Evaluation in vitro du potentiel d'agents de blanchiment dentaire OTC sans peroxyde

Grillon, Marlène

Clinique universitaire de médecine dentaire
Division de cariologie et d'endodontie

Thèse préparée sous la direction du Docteur Stefano ARDU et du Professeur Ivo KREJCI

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Marlène Grillon

de

Corcelles-Cormondrèche (Neuchâtel)

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Thèse de :

Marlène GRILLON
originaire de Corcelles-Cormondrèche (NE), Suisse

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I. PARTIE FRANÇAISE

Résumé

L'objectif de cette recherche a été d'évaluer l'efficacité de différents blanchiments dentaires sans peroxyde disponibles en vente libre (OTC), ainsi que celle d'une solution expérimentale contenant 0,1 % de peroxyde d'hydrogène complexé à des agents dopants. Ces résultats ont été comparés avec ceux du blanchiment dentaire *gold standard* (contrôle positif) contenant 16 % de peroxyde de carbamide.

Pour ce faire, l'évaluation du pouvoir blanchissant de ces différents produits a été élaborée sur quatre-vingt incisives permanentes bovines extraites, exposées au préalable à quatre agents de coloration, à savoir, thé, café, vin rouge, et curry. Le temps et la méthodologie d'application des différents produits ont été effectués suivant les recommandations fournies par chaque fabricant. Six mesures CIE L*a*b ont été réalisées sur chaque dent sur un fond blanc et noir à l'aide d'un spectrophotomètre calibré utilisant une méthode numérique quantitative. Les changements de couleur ont été calculés sur la base du score L*a*b, utilisant la formule CIEDE 2000 $\Delta E00$.

Les résultats de cette étude ont permis de démontrer une variation de couleur favorable pour tous les agents de blanchiment dentaires OTC, excepté un qui a, quant à lui, présenté des résultats défavorables. Cette variation favorable est cependant nettement inférieure à celle obtenue par le contrôle positif contenant 16 % de peroxyde de carbamide. La solution expérimentale a, quant à elle, présenté le meilleur résultat de tous les produits OTC testés, son résultat se rapproche de celui obtenu par le contrôle positif.

Introduction

La médecine dentaire esthétique a gagné en popularité et l'esthétique dentaire est une priorité d'importance grandissante auprès des patients, elle est souvent associée à la santé et à la beauté (Samorodnitzky-Naveh et al., 2007) (Bruhn et al., 2012). Cette récente évolution s'explique, entre autres, par la démographie et certains facteurs socioculturels. Alors que, dans le passé, la santé était considérée comme l'absence de maladie, elle est aujourd'hui vue comme une qualité multidimensionnelle composée de bien-être social, mental et psychologique (Slade, 1997) (Kothari et al., 2019). Selon les perspectives de la population mondiale 2019 des Nations unies : « *D'ici 2050, 1 personne sur 6 dans le monde sera âgée de plus de 65 ans, contre 1 sur 11 en 2019. »* (United Nations, 2019). Alors que la population mondiale vieillit plus que jamais, davantage de ressources sont allouées à la quête de la jeunesse éternelle afin de lutter contre les effets négatifs que le vieillissement peut avoir sur l'apparence physique (Smirnova, 2012). De nombreuses industries ont été impactées par cette évolution. Par exemple, la demande accrue de lotions capillaires et de sérums pour la peau a alimenté la croissance de l'industrie cosmétique. De même, les traitements chirurgicaux et non chirurgicaux sont plus fréquemment demandés pour compléter des solutions plus traditionnelles et moins invasives (Market et al., 2018). La médecine dentaire esthétique n'échappe pas à cette tendance. Au contraire, le sourire joue un rôle central dans la beauté globale et les « *smile makeover* » sont très fortement demandés auprès de la population. D'une part, la population âgée doit faire face à un assombrissement naturel des dents qui accompagne le vieillissement, d'autre part, de l'autre côté de la pyramide démographique, les jeunes générations sont de plus en plus influencées par internet et les réseaux sociaux qui véhiculent l'image des dents blanches comme un indispensable. Le *hashtag#smile* est l'un des plus populaires sur Instagram avec plus de 347 millions de poste (Instagram, 2019). De récentes études ont démontré une corrélation entre les réseaux sociaux et les problèmes d'image de soi (Fardouly et al., 2016). La population a accès

à un nombre illimité d'images et les individus partagent davantage de clichés d'eux-mêmes sur les plateformes numériques. Avec une moyenne de dix millions de nouvelles photos publiées sur Facebook toutes les heures, les utilisateurs sont constamment exposés et donc enclins à se comparer (Mayer-Schönberger et al., 2013) (Fardouly et al., 2016). Parallèlement, le secteur de la distribution exploite le pouvoir du marketing des influenceurs pour promouvoir des standards de beauté élevés et mettre à disposition des produits qui peuvent aider à obtenir une image parfaite. Ce flux constant d'informations remet en question les perceptions générales et transforme la façon de penser et de consommer. Lorsqu'il s'agit de l'esthétique dentaire, de nombreux facteurs peuvent affecter un sourire parfait : la carie dentaire causée par une hygiène bucco-dentaire sous-optimale peut avoir un impact particulièrement important, surtout lorsque les dents touchées sont situées dans une zone visible. Des pathologies telles que les traumas ou les problèmes orthodontiques peuvent avoir un impact important sur la forme et l'aspect du sourire. Enfin, une coloration ou une translucidité anormale des dents peut péjorer l'apparence générale du sourire. Le sujet des colorations dentaires, des principaux facteurs de coloration et des méthodes de blanchiment dentaire seront abordés et développés plus loin dans ce manuscrit. Les principales causes de coloration dentaire sont généralement classées en deux grandes catégories comprenant : les causes extrinsèques et les causes intrinsèques (Hattab et al., 1999). Les causes extrinsèques sont celles qui découlent des comportements individuels, tels que les choix alimentaires. Une personne dont le régime alimentaire est riche en café noir aura comme conséquence une accumulation d'agents colorants à la surface des dents (Bazzi et al., 2012). A l'inverse, les facteurs intrinsèques sont, quant à eux, non liés à des comportements spécifiques, mais dus à différentes pathologies survenant avant ou après la naissance, telles que la fluorose, les colorations liées aux tétracyclines et autres. Les patients insatisfaits de la couleur de leurs dents peuvent avoir recours à un blanchiment vital (*vital bleaching*) (Fiorillo et al., 2019). Selon l'*American Academy of Cosmetic Dentistry*, le blanchiment vital (*vital*

