

Where; Δ L = L Final – L Initial; Δ a = a Final - a Initial; Δ b = b Final – b Initial and Δ E = E Final – E Initial.

Due to Levene statistic showed that homogeneity of variance of Δb between ZOOM!® Whitespeed ™ groups was violated. (p=0.010) Tamhane's test was used instead of Tukey's homogeneous test. Table 9 is given the results of Tamhane's test for Δb of ZOOM!® Whitespeed ™ groups. Δb of the C and Fe100% groups were significantly different from Fe50% group.

Table 9 *Results of Tamhane's test of Δb of ZOOM!® Whitespeed™ groups.*

Group	Mean of Δb (SD)	Tamhane's test
C	-6.68 (2.78)	A
Fe100%	-5.07 (1.70)	A
Fe75%	-2.34 (5.60)	A B
Fe50%	-0.04 (3.47)	B

The groups accompanied by the same letters do not present significant differences. Where; Δ b = b Final – b Initial.

Mean of ΔE of experimental groups are shown in Figure 13. The product that

got the highest color change was ZW, followed by OB and PO respectivety. However, there was no statistically significant difference in ΔE between experimental groups in each product.

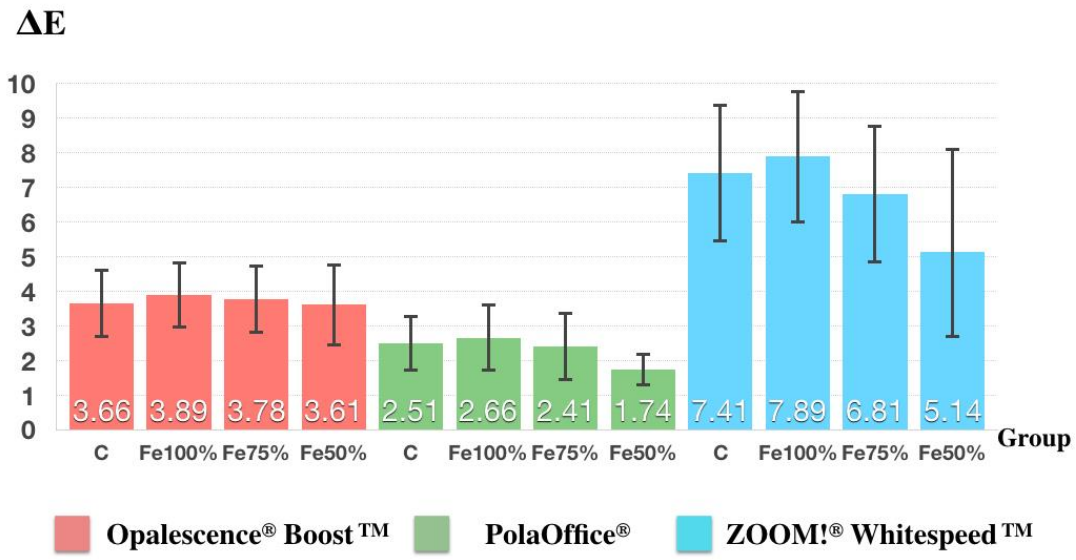


Figure 13

Graph of mean ΔE of experimental groups

Discussion

The first null hypothesis was accepted due to no significant difference in tooth color between the Fe100% and C groups. The second null hypothesis was partially accepted, as the results show no significant difference in tooth color between the Fe75%

and C groups, however, Δb of the ZW Fe50% group was significantly different from that of the C group.

If the ΔE resulting from increased ΔL and decreased Δb is 2 or greater, the bleaching efficacy of the product is deemed to be acceptable, per ISO 28399.[61] Our results demonstrated a ΔE of more than 2 for every commercial product used in this study, indicating that every product is acceptable to use for tooth bleaching.

ΔE results of the Fe100%, Fe75% and Fe50% groups were not significantly different compared with the C groups of all products (Fig. 13). The data showed that for the OB, PO and ZW materials, ΔE of the Fe100% group was little higher compared with that of the C group at 6.3%, 5.9% and 6.5% respectively, similar to that found by Travassos *et al.*[38] In contrast to the results of the present study, other studies found significantly improved ΔE results when adding $FeSO_4$ as a chemical activator.[6, 39] It should be noted that most studies related to chemical activators did not use or use limited brand of commercial whitening product in their investigations.[6, 32-37] Every commercial bleaching product has different composition. Even products, labeled as having the same concentration of hydrogen peroxide, may provide different bleaching effects.[62, 63] Furthermore, some studies used a coloring solution to darken the tooth samples before bleaching, which may have facilitated the observation of significant differences.[36, 38, 39]

Our results showed that ΔE of the Fe75% and Fe50% groups in each product were not statistically different from that of the C groups. The use of an iron derivative catalyzer resulted in a reaction between the Fe^{2+}ion from $FeSO_4$ with hydrogen peroxide, known as the Fenton Reaction (Fig. 14). [40, 64] Addition of an iron ion

caused an increase in the oxidative force of hydrogen peroxide, resulting in an increase in its degradation speed, causing free radicals, a hydroxyl radical and a peroxide radical, to form more rapidly.[40, 65] The hydroxyl radicals are strongly oxidizing, leading to the breakdown of chromogens.[6, 38, 65] From the three products evaluated in our study the results suggested that $FeSO_4$ increased the speed of hydrogen peroxide decomposition. $FeSO_4$ increased the formation of free radicals on the tooth surface more rapidly.[6, 38-40] Therefore, these results implied that addition of $FeSO_4$ could reduce treatment time. We had not any groups without $FeSO_4$ using reduced time because the manufacturer's recommended time should represent the best outcome of each product. Reducing treatment time produced significantly lower ΔE value than the traditional in-office protocol.[66, 67]

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO\bullet + OH^-$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HOO\bullet + H^+$$

Figure 14

Fenton's reaction: $Fe^{2+}$is oxidized by hydrogen peroxide to Fe^{3+}, forming a hydroxyl radical and a hydroxyl anion. Fe^{3+} is then reduced back to Fe^{2+} by another molecule of hydrogen peroxide, forming a peroxide radical and a proton.

The ΔE results of the PO Fe75% and Fe50% groups were not significantly different compared with the C group. However, the Fe50% group, which treatment time was reduced by half, showed a ΔE of 1.74±0.49, which is not acceptable according to

ISO 28399. We also found that L* in this group did not increase after bleaching. Although ΔL of the Fe50% group was significantly different from that of the Fe100% group, it was not significantly different compared with the C group. The PO 75% group demonstrated ΔL, Δa, Δb and ΔE results that were not significantly different from that of the C group. Our data suggested that if $FeSO_4$ was added to PO as a chemical activator, the treatment time may be reduced by 25%, from 32 minutes to 24 minutes. The ΔE of the ZW Fe50% group was not significantly different from that of the C group. However, Δb of the Fe50% group was significantly different from that of the C group. This result indicated that the bleaching agent in this group did not decrease b*. We also found that L* in the Fe50% group decreased. Thus, the bleaching procedure in this group was inefficient. However, the ZW Fe75% group ΔL, Δa, Δb and ΔE were not significantly different from those of the C group. These data suggested that when adding $FeSO_4$ as a chemical activator to ZW, the treatment time may be reduced by 33% from 45 minutes to 30 minutes. It was also found that the OB Fe75% and 50% groups demonstrated the values of ΔL, Δa, Δb and ΔE that were not significantly different compared with the C group. These data suggested that addition of $FeSO_4$ as a chemical activator in OB, facilitated treatment time reduction from 40 minutes to 20 minutes or 50% of treatment time reduction.