bleaching) est le traitement le plus souvent demandé dans les cabinets dentaires aux États-Unis d'Amérique (Schmidt et al., 2006). Le diagnostic joue un rôle crucial lors de la proposition de la bonne approche de traitement. De plus, selon la cause de la coloration, le traitement peut être différent. Historiquement, le blanchiment dentaire était effectué dans des cabinets dentaires ou à domicile sous la supervision d'un spécialiste. Dès les années 1990, une nouvelle forme de blanchiment dentaire est apparue, vendue en vente libre OTC (Demarco et al., 2009) (Greenwall et al., 2001). Ces dernières années, les produits de blanchiment OTC, notamment plus abordables que les produits traditionnels, ont connu un grand succès, en particulier auprès des jeunes adultes (Kugel, 2003). Ces blanchiments dentaires sont en vente libre et peuvent être utilisés à domicile sans la supervision d'un professionnel. Bien qu'ils soient librement disponibles sur le marché, ils ne sont pas sans risque pour la santé et peuvent créer des effets secondaires tels que des hypersensibilités dentaires et des irritations gingivales (Pintado-Palomino et al., 2015). Après des années sans claire réglementation, le Conseil européen a promulgué en 2011 une nouvelle loi concernant les produits cosmétiques. Les solutions de blanchiment dentaire contenant du peroxyde d'hydrogène se sont également trouvées visées par cette nouvelle loi. A la suite de l'introduction de cette règlementation, le niveau de concentration pour le blanchiment dentaire effectué par des dentistes ou sous la supervision de spécialistes a été limité à 6 %, tandis que la concentration maximale acceptée pour les produits en vente libre OTC est, quant à elle, descendue à 0,1 % (Council Directive 2011/84/EU, 2011). A la suite de ces nouvelles dispositions légales, l'industrie s'est rapidement adaptée et a commencé à commercialiser de nouveaux produits alternatifs sans peroxyde d'hydrogène lequel a été remplacé par de nouveaux principes actifs tels que l'acide phtalimidoperoxycaproïque (PAP) ou le chlorure de sodium (Bizhang et al., 2017). Quelques années seulement après ce changement législatif et la constante introduction de nouveaux produits sur le marché, les études scientifiques ne peuvent suivre le rythme. Aujourd'hui, le

nombre de recherches scientifiques analysant l'efficacité et les effets secondaires de ces produits n'est pas assez exhaustif pour permettre de tirer une conclusion concrète quant à l'efficacité réelle de ces nouveaux produits de blanchiment OTC. De plus, l'absence de diagnostic et de supervision professionnelle peut conduire, dans certains cas, à des utilisations inappropriées de la part de consommateurs inexpérimentés.

Agents de coloration

Les colorations dentaires peuvent provenir de diverses origines. Watts et Addy ont classé ces origines en deux catégories : intrinsèques et extrinsèques (Watts et al., 2001). Ces deux catégories sont basées sur le type et la localisation de la coloration (Addy et al., 1995) (Nathoo, 1997). Les colorations intrinsèques sont liées aux changements structurels de la dentine et de l'émail ainsi qu'aux propriétés de transmission de la lumière (Ten Bosch et al., 1995). Elles font référence à une modification interne de la substance dentaire résultant d'un trouble pré ou postnatal. Par exemple, l'absorption d'antibiotiques de type tétracycline pendant la grossesse entraîne une altération de la structure interne des dents du fœtus (Sánchez et al., 2004). Cela se produit au vu du fait que ces antibiotiques traversent la barrière hémato-placentaire, fusionnent avec le calcium présent dans les dents et créent un complexe insoluble qui modifie la structure de la dentine et de l'émail (Sánchez et al., 2004). En conséquence, les dents touchées présentent une coloration grisâtre. Une coloration intrinsèque appelée fluorose peut être induite par une ingestion excessive de fluorure pendant le développement dentaire (Akuno et al., 2019). Une corrélation entre l'eau potable contenant plus de 1,5 ppm de fluorure et l'apparition de fluorose a été prouvée (Akuno et al., 2019). Cette pathologie provoque des colorations blanches opaques et, dans les cas les plus graves, des taches brun foncé à la surface de l'émail. Les colorations intrinsèques sont également associées à un trouble métabolique tel que l'hyperbilirubinémie congénitale qui apparaît lorsque le foie n'élimine pas correctement la bilirubine (De Oliveira

Melo et al., 2015) (Watanabe et al., 2007). Un niveau élevé de bilirubine dans le sang va impacter la peau et les dents, ces dernières présentent un aspect jaune-vert communément appelé jaunisse. Les troubles génétiques tels que l'amélogénèse et la dentinogénèse imparfaite affectent les dentitions de lait et permanentes, elles présentent soit des taches blanc-jaune-brun ou une dentine gris-brun et un émail translucide (Witkop, 1988) (De La Dure-Molla et al., 2015). Un trouble idiopathique décrit comme « *Molar Incisive Hypomineralisation* » (MIH) peut également affecter la couleur des dents en raison d'un défaut qualitatif de la structure de l'émail (Weerheijm, 2004). Les dents hypominéralisées apparaissent avec des taches blanchâtres et opaques à leur surface, conséquence des traumatismes ou infections survenant lors de la dentition lactéale qui affectent la minéralisation de l'émail des dents permanentes. Lorsqu'un traumatisme entraîne une hémorragie dans la chambre pulpaire, l'apparence de la dent affectée peut devenir rouge-gris. Finalement, la cause la plus courante et physiologique des colorations intrinsèques reste le vieillissement dentaire pendant lequel les dents développent de la dentine secondaire et la couche d'émail devient plus fine en raison de l'usure. Ces deux phénomènes conduisent à des dents plus foncées (Solheim, 1992) (Paewinsky et al., 2005) (Kwon et al., 2015) (Watts et al., 2001). Contrairement aux taches intrinsèques, les taches extrinsèques sont fortement liées aux habitudes et au comportement individuel. L'émail, la couche externe de la dent, est un tissu translucide, fortement minéralisé, formé principalement de cristaux d'hydroxyapatite (Boyde A., 1989). En raison de la surface structurée de cette barrière protectrice, les pigments chromogènes peuvent s'agréger à la surface de l'émail et créer des taches superficielles. Il est également rapporté que les protéines de la pellicule dentaire qui recouvrent l'émail peuvent réagir avec les pigments colorants et, de ce fait, contribuer à la création de coloration (Nathoo, 1997). Une consommation fréquente d'aliments ou de boissons contenant du tanin peut provoquer des taches. Par exemple, la consommation de curry, une épice à forte pigmentation jaune, peut entraîner une coloration

jaunâtre. Les boissons comme le café, le thé ou le vin rouge ont des pigments forts qui peuvent modifier la couleur des dents à long terme (Prayitno et al., 1979) (Karadas et al., 2014). Le café, une boisson très répandue et populaire, est reconnu comme l'un des agents colorants le plus puissant (Bazzi et al., 2012) (Türkün et al., 2004) (Liporoni et al., 2010) (Mundim et al., 2010). Les colorations indirectes sont également le résultat d'une utilisation prolongée de bains de bouche antiseptiques contenant des composants tels que la chlorhexidine ou des sels métalliques (Addy et al., 1985). La chlorhexidine, un antiseptique di-cationique, crée une réaction chimique avec les chromogènes alimentaires présents sur les surfaces dentaires et la langue, ce qui entraîne des colorations brunes (Addy et al., 2005). L'exposition fréquente à des composants chimiques tels que le tabac, une hygiène bucco-dentaire inadéquate sont des raisons courantes de colorations dentaires extrinsèques. L'accumulation de pellicule dentaire ou de tartre se traduit par une couche jaune-brun à la surface dentaire, là où les pigments adhèrent et modifient la coloration. En parcourant ces descriptions, il devient évident à quel point les causes des colorations sont hétérogènes. Un diagnostic précis de la cause de la coloration est crucial, car il influence l'approche thérapeutique et détermine par conséquent le succès du traitement.