Our study indicated that the use of $FeSO_4$ as a chemical activator may be an alternative method for increasing the speed of hydrogen peroxide decomposition. Because only 0.004% of $FeSO_4$ was required, this method could, therefore, be considered inexpensive. This metal salt is not toxic when used in the right concentration, since it is used as nutritional supplement in human diet.[6] Duque *et al.* evaluated the effect of $FeSO_4$ on the transenamel and transdentinal cytotoxicity of the

bleaching gel to two culture lineages obtained from dental pulp in an *in vitro* study. The viability of odontoblast-like cells (MDPC-23) and Human dental pulp cells (HDPCs) from tooth bleached with 35% hydrogen peroxide gel mixed with $FeSO_4$ was not significant different from those bleached with 35% hydrogen peroxide gel alone.[39] However, when using this method a large amount of hydroxyl free radicals and hence a large amount of oxygen are released in a very short time. One concern is that the active generation of oxygen bubbles may result in gel contacting gingiva, leading to irritation.[33]

There are many benefits of reducing bleaching time. Patients undergoing bleaching procedures frequently complained of painful or uncomfortable sensations arising in the treated teeth. This long duration of pain had affected patient's satisfaction with their bleaching experience and might be so severe that patient's discontinue treatment.[68, 69] Shorten treatment time reduced tooth sensitivity.[66, 69, 70] It also reduced the amount of hydrogen peroxide penetrating into pulp chamber, associated with decreased adverse effects to pulp cells.[66, 67]

In the present study, ZW groups surprisingly showed the highest ΔE results, despite the lowest concentration of hydrogen peroxide among the three products. The gels containing higher hydrogen peroxide concentrations did not appear to have a greater effect on whitening[63], in contrast to other studies.[11, 17, 71] The reasons why ZW generated a higher ΔE may be due to its longer contact time, high pH and the effect of light activation. Studies have shown a trend toward improved whitening with increased contact time.[63, 66, 71, 72] However, this effect would be limited by the activity of the peroxide. In a high pH (>7) environment, hydrogen peroxide became

destabilized and released hydroxyl and peroxide free radicals and oxygen gas.[33] The pH of OB is 7, while those of PO and ZW are 5.5 and 8.9, respectively. Thus, only the pH of ZW is greater than 7. A number of studies demonstrated that light activation increased bleaching gel efficacy.[10, 31, 73-75] However, some studies showed that the increased bleaching gel efficacy from light activation was limited or were not significant.[2, 3, 76, 77] Studies have speculated that light may cause temporary dehydration, resulting in lighter appearance of teeth that relapsed over time.[63, 77] More research is required to resolve this issue. The manufacturer of OB informed that teeth would continually be whitened 12 to 24 hours after whitening treatment is completed. However, our study focused only on immediate color change after whitening treatment.

Because the ΔL and Δb raw data in each group was quite varied, the SD was higher than the mean. These results may have occurred because of tooth color variation. Evaluation of the effect of LASER tooth whitening revealed that teeth with hue A showed greater shade improvement than those of teeth with hue C or D.[78] However, in our study we used human upper premolar teeth of hue A or B to mitigate this issue. We also found that even samples of the same hue, samples with high chroma showed greater ΔE results than those with low chroma. Somehow further studies should consider using teeth of the same initial tooth color, both hue and chroma.

Visual color matching is subjective and influenced by variety of factors. Observer-related variables such as sex, color blindness, experience and eye fatigue could influence the final results.[41, 42] More accurate results were reported with the use of dental spectrophotometer compared with visual methods.[53-57] Furthermore,

studies have demonstrated that the performance of the VITA Easyshade® spectrophotometer was more accurate than that of a naked eye.[53, 59, 60] One problem with shade matching, especially for bleaching studies, is positioning the tip of the measuring device. A flat surface is required for accurate placement of measuring tip. Therefore, the present study used 2 mm thick enamel/dentin specimens, which were grounded flat on both sides, to eliminate problem of tooth convexity. In addition, 5 mm x 5mm specimens were used in order to accommodate the 5 mm diameter probe tip of the measuring device.

The purpose of this study was only to improve material efficacy. However, the pulpal reaction is still the most important aspect that should be concerned. This is to prevent misuse of the report. Further studies should include groups using reduced time with and without $FeSO_4$ in order to confirm role of $FeSO_4$. Additional studies should also focus on the safety and efficiency when using a bleaching agent mixed with $FeSO_4$ *in vivo.* Studies concerning color relapse and the exact amount of time that can be reduced when adding $FeSO_4$ to other commercial bleaching products should also be conducted. This information will allow for greater understanding of the effects of $FeSO_4$ as a chemical activator. Furthermore, there are other potential chemical activators that may improve bleaching results and simultaneously reduce treatment time such as manganese gluconate, manganese chloride, etc.

Conclusion

Within the limitations of this study, it can be concluded that adding 0.004% w/w $FeSO_4$ as a chemical activator, reduced the treatment time of these commercial

bleaching products, while obtaining the same bleaching results. For PolaOffice®, the treatment time can be reduced by 25%, from 32 minutes to 24 minutes. For ZOOM!® Whitespeed ™, the treatment time can be reduced by 33%, from 45 minutes to 30 minutes. The treatment time can be reduced by 50% for Opalescence® Boost ™, from 40 minutes to 20 minutes.

Clinical Significance

Adding ferrous sulphate as a chemical activator to some commercial bleaching gels may reduce chair time when doing an in-office tooth whitening. This method is considered inexpensive, because small amount of ferrous sulphate is used (0.004% by weight).

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APPENDIX

Data of ΔL, Δa, Δb and ΔE from experimental groups

Table 1 Data of ΔL, Δa, Δb and ΔE from the Opalescence® Boost™ C group

	ΔL	Δa	Δb	ΔE
1	2.5	-1.7	-3.5	4.62
2	2.3	-0.2	-3.3	4.03
3	2.9	-0.5	-1.4	3.26
4	0.1	-1.0	-3.6	3.74
5	1.7	-0.3	-2.6	3.12
6	1.0	-0.2	-1.9	2.16
7	5.3	-0.8	-0.2	5.36
8	2.5	-0.9	-3.1	4.08
9	4.4	-0.2	-1.9	4.80
10	1.3	0.0	-0.6	1.43
mean	2.40	-0.58	-2.21	3.66
SD	1.55	0.52	1.21	1.21

Where; $\Delta L = L_{Final} - L_{Initial}$; $\Delta a = a_{Final} - a_{Initial}$; $\Delta b = b_{Final} - b_{Initial}$ and $\Delta E = E_{Final} - E_{Initial}$.

Table 2 Data of ΔL, Δa, Δb and ΔE from the Opalescence® Boost ™ Fe100% group

	ΔL	Δa	Δb	ΔE
1	-0.7	-0.7	-3.3	3.45
2	3.1	-1.1	-1.6	3.66
3	0.9	-0.4	-4.9	5.00
4	1.0	-0.2	-2.6	2.79
5	-1.6	-0.7	-2.7	3.22
6	1.0	-1.0	-2.5	2.87
7	2.7	-0.2	-1.7	3.18
8	-1.3	0.0	4.1	4.29
9	-3.7	-0.4	3.2	4.85
10	5.5	-0.4	-1.1	5.59
mean	0.69	-0.51	-1.31	3.89
SD	2.65	0.36	2.81	0.98

Where; Δ L = L Final – L Initial; Δ a = a Final - a Initial; Δ b = b Final – b Initial and Δ E = E Final – E Initial.

Table 3 Data of ΔL, Δa, Δb and ΔE from the Opalescence® Boost ™ Fe75% group

	ΔL	Δa	Δb	ΔE
1	2.8	-1.3	-3.5	4.67
2	-1.0	0.0	-2.4	2.60
3	1.1	-1.3	-2.8	3.28
4	1.6	-0.8	-2.9	3.41
5	-0.3	-0.7	-2.7	2.81
6	1.2	-0.4	-2.8	3.07
7	-1.1	-0.1	-2.5	2.73
8	3.7	-0.2	-0.7	3.74
9	5.1	-0.4	-0.8	5.18
10	6.2	-0.2	-1.3	6.34
mean	1.93	-0.54	-2.24	3.78
SD	2.50	0.47	0.97	1.23

Where; Δ L = L Final – L Initial; Δ a = a Final - a Initial; Δ b = b Final – b Initial and Δ E = E Final – E Initial.