*La chimie du blanchiment vital (*vital bleaching*)*

Le blanchiment dentaire consiste en une réaction d'oxydation-réduction des pigments dont les molécules se désagrègent et résultent en de petits fragments incolores. Cela modifie la réflexion et l'absorption de la lumière des dents (Bizhang et al., 2017) (Llena et al., 2018). Les procédures de blanchiment non vital (*non vital bleaching*) ont été mentionnées pour la première fois au milieu du XIX^{e} siècle (Rodríguez-Martínez et al., 2019) (Haywood, 1992). Cependant, à partir de 1862, Atkinson a décrit un nouvel agent oxydant, l'acide oxalique, comme une solution de blanchiment vital (*vital bleaching*) appliquée directement sur la surface externe des dents.

D'autres oxydants comme le pyrozone (Joshi, 2016) ou le peroxyde d'hydrogène ont été décrits en 1885. En 1918, Abbot a mentionné pour la première fois le superoxol, contenant 35 % de peroxyde d'hydrogène. A partir de 1910, plusieurs auteurs ont décrit l'utilisation de la lumière et de la chaleur pour accélérer les réactions chimiques et amplifier l'oxydation des agents de blanchiment (Joshi, 2016). Le peroxyde d'hydrogène est l'agent oxydant le plus couramment utilisé pour le blanchiment vital (*vital bleaching*), car il peut oxyder de nombreux composants chromatiques (Tredwin et al., 2006). Bien que le blanchiment dentaire soit devenu une procédure très courante, le mécanisme exact de l'oxydation du peroxyde d'hydrogène n'est, à ce jour, pas bien connu. Le peroxyde d'hydrogène est une molécule très instable et réactive à forte capacité d'oxydation. Il pénètre dans la dent en quelques minutes, libère de l'oxygène ainsi que des ROS. Cette réaction résulte en la production de radicaux libres. Les composants organiques causant des colorations sont des molécules à longues chaînes contenant des pigments très chromatiques. Les radicaux libres instables attaquent et décomposent ces chaînes en fragments plus petits et incolores et, de ce fait, entraînent le blanchiment dentaire (Dahl et al., 2003) (Joiner, 2006). En raison de sa formation très rapide et de son temps d'action limité, l'utilisation du peroxyde d'hydrogène se borne au blanchiment au fauteuil (*chair side bleaching*). De plus, il est utilisé à concentration élevée durant une courte durée. Du fait de ses propriétés mentionnées précédemment, le peroxyde d'hydrogène est remplacé par le peroxide de carbamide qui, lui, est une molécule stable. Le peroxyde de carbamide se décompose en 70 % d'urée et 30 % de peroxyde d'hydrogène (Aschheim, 2014). L'une des principales préoccupations concernant le blanchiment dentaire sont ses effets secondaires, car ils peuvent affecter les tissus mous et durs (Goldberg et al., 2010). Par exemple, l'application d'une concentration excessive de l'agent oxydant peut avoir un impact sur les tissus et les matériaux de restauration. L'effet secondaire le plus fréquent est lié à l'hypersensibilité dentaire, elle peut provoquer des sensations douloureuses intenses et affecter la vie quotidienne des patients. La

douleur liée à l'hypersensibilité dentaire représente la principale raison de l'arrêt d'un traitement en cours (Leonard et al., 2001). Dans certains cas, la douleur est si forte qu'elle rend le brossage des dents difficile, ce qui a des conséquences sur l'hygiène bucco-dentaire des patients (Sulieman, 2004) (Hannig et al., 2007). L'hypersensibilité dentaire peut être expliquée par trois théories : la théorie de l'innervation directe, la théorie des récepteurs odontoblastiques et la théorie du mouvement des fluides, qui est la théorie la plus avancée aujourd'hui. Pour réduire et éviter les hypersensibilités, des molécules comme le nitrate de potassium, le fluorure de sodium ou le phosphate de calcium amorphe (ACP) sont utilisées pendant ou après le traitement, car elles bloquent les tubuli dentinaires en formant des cristaux à l'intérieur de ceux-ci (Joshi, 2016) (Davari et al., 2013). Enfin, il est essentiel pour la santé gingivale d'utiliser la bonne quantité d'agent blanchissant et de limiter son utilisation aux surfaces dentaires. Une concentration excessive de peroxyde d'hydrogène ainsi que son utilisation inappropriée peuvent affecter les tissus mous et entraîner des irritations gingivales ou des sensations de brûlure au niveau des gencives (Howard, 1992) (Alqahtani, 2014).

*Des techniques de blanchiment vital (*vital bleaching*)*

Le blanchiment vital (*vital bleaching*) est disponible sur le marché principalement sous trois formes : (a) le blanchiment au fauteuil (*in office bleaching*), (b) le blanchiment à domicile (*home bleaching*) et (c) les produits de blanchiment en vente libre (*over the counter* - OTC) (Haywood, 1992). Le blanchiment vital au fauteuil, également appelé « *power bleaching »*, est la première technique et la plus traditionnelle. Ce type de blanchiment est effectué dans un cabinet dentaire, sous la supervision d'un dentiste. Cette technique utilise une forte concentration de l'agent oxydant (généralement l'équivalent de 25 à 48 % de peroxyde d'hydrogène) pendant une période relativement courte (de 20 à 60 minutes) (Joshi, 2016). Alors que la concentration élevée permet la libération d'un grand nombre de radicaux libres oxydants,

les tissus mous vont, quant à eux, souffrir d'effets secondaires plus importants. Pour éviter autant que possible ces effets secondaires, il est recommandé de protéger les tissus mous avec une digue liquide en caoutchouc pendant le traitement. Le produit de blanchiment est appliqué par le dentiste sur les surfaces vestibulaires des dents, certains praticiens utilisent de la lumière ou de la chaleur comme catalyseur pour accélérer la réaction chimique du processus de blanchiment (Marson et al., 2008). L'*American Dental Academy* (ADA) souligne cependant qu'il n'existe à ce jour aucune étude n'ayant démontré que la lumière ou la chaleur contribuent positivement au résultat du blanchiment. Leur seule utilité serait d'accélérer la réaction chimique afin de réduire la durée d'exposition au produit (Marson et al., 2008). Le blanchiment dentaire au fauteuil est particulièrement indiqué pour les patients qui souhaitent un résultat immédiat après la première séance, sans avoir à prendre plusieurs rendez-vous. Le blanchiment dentaire au fauteuil est également recommandé pour les patients ne tolérant pas les gouttières dentaires, ayant une dextérité réduite ou ne voulant pas faire d'autotraitement à la maison. Les techniques au fauteuil offrent une plus grande flexibilité au dentiste pour adapter le traitement en vue d'un résultat optimal. D'autre part, le coût élevé et les effets secondaires succédant la procédure représentent les principaux inconvénients qui pourraient dissuader un patient de choisir ce traitement. En outre, le blanchiment au fauteuil cible surtout les décolorations de l'émail et est moins efficace pour les colorations situées dans la dentine. Une alternative au blanchiment au fauteuil consiste en la technique du « blanchiment à domicile » (*home bleaching*). Le blanchiment à domicile est le résultat d'une découverte accidentelle faite en 1960, lorsque l'orthodontiste Dr Klusmier a demandé à un patient d'appliquer une solution antiseptique pendant la nuit pour guérir des inflammations gingivales. Cette solution antiseptique contenant 10 % de peroxyde de carbamide a non seulement eu un effet antibactérien, mais a également résulté à l'obtention de dents plus blanches (Haywood et al., 1990). A la suite de cette découverte, de nombreuses recherches ont été menées et, en 1989,

Haywood et Heyman ont décrit la méthode du « *nightguard vital bleaching* » comme étant le traitement de blanchiment à domicile de dents vitales (Haywood et al., 1989). Cette technique de blanchiment nécessite un partenariat entre le patient et le dentiste. Le dentiste initie le traitement demandé et donne des instructions claires au patient qui exécute les étapes nécessaires de manière indépendante à la maison. Des gouttières de blanchiment dentaire sur mesure sont fournies au patient qui, pendant deux semaines, devra les remplir avec l'agent de blanchiment et les porter durant la nuit. Pour s'assurer que le traitement se déroule comme prévu, le dentiste rencontre régulièrement le patient pour suivre la situation. Le blanchiment vital à domicile (*home vital bleaching*) est bien décrit dans des articles scientifiques et son efficacité a été démontrée depuis de nombreuses années. L'un des avantages les plus importants de cette méthode provient de la concentration plus faible de peroxyde d'hydrogène (3 à 6 %) par rapport à la concentration beaucoup plus élevée utilisée pour le blanchiment en cabinet. La concentration plus faible de peroxyde d'hydrogène entraîne une diminution des effets secondaires de type irritations gingivales ou hypersensibilités dentaires (Fiorillo et al., 2019). D'autre part, le blanchiment à domicile peut être une procédure exigeante, car elle nécessite de la compliance, de la motivation et de la constance dans le port des gouttières de blanchiment de la part du patient. Finalement, une troisième et relativement nouvelle méthode de blanchiment dentaire est représentée par les produits de blanchiment en vente libre (OTC). Ces produits, disponibles dans les commerces de détail et en ligne, sont utilisés sans la supervision d'un dentiste. Ils connaissent une popularité croissante depuis la fin des années 1990 (Demarco et al., 2009). L'utilisation généralisée de ce type de produits peut être, entre autres, expliquée par sa facilité d'accès et son prix comparativement bas. Jusqu'en 2011, les produits de blanchiment OTC n'étaient soumis à aucune réglementation ou restriction significative concernant leur quantité de peroxyde d'hydrogène. Cette situation a changé de manière importante avec l'introduction d'une nouvelle directive européenne (Council Directive

2011/84/EU 2011) qui a introduit des restrictions claires concernant la concentration de peroxyde d'hydrogène. Cette réglementation a réduit la concentration maximale de l'équivalent en peroxyde d'hydrogène lors d'un usage non professionnel à un maximum de 0,1 %. Cette régulation a engendré une réduction de la concentration du principe actif et, de ce fait, une diminution du pouvoir blanchissant de ces produits par rapport aux méthodes plus traditionnelles. Bien qu'une concentration plus faible de l'agent de blanchiment crée moins d'effets secondaires au niveau des tissus mous et durs, elle prolonge de manière exponentielle le temps nécessaire pour produire un résultat significatif (Fiorillo et al., 2019). En réponse à cette nouvelle réglementation, les fabricants ont adapté leurs approches et ont commencé à commercialiser des produits de blanchiment sans peroxyde d'hydrogène. De nouveaux ingrédients actifs tels que l'acide phtalimidoperoxycaproïque (PAP), le chlorite de sodium ou le peroxyde de carbonate de sodium ont fait leur apparition sur le marché pour contrer la nouvelle restriction (Bizhang et al., 2017). La commercialisation de ces nouveaux produits de blanchiment OTC a suscité des inquiétudes quant à leur sécurité, car seules quelques rares études scientifiques indépendantes et des tests complets ont été réalisés pour évaluer leur efficacité. L'utilisation du blanchiment dentaire OTC, sans diagnostic professionnel et sans analyse précise des causes de la coloration, soulève des questions quant à leur utilisation correcte (Li, 2011). À cet égard, la commission de l'Association dentaire française (ADF) a souligné que l'utilisation répétitive et excessive de produits en vente libre sans contrôle professionnel pouvait être préoccupante et représenter un problème de santé publique (Demarco et al., 2009). Les blanchiments dentaires OTC peuvent être trouvés sous plusieurs formes sur le marché : dentifrices blanchissants, gums, gels, solutions de rinçage, bandes et gouttières blanchissantes préchargées (Demarco et al., 2009) (Hasson et al., 2007). Les agents oxydants tels que le peroxyde d'hydrogène sont rarement utilisés comme composés dans les dentifrices, c'est pourquoi, l'effet éclaircissant est induit ou potentiellement induit par des

particules abrasives destinées à éroder la surface de l'émail pour éliminer le composant externe provoquant la coloration (Joiner, 2010). En raison de sa forte abrasivité, l'utilisation excessive et inappropriée d'un dentifrice blanchissant peut endommager la surface externe de l'émail (Joiner, 2010). En ce qui concerne les dentifrices et les gels, jusqu'à présent, aucune étude ou recherche n'a pu démontrer une quelconque efficacité de blanchiment. Les bains de bouche contenant un plus faible pourcentage de peroxyde d'hydrogène ne présentent aucun effet significatif en termes de blanchiment, mais ils peuvent néanmoins provoquer une irritation des tissus mous et des hypersensibilités. Les gouttières disponibles en vente libre sont plus ou moins similaires à celles utilisées pour les traitements de blanchiment à domicile, cependant, elles contiennent une forme standard (non personnalisée) et sont préremplies avec l'agent de blanchiment. Du point de vue pratique, du fait de la forme « standardisée » des gouttières, ces dernières ne vont être que très approximativement adaptées à la forme individuelle des dents et des gencives du patient. C'est pourquoi, il est important de noter qu'une mauvaise adaptation de la gouttière peut avoir comme conséquence l'application d'une dose excessive d'agent de blanchiment, ainsi qu'un excès de produit au niveau des gencives qui peut conduire à des irritations gingivales (Bizhang et al., 2017). Finalement, les bandes de blanchiment (*whitening strips*) sont une alternative pour appliquer l'agent de blanchiment directement sur les dents de manière ciblée, pour cette raison ces produits ont tendance à créer moins d'irritations gingivales et d'hypersensibilité. En revanche, les bandes de blanchiment (*whitening strips*) sont considérées comme moins efficaces que les gouttières de blanchiment standard (Oldoini et al., 2018). Le défi pour les fabricants actuels est de trouver le bon équilibre entre l'efficacité du principe actif (capacité d'oxydation), la durée d'exposition et les effets secondaires (Martín et al., 2015). Étant donné la popularité croissante des produits de blanchiment dentaire OTC et l'absence de recherche scientifique approfondie pour évaluer leur efficacité, le présent document vise à comparer certains des produits de blanchiment les plus fréquemment utilisés

ne contenant pas de peroxyde d'hydrogène. Ces blanchiments sont-ils aussi efficaces qu'ils le prétendent ? Pour répondre à cette question, la présente étude testera l'efficacité de produits de blanchiment sur des dents bovines extraites, exposées à des colorations extrinsèques par différents agents colorants tels que le thé, le café, le vin rouge et le curry. Tous les résultats seront ensuite comparés à la procédure de blanchiment *gold standard* contenant 16% de carbamide peroxyde. Pour terminer, l'étude comparera ces résultats à ceux d'une solution de blanchiment expérimentale supplémentaire composée de 0,1% de peroxyde d'hydrogène, complexée à un agent dopant. L'hypothèse nulle est qu'il n'y a pas de changement de couleur statistiquement significatif avant ou après le traitement pour tout liquide de coloration.