Table 4 Data of ΔL, Δa, Δb and ΔE from the Opalescence® Boost ™ Fe50% group

	ΔL	Δa	Δb	ΔE
1	1.9	-0.1	-3.2	3.72
2	2.2	-0.3	-0.9	2.40
3	-0.7	-1.4	-3.7	4.02
4	5.4	-0.6	-4.0	6.75
5	4.9	-0.7	-1.1	5.07
6	0.6	-0.4	-2.2	2.32
7	-0.3	-0.9	3.4	3.48
8	1.0	-0.6	3.6	3.77
9	-2.4	-0.5	0.7	2.57
10	1.2	0.1	1.6	1.99
mean	1.38	-0.54	-0.58	3.61
SD	2.40	0.41	2.80	1.45

Where; Δ L = L Final – L Initial; Δ a = a Final - a Initial; Δ b = b Final – b Initial and Δ E = E Final – E Initial.

Table 5 Data of ΔL, Δa, Δb and ΔE from the PolaOffice® C group

	ΔL	Δa	Δb	ΔE
1	3.2	0.3	0.8	3.31
2	1.1	0.0	-1.6	1.94
3	-1.0	-0.2	-0.9	1.36
4	2.1	0.0	0.3	2.12
5	1.7	-0.1	-0.6	1.81
6	3.4	-0.2	1.3	3.65
7	0.8	-0.4	-2.0	2.19
8	2.5	-0.5	2.2	3.37
9	1.9	0.3	0.6	2.01
10	3.1	-0.1	-1.2	3.33
mean	1.88	-0.09	-0.11	2.51
SD	1.34	0.26	1.36	0.82

Where; Δ L = L Final – L Initial; Δ a = a Final - a Initial; Δ b = b Final – b Initial and Δ E = E Final – E Initial.

Table 6 Data of ΔL, Δa, Δb and ΔE from the PolaOffice® Fe100% group

	ΔL	Δa	Δb	ΔE
1	3.1	0.6	2.0	3.74
2	1.7	-0.1	-1.5	2.27
3	2.3	0.2	-0.3	2.33
4	2.2	0.0	0.0	2.20
5	0.2	-0.8	-1.2	1.46
6	-0.9	-0.3	-1.2	1.53
7	3.5	-0.2	1.4	3.77
8	3.4	0.6	2.4	4.20
9	2.9	-0.2	-1.1	3.11
10	1.4	0.4	1.3	1.95
mean	1.98	0.02	0.18	2.65
SD	1.43	0.44	1.47	0.98

Where; Δ L = L Final – L Initial; Δ a = a Final - a Initial; Δ b = b Final – b Initial and Δ E = E Final – E Initial.

Table 7 Data of ΔL, Δa, Δb and ΔE from the PolaOffice® Fe75% group

	ΔL	Δa	Δb	ΔE
1	-3.8	-0.6	-3.6	5.27
2	-1.3	-0.9	-1.9	2.47
3	-0.8	-0.5	-1.4	1.69
4	1.6	0.2	-0.5	1.69
5	1.3	-0.1	-1.3	1.84
6	-0.8	-0.3	-1.0	1.32
7	2.4	0.4	1.7	2.97
8	2.3	0.3	0.0	2.32
9	2.7	-0.1	0.3	2.72
10	0.5	0.3	1.7	1.80
mean	0.41	-0.13	-0.60	2.41
SD	2.07	0.44	1.62	1.13

Where; Δ L = L Final – L Initial; Δ a = a Final - a Initial; Δ b = b Final – b Initial and Δ E = E Final – E Initial.

Table 8 Data of ΔL, Δa, Δb and ΔE from the PolaOffice® Fe50% group

	ΔL	Δa	Δb	ΔE
1	-0.1	-0.2	-1.0	1.02
2	2.1	0.0	0.2	2.11
3	0.3	-0.1	-1.3	1.34
4	-1.4	0.0	0.7	1.57
5	0.0	1.0	-1.1	1.49
6	1.9	-0.2	1.4	2.37
7	1.1	0.4	-0.7	1.36
8	0.3	1.3	0.8	1.55
9	-2.1	-0.2	0.6	2.19
10	-2.2	0.0	-1.0	2.42
mean	-0.01	0.20	-0.14	1.74
SD	1.51	0.54	0.98	0.49

Where; Δ L = L Final – L Initial; Δ a = a Final - a Initial; Δ b = b Final – b Initial and Δ E = E Final – E Initial.