In vitro evaluation of tooth whitening potential of peroxide-free OTC dental bleaching agents

II. PARTIE ANGLAISE

Introduction

Aesthetic dentistry has grown in popularity and the appearance of teeth is an increasingly important priority among patients and often associated with health and beauty (Samorodnitzky-Naveh et al., 2007) (Bruhn et al., 2012). There are numerous reasons behind this recent development, related to demographics and other sociocultural factors. While in the past health was considered to be the absence of disease, we now think about health as a multidimensional quality composed of social, mental and psychological well-being (Slade, 1997) (Kothari et al., 2019). According to the UN World Population Prospects 2019: "By 2050, 1 in 6 people in the world will be over the age of 65, up from 1 in 11 in 2019" (United Nations, 2019). As the world population is aging more than ever, more resources are allocated to the quest of eternal youth, to fight against the negative effects that aging has on the physical appearance (Smirnova, 2012). Many industries have been impacted by this development. For example, the increased demand for hair lotions and skin serums fueled the growth of the cosmetic industry. Similarly, surgical and non-surgical treatments are more frequently requested to supplement more traditional and less invasive solutions (Market et al., 2018). Aesthetic dentistry is not immune to this trend. On the contrary, smile takes a central role in the overall beauty and smile makeovers are highly demanded by the population. On one side the elderly population is coping with the natural teeth discoloration that comes with aging, and on the other end of the demographic pyramid, the younger generations are increasingly influenced by internet and social media suggesting that white teeth are a must. The hashtag #smile is one of the most popular ones on Instagram with more that 347 million posts (Instagram, 2019). New studies have shown a correlation between

social media and body image concerns (Fardouly et al., 2016). We share more images of ourselves on digital platforms and we also have access to unlimited number of images. With an average of ten million new pictures published on Facebook every hour, users are constantly exposed and therefore prone to compare themselves with others (Mayer-Schönberger et al., 2013) (Fardouly et al., 2016). At the same time, the distribution industry leverages the power of influencers' marketing to promote high standards of beauty and make readily available products that can help us to achieve the perfect image. The constant flow of information is resetting the general perceptions and transforming the way we think and consume. When it comes to dental beauty, there are many factors that can affect the perfect smile: Tooth decay caused by suboptimal oral hygiene can be particularly impactful, especially when the teeth affected are located in the visible area. Pathologies like traumas or orthodontics complications might have high impact on the form and shape of the smile. Finally, an abnormal teeth coloration or translucency might cause distress and affect the overall appearance of the smile. The subject of teeth discoloration will be further developed in this paper which will also touch upon the key staining factors and the most relevant bleaching methods. The key staining causes are typically classified into two main categories: extrinsic causes and intrinsic causes (Hattab et al., 1999). The extrinsic causes are the ones driven by the behaviors of the individual such as particular dietary choices. A person whose diet is rich on black coffee might lead to an accumulation of staining agents on the teeth surface (Bazzi et al., 2012). On the other hand, the intrinsic factors are completely unrelated to specific behaviors, but driven by different health pathologies occurring before or after birth such as fluorosis, tetracycline stains and others. Patients unsatisfied with their teeth color appearance may resort to a tooth vital bleaching (Fiorillo et al., 2019). According to the American Academy of Cosmetic Dentistry, vital bleaching is the most commonly requested treatment in dental offices across the Unites States of America (Schmidt et al., 2006). Diagnosis plays a crucial role in proposing the right

approach and depending on the staining cause, the treatment might be different. Historically dental bleaching has been performed in dentals offices or at home under supervision. Starting from 1990, a new form of dental bleaching emerged, sold over-the-counter (OTC) (Demarco et al., 2009) (Greenwall et al., 2001). In recent times OTC products, more affordable than traditional dental office breaching, proved to be very successful especially among young adults (Kugel, 2003). Despite being freely available on the market (over-the-counter solution can be used at home without the supervision of a professional), these products are not risk-free and might generate side effects like hypersensitivity and gingival irritations (Pintado-Palomino et al., 2015). After years without clear regulations, in 2012 the European council enacted a new law concerning the cosmetic products and targeting also dental bleaching solutions containing hydrogen peroxide (Council Directive 2011/84/EU 2011). The concentration level for bleaching performed by dental practitioners or under specialists' supervision was limited to 6%, while the maximum concentration accepted for products over-the-counter went down to 0,1% (Council Directive 2011/84/EU 2011). Following the introduction of these new legal provisions, the industry had quickly adapted and started to introduce new alternative products without hydrogen peroxide. Hydrogen peroxide was replaced by new active principles such as phthalimidoperoxycaproic acid (PAP) or sodium chloride (Bizhang et al., 2017). With only few years past from this change and new products introduced in the marketplace almost every year, the scientific studies cannot keep the pace. Today the number of research papers analyzing the efficiency and side effects of these products is not exhaustive enough to draw a firm conclusion. At the same time, we know that the lack of professional diagnosis or supervision might lead, in some cases, to an inappropriate use of these products from unexperienced consumers.

Staining agents

Teeth discoloration can come from various origins. Watts and Addy classified the origins in two categories: extrinsic and intrinsic (Watts et al., 2001). These two categories are based on the type and the localization of the discoloration (Addy et al., 1995) (Nathoo, 1997). Intrinsic discoloration is related to the dentine and enamel structural changes as well as to the light transmission properties (Ten Bosch et al., 1995). It refers to an inner modification of the tooth substance resulting from a pre- or post-natal disorder. For example, the absorption of tetracycline-type antibiotics during pregnancy leads to an alteration of the inner teeth structure of the fetus (Sánchez et al., 2004). This happens because these antibiotics cross the blood-placental barrier and merge with the calcium present in teeth to create an insoluble complex that alters the dentine and enamel structure (Sánchez et al., 2004). As a result, the affected teeth show a greyish discoloration. Intrinsic teeth coloration called fluorosis can be induced by an excessive ingestion of fluoride during the tooth development (Akuno et al., 2019). A correlation between drinking water complemented with more than 1.5ppm of fluoride and fluorosis has been proved (Akuno et al., 2019). With this pathology the teeth will turn white opaque, and, in more severe cases, with dark brown spots on the surface of enamel. Intrinsic staining is also associated with metabolic disorder such as congenital hyperbilirubinemia, which appears when the liver does not eliminate the bilirubin properly (De Oliveira Melo et al., 2015) (Watanabe et al., 2007). A higher level of bilirubin in the bloodstream will impact the skin and the teeth with a yellow-green appearance commonly known as jaundice. Genetic disorders such as amelogenesis and dentinogenesis imperfecta affect both deciduous and permanent teeth with either white-yellow-brown spots or grey-brown dentin and translucent enamel (Witkop, 1988) (De La Dure-Molla et al., 2015). Idiopathic disorder described as Molar Incisive Hypomineralization may also affect the color of the teeth because of a qualitative defect of the enamel structure (Weerheijm, 2004). The hypomineralized teeth will appear with

whitish and opaque spots on their surface. Traumas and infections in primary teeth can also affect the mineralization of enamel in permanent teeth resulting in white opaque spots. When a trauma leads to a hemorrhage in the pulp chamber, the appearance of the affected tooth might turn red-grey. Finally, the most common and physiologic cause of intrinsic discoloration remains aging. With aging, the teeth develop secondary dentine, and due to wear, the enamel layer becomes thinner. This two phenomena leads to darker teeth (Solheim, 1992) (Paewinsky et al., 2005) (Kwon et al., 2015) (Watts et al., 2001).

As opposed to intrinsic stains, extrinsic stains are strongly linked to individual habits and behaviors. The external layer of the tooth, the enamel, is a translucent and highly mineralized tissue formed from hydroxyapatite crystals mainly (Boyde A., 1989). As this protective barrier has a structured surface, the chromogenic pigments can aggregate on the surface and create superficial staining. It is also reported that the protein of the pellicle covering enamel can react with the staining pigments and contribute to the discoloration (Nathoo, 1997). A frequent consumption of tanning food or drinks may cause staining. For example, the frequent consumption of curry, a spice with a strong yellow pigmentation, may lead to yellowish color. Beverages like coffee, tea or red wine have strong pigments that can modify the color of the teeth on the long term (Prayitno et al., 1979) (Karadas et al., 2014). Coffee, a widespread and popular beverage, is by far recognized as one of the most powerful staining agents (Bazzi et al., 2012) (Türkün et al., 2004) (Liporoni et al., 2010) (Mundim et al., 2010). Indirect staining is the result of prolonged usage of antiseptics mouth wash containing component such as chlorhexidine or metal salts (Addy et al., 1985). Chlorhexidine, a di-cationic antiseptic creates a chemical reaction with dietary chromogen present in dental surfaces and in the tongue resulting in brown colorations (Addy et al., 2005). The frequent exposure to chemical components like tobacco smoking is also associated with extrinsic discoloration. A very common reason for extrinsic tooth discoloration is an inadequate oral hygiene. The

accumulation of pellicle or calculus result in a yellow-brown layer on the surface, where pigments adhere and alter the coloration. When scrolling through these descriptions, it becomes obvious how heterogeneous the staining causes are. A precise diagnosis of the staining cause is crucial as it influences the therapeutic approach and consequently determines the success of the treatment.

Chemistry of vital bleaching

Dental bleaching consists of an oxidation-reduction reaction of the pigments of which the molecules are breaking apart resulting in small colorless fragments. This modifies the reflexion and light absorption of the teeth (Bizhang et al., 2017) (Llena et al., 2018). Non-vital bleaching procedures were first mentioned in the middle of the 19th century (Rodríguez-Martínez et al., 2019) (Haywood, 1992). However, starting from 1862, Atkinson described a new oxidative agent, the oxalic acid, as a vital bleaching solution applied directly on the external surface of the teeth. Other oxidizers like pyrozone or hydrogen peroxide were described in 1885 and Abbot mentioned for the first time superoxol, which contains 35% of hydrogen peroxide, in 1918 (Joshi, 2016). Starting from 1910, several authors described the use of light and heat to accelerate the chemical reactions and amplify the oxidation of the bleaching agents (Joshi, 2016). Hydrogen peroxide is the most commonly used oxidative agent for vital bleaching because it can oxidize many chromatic components (Tredwin et al., 2006). Even though bleaching became a very common procedure, the exact mechanism of hydrogen peroxide oxidation is still not well-known today. It is a very unstable and reactive molecule with a strong oxidative capacity. Hydrogen peroxide penetrates the tooth within minutes, releases oxygen as well as ROS and the result of this reaction produces free radicals. The organic components that cause stains are molecules with long chains and very chromatic pigments. The unstable free radicals attack and break down these chains into smaller and colorless fragments and will

result in bleaching the teeth (Dahl et al., 2003) (Joiner, 2006). Because of its very rapid formation and thus limited time of action, hydrogen peroxide is limited to the application in high concentration but short time chair side bleaching methods. Carbamide peroxide is used instead as it present a stable molecule which breaks down when activated to 70% urea and 30% hydrogen peroxide (Aschheim, 2014). One of the main concern with the bleaching process is its side effects which might affect soft and hard tissues (Goldberg et al., 2010). For example, the application of an inappropriate concentration of the oxidative agent might impact tissues and restoratives materials. The most frequent side effect however is related to teeth hypersensitivity, which might cause intensely painful sensations and affect the everyday life of the patients. It comes with no surprise that this is reported to be the main breakout reasons to halt an ongoing treatment (Leonard et al., 2001). In some cases, pain coming from hypersensitivity is so intense that it makes it difficult to brush teeth with consequent implication for the oral hygiene (Sulieman, 2004) (Hannig et al., 2007). Teeth hypersensitivity can be explained with three theories: the direct innervation theory, the odontoblast receptor theory and the fluid movement theory, which is the most advanced theory today. To reduce and avoid hypersensitivities, molecules like potassium nitrate, sodium fluoride or amorphous calcium phosphate (ACP) are used during or after the treatment, as they block the dentinal tubuli by forming crystals within these tubuli (Joshi, 2016) (Davari et al., 2013). Finally, it is critical for the gingival health to use the right amount of bleaching agent. An excessive concentration of hydrogen peroxide or an inappropriate use might affect the soft tissues and lead to gingival irritations or burning sensation in the gums (Howard, 1992) (Alqahtani, 2014).

Vital bleaching techniques

Vital bleaching treatments are available on the market mainly in three forms: (a) In office bleaching, (b) home bleaching and (c) over-the-counter bleaching products (Haywood, 1992). In office vital bleaching, also known as "power bleaching", is the first and most traditional technique. It is carried out in a dental office under the supervision of a professional dental practitioner. This technique employs high concentration of the oxidative agent (usually the equivalent of 25-48% hydrogen peroxide) for a relatively short span of time (from 20 to 60 minutes) (Joshi, 2016). While the high concentration allows the release of a high number of free oxidative radicals, the soft tissues will suffer from bigger side effects. To avoid as much as possible these side effects it is recommended to protect the soft tissues with a liquid rubber dam during the treatment. Generally, the bleaching product is applied by the dentist on the vestibular tooth surfaces and certain practitioners use light or heat as a catalyzer to accelerate the chemical reaction of the whitening process (Marson et al., 2008). As the American Dental Academy (ADA) pointed out, there are no studies demonstrating that light or heat provide any positive contribution to the bleaching outcome so far. Their only utility is said to accelerate the chemical reaction in order to reduce the time of exposure to the produce (Marson et al., 2008). In office dental bleaching is particularly indicated for patients who want an immediate result after the first session, without the need of multiple appointments. It is also recommended for patients that do not tolerate night dental trays, have reduced dexterity, or do not want to do any self-treatment at home. In office techniques provide greater flexibility to the dentist to adapt the treatment for an optimal outcome. On the other hand, the high cost of the procedure and the side effects represent the major downside that might deter a patient from choosing this treatment. In addition, chairside bleaching targets especially discolorations in enamel and is less efficient on colorations located in dentine. An alternative to chairside bleaching consists in the "home bleaching" technique. Home bleaching is the result of an accidental discovery