Table 9 Data of ΔL, Δa, Δb and ΔE from the ZOOM!® Whitespeed ™ C group

	ΔL	Δa	Δb	ΔE
1	4.60	1.10	-2.50	5.35
2	0.40	-1.00	-12.30	12.35
3	3.60	-1.10	-9.40	10.13
4	1.40	-0.80	-7.00	7.18
5	-4.90	-0.30	-5.30	7.22
6	-2.10	-0.50	-5.80	6.19
7	1.60	-0.50	-6.50	6.71
8	2.40	-0.20	-4.60	5.19
9	-1.60	-0.40	-5.00	5.26
10	-1.10	0.70	-8.40	8.50
mean	0.43	-0.30	-6.68	7.41
SD	2.88	0.70	2.78	2.33

Where; Δ L = L Final – L Initial; Δ a = a Final - a Initial; Δ b = b Final – b Initial and Δ E = E Final – E Initial.

Table 10 Data of ΔL, Δa, Δb and ΔE from the ZOOM!® Whitespeed ™ Fe100% group

	ΔL	Δa	Δb	ΔE
1	3.10	0.10	-4.90	5.80
2	-9.50	1.30	-5.60	11.10
3	-5.60	0.30	-6.70	8.74
4	-2.50	-1.20	-6.90	7.44
5	7.60	-1.00	-3.50	8.43
6	4.10	0.70	-3.60	5.50
7	4.30	-0.10	-7.80	8.91
8	-6.18	0.70	-4.40	8.13
9	-9.30	1.70	-2.40	9.75
10	1.50	-0.10	-4.90	5.13
mean	-1.25	0.24	-5.07	7.89
SD	6.16	0.92	1.70	1.94

Where; Δ L = L Final – L Initial; Δ a = a Final - a Initial; Δ b = b Final – b Initial and Δ E = E Final – E Initial.

Table 11 Data of ΔL, Δa, Δb and ΔE from the ZOOM!® Whitespeed ™ Fe75% group

	ΔL	Δa	Δb	ΔE
1	-4.70	-0.40	-5.80	7.48
2	-4.70	-0.60	2.80	5.50
3	-3.70	0.50	1.30	3.95
4	-3.00	-0.40	8.60	9.12
5	-4.50	0.70	0.00	4.55
6	2.10	-2.20	-10.40	10.84
7	7.80	-0.40	-3.90	8.73
8	0.80	-1.20	-8.10	8.23
9	1.50	0.50	-3.70	4.02
10	3.80	-0.40	-4.20	5.68
mean	-0.46	-0.39	-2.34	6.81
SD	4.32	0.87	5.60	2.40

Where; Δ L = L Final – L Initial; Δ a = a Final - a Initial; Δ b = b Final – b Initial and Δ E = E Final – E Initial.

Table 12 Data of ΔL, Δa, Δb and ΔE from the ZOOM!® Whitespeed ™ Fe50% group

	ΔL	Δa	Δb	ΔE
1	2.10	0.20	1.50	2.59
2	0.50	-0.50	4.70	4.75
3	-1.30	0.10	4.90	5.07
4	-2.70	0.10	3.50	4.42
5	-1.10	0.80	-3.10	3.39
6	-4.30	0.90	-1.20	4.55
7	0.40	0.30	-1.40	1.49
8	-0.90	0.40	-4.10	4.22
9	-12.60	1.10	-4.10	13.30
10	-7.50	1.00	-1.10	7.65
mean	-2.74	0.44	-0.04	5.14
SD	4.40	0.50	3.47	3.29

Where; Δ L = L Final – L Initial; Δ a = a Final - a Initial; Δ b = b Final – b Initial and Δ E = E Final – E Initial.

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