made in 1960 when the orthodontist Dr. Klusmier asked a patient to apply an antiseptic solution overnight to heal inflamed gums. This antiseptic solutions which contained 10% carbamide peroxide not only had an antibacterial effect, but resulted in whiter teeth (Haywood et al., 1990). Following this finding, a lot of research has been conducted and in 1989 Haywood and Heyman described the "nightguard vital bleaching" method as the home bleaching treatment for vital teeth (Haywood et al., 1989). This bleaching technique requires a partnership between the patient and the dentist. The dentist initiates the requested treatment and provides clear instructions to the patient which executes the necessary steps independently at home. A custom-made dental whitening tray is provided to the patient. For two weeks the patient has to fill the tray with the bleaching agent and apply it during the night. To make sure the treatment progresses as expected, the dentist meets regularly with the patient to monitor the situation. Home vital bleaching is well described in scientific papers and its effectiveness has been demonstrated over many years. One of the most relevant benefit of this method comes from the lower concentration of hydrogen peroxide (3% to 6%) compared to the much higher concentration used for the in-office bleaching, which leads to fewer side effects like irritations or tooth hypersensitivities (Fiorillo et al., 2019). On the other hand, home bleaching might be a demanding procedure as it requires compliance, motivation and consistency in wearing the whitening tray every night. Finally, a third and relatively new tooth bleaching method is represented by over-the-counter bleaching products. These products, freely available in retail stores and online, are usually used without the supervision of a dentist and have experienced an increased popularity starting from the late 1990 (Demarco et al., 2009). The widespread consumption of this kind of products can be reconducted to the ease of access and comparatively low prices. Until 2012, over-the-counter bleaching products were not subject to any significative regulation or restriction regarding the amount of hydrogen peroxide. This situation changed in an important way with the introduction of a new European directive

(Council Directive 2011/84/EU 2011) which introduced among others a clear restriction on the concentration of hydrogen peroxide. This regulatory amended the maximum concentration of hydrogen peroxide equivalent for non-professional use to maximum 0.1%. With the reduction of concentration of the bleaching agent the whitening power decreased in comparison to the more traditional methods. While a lower concentration ensures fewer side effects on the soft and hard tissues, it also extends exponentially the time required to produce a meaningful result (Fiorillo et al., 2019). In response to this new regulation, manufacturers adapted their approach and started to commercialize bleaching products without hydrogen peroxide. New active ingredient such as phthalimidoperoxycaproic acid (PAP), sodium chlorite or sodium carbonate peroxide appeared in the market to counter the restriction (Bizhang et al., 2017). Safety concerns has been raised in some instances regarding the commercialization of these new over the counter bleaching product since only few independent scientific studies and comprehensive testing have been completed to assess their effectiveness. The use of over the counter products without professional diagnosis and precise analysis on the causes of the discoloration raise questions on whether these bleaching solution are used correctly (Li, 2011). In this regards, the Association Dentaire Française (ADF) commission highlighted how the repetitive and excessive use of over the counter products without a professional supervision might be of public health concern (Demarco et al., 2009). Over the counter bleaching products can be found in several forms on the market: whitening toothpaste, gums, gels, rinses solutions, strips and whitening preloaded trays (Demarco et al., 2009) (Hasson et al., 2007). Oxidative agent such as hydrogen peroxide are not usually used for whitening toothpaste, therefore the lightening effect is inducted or potentially inducted with abrasives particles meant to erode the surface of the enamel to remove the external staining component (Joiner, 2010). Due to its high abrasiveness, the excessive and unappropriated utilization of whitening toothpaste can damage the external surface of enamel (Joiner, 2010). When looking at toothpastes and gels, so far, no

study or research was able to demonstrate any bleaching effectiveness. Mouth rinses containing a lower percentage of hydrogen peroxide do not show any significant effect in terms of bleaching, however, they still may cause soft tissues irritation and hypersensitivities. Over the counter trays are similar to the ones used for home bleaching treatments, but they use a standard shape tray and are pre-filled with the bleaching agent. From a practical point of view, the standard shape of the tray might be more or less adaptable to the individual shape of the teeth of the patient. For this reason it is important to note that a mismatch might cause an excessive dose of bleaching agent to be applied and therefore cause gingival irritations (Bizhang et al., 2017). Finally, whitening strips are an alternative to apply the bleaching agent directly to the teeth in a targeted way and for this reason tend to generate less irritations and hypersensitivities. At the same time, they are considered less effective compared to the standard bleaching trays (Oldoini et al., 2018). The challenge for the manufacturers today is to find the right balance between effectiveness (oxidative capacity), time of exposure and side effects (Martín et al., 2015). Given the growing popularity of the OTC products and the lack of comprehensive scientific research to assess their effectiveness, this paper aims to compare some of the most frequently used bleaching products that do not contain hydrogen peroxide. Are these products as effective as they claim? To answer that question the study will test the bleaching effectiveness of these products on extracted bovine teeth exposed to extrinsic discolorations by different staining agents such as tea, coffee, red wine and curry. All the results will then be compared to the gold standard of a traditional bleaching procedure containing 16% of peroxide carbamide. Finally, the study will compare these results to the results of an additional experimental bleaching solution composed of 0.1% hydrogen peroxide, complexed with a doping agent. The null hypothesis is that there is no statistically significant color change before or after the treatment for any staining liquid.

Materials and Method

Sample selection and preparation

This study was done on bovine teeth. Eighty permanent incisors were extracted, stored in water and randomly allocated to eight different groups with 10 teeth each. The number of teeth was determined based on a power analysis (Appendix 1). The roots were sectioned 1mm below the cementoenamel junction (JEC) using a slow speed water-cooled diamond saw (Minitom, Struers Type 04436216, Serial No. 44310284). All teeth were carefully cleaned with pumice and numbered in the palatal area with a bur. All operations were executed by the same operator. Sample selection and preparation were performed following the methodology described by (Dietschi et al., 2006).

Staining procedure

Five different staining solution were readied for this study in plastic bottles (Table 1): Group A: 60ml coffee (Ristretto, Nespresso, Nestle Switzerland); Group B: 3 tea bags in 60ml of boiling water (Twinings Earl Gray tea, London, England); Group C: 60ml red wine (Côte du Rhône (DOC), Les arénes, Vacqueyras, France); Group D: 5g of curry mixed in 60 ml of warm oil (Curry Bio Natura plan Coop, Switzerland); Group E: 60ml distilled water (control group). Two teeth from each group were immerged into the respective staining colorant and stored at 37°C during 28 days in an incubator (INP-500, Memmert GmbH & Co.KG, D-91107 Schwabach, Germany). The staining solution were renewed every 7 days to avoid bacteria or yeast contamination. After 28 days of storage, the teeth surfaces were cleaned using high pressure hot water airbrush (0.4 MPa 135°C, Minivapor 93, Effegi Brega s.r.l., 29010 Sarmato, PC- Italy) and briefly air dried. All details regarding the staining methodology were described in previous publications (Gregor et al., 2016) (Ardu et al., 2010).

Bleaching procedure

After the staining procedure, the stained teeth were reallocated to the eight groups, resulting in 10 samples per group, and each group was matched with a bleaching product (Table 2): Group 1: MeaWhite kit teeth whitening (MEA); Group 2: iWhite instant teeth whitening (IWH); Group 3: PAP pure (PAP pure); Group 4: Opalescence PF 16% (OPL); Group 5: Experimental bleaching agent (EXP1); Group 6: Hismileteeth (HST); Group 7: Placebo (GLY); Group 8: oZoral gel oral (OZG). The bleaching procedure consisted in applying approximately 1mm thick layer of the bleaching agent onto the surface of the enamel for the defined period of bleaching, then rinse with water for 30 s, and clean the surface with paper tissue. All applications were performed following the manufacturers' recommendations (Table 3). For each group, the bleaching gel was applied for 60, 100 and 200 consecutive minutes. During the bleaching periods, samples were kept at ambient temperature and 100% humidity. Following the manufacturer's recommendations, the application of MEA/IWH/PAP/OPL/GLY/OZG were repeated every 20 min, EXP1 every 60-100-200 minutes and HST every 10 minutes. Moreover, the bleaching procedures for MEA, PAP and HST were always combined with light activation (standardized distance of 2 mm) in line with the manufacturer's recommendations.

Color change measurements and data collection

Color of each sample was recorded on black and on white background using a quantitative numerical measurement approach with a calibrated spectrophotometer (Spectro-Shade, Handy Dental Type 713000, Serial No. HDL0090 MHT). The classic CIEDE 2000 (ΔE00) formula based on lightness (ΔEL), chroma (ΔEC) and hue (ΔEH) was used to determinate color changes (Ardu et al., 2014) (Ardu et al., 2010). Spectrophotometric measurements were performed after exposing the teeth to the bleaching agent for 60 min (T_2), 100 min (T_3), and 200 min (T_4), respectively. Before every spectrophotometric measurement, the samples were stored in

distilled water at room temperature for 24 hours to avoid dehydration. An integrated detection function within the spectrophotometer guaranteed equal measurement conditions for all measurements due to reproducible positioning, perpendicular to the sample surface. Before every measurement, the spectrophotometer was calibrated using the green and white calibration standard provided by the manufacturer. AD65 (6500 °K) light source illuminating simultaneously from both sides at a 45° angle was used for the measurements and the system's detector area received a 0° angle reflected light. Data generated from the spectrophotometer were stores in a proprietary image file format (Ardu et al., 2010). For each tooth image file, six measurements were taken on different zones based on a clockwise sequential localisation in order to generate details CIE L*a*b data. CIE L*a*b values were recorded at the beginning of the study on the unstained extracted teeth (T_0). Another measurement was taken after the staining procedures in order to evaluate the staining susceptibility (T_1). Finally, measurements were taken after each bleaching step (T_2, T_3 and T_4). Based on the L*a*b scores, color changes were calculated using the classical CIEDE 2000 (ΔE00) formula (Appendix 2) (Ardu et al., 2017) (Paravina et al., 2015).

Statistical Analysis

Statistically significant CIEDE 2000 color changes over time for the 8 groups and 5 staining solutions were assessed using repeated ANOVA (Table 4 and 5) measures with sigma-restricted parametrisation to account for categorical predictors in the model, followed by Fisher's LSD test (p-value < 0.01). Samples ranked with the same letter were considered equivalent in terms of color change. Normality assumptions were checked using the Shapiro-Wilk normality test on the within-cells residuals of the ANOVA analysis (p-value > 0.1). All statistical analyses were done in Statistica 13 (Tibco Software Inc., Palo Alto, USA). CIEDE00 color differences have been computed in MATLAB 2017b (The Mathworks, Inc., USA)

Results

Six CIE L*a*b measurements were recorded on each of the 80 teeth, resulting in 480 measurements per time interval, totaling 2400 measurements for the five times intervals. Tables 6 and 7 provide the mean and standard deviation CIEDE00 color changes over time for all the groups and staining solution, on both black and white background, respectively. The overall color change considers the data pooled together per bleaching product but without distinction per staining liquid. Superscripts denote the samples' ranking for each staining solution and time. Superscript A corresponds to the best and D corresponds to the worst ranking. Results with the same superscript are not significantly different according to Fisher's LSD test; p value < 0.01. The highest ΔE00 value represent the highest color change difference.

On the white background, when stained by distilled water, values ranged from ΔE00 3.26 (Opalescence PF) to 1.04 (oZoral Gel) with no significant differences between the bleaching products. On the white background, when stained with coffee, bleaching susceptibility values ranged from ΔE00 3.69 (EXP1) and 1.54 (Glycerine) with no meaningful statistical differences observed. On the white background, when stained with curry mixed with oil, bleaching values

ranged from ΔE00 5.07 (EXP1) to 2.13 (PAP pure), with significant differences observed. On the white background, when stained with red wine, bleaching values ranged from ΔE00 11.2 (Opalescence PF) to 2.86 (oZoral Gel), with significant differences being present. On the white background, when stained with tea, bleaching values ranged from ΔE00 10.17 (Opalescence PF) to 2.09 (Glycerine), and here again, significant differences were observed. The overall color change on white background ranged from ΔE00 6.32 (Opalescence PF) to 2.14 (Glycerine), with significant differences between the products. On the black background, when stained with distilled water, bleaching values ranged from ΔE00 4.83 (Opalescence PF) to 1.25 (oZoral Gel) with significant differences. When stained by coffee and measured on the black background, bleaching values ranged from ΔE00 4 (iWhite) to 1.73 (Glycerine), without being significantly different form each other. On the black background, when stained by curry mixed with oil, bleaching values ranged from ΔE00 6.02 (EXP1) to 2.36 (PAP pure) and the differences were statistically significant. When stained with red wine, bleaching values ranged from ΔE00 9.39 (Opalescence PF) to 3.03 (Glycerine) on the black background the differences were also statistically significant. When stained with tea, bleaching values on black background ranged from ΔE00 7.73 (Opalescence PF) to 1.77 (Glycerine) and the differences were statistically significant. The overall color change measured on black background was significantly different between the bleaching products and ranged from ΔE00 5.78 (Opalescence PF) to 2.31 (Glycerine).

Tables 8, 9 and Figures 1, 2 provide the mean and standard deviation CIEDE00 of the color difference over time among different staining liquids on a white and black background respectively. The total value color change considers the data pooled together over time without distinction per staining liquid. On a white background, the mean ranged from ΔE00 5.96 (red wine) to 2.30 (distilled water) with statistically significant differences observed and a total value of ΔE00 3.67. On a black background, the mean ranged from ΔE00 5.61 (red wine) to

2.84 (distilled water) with statistically significant differences observed and a total value of ΔE00 3.96. Table 10 and Figures 3, 4 provide the mean and standard deviation CIEDE00 of the color difference over initial time among different staining liquids on a white and black background respectively. On a white background, the mean ranged from ΔE00 21.67 (red wine) to 1.85 (distilled water) with significant differences observed. On a black background, the mean ranged from ΔE00 20.30 (red wine) to 2.42 (distilled water) with significant differences observed. Initial and final L*a*b values of the samples are illustrated in Table 11 and Figure 5.

Discussion

The demand and supply for over-the-counter whitening kit has largely increased in recent years. However, data about their effectiveness is still scarce. The aim of this research was to evaluate the effectiveness of different non-hydrogen peroxide based over-the-counter whitening products and benchmark them with a "traditional" hydrogen peroxide based bleaching product. This study was performed on extracted bovine teeth because of their large size and their similarities in dental hard tissue and color which allows for better standardization (Al-Harbi et al., 2013). Their relative flat vestibular surface allowed for homogeneous application of the bleaching and for standardized spectrophotometric measurements. Tooth discoloration may come from various origins, and this study focuses on the effect of discoloration due to dietary factors. Foods and beverages like coffee, red wine and curry are frequently present in everyday diets and they are well-known for their capacity to stain (Addy et al., 1985) (Prayitno et al., 1979). The microstructure of the substrate influences the staining susceptibility (Liporoni et al., 2010). In order to examine the teeth under natural conditions, we decided to maintain the natural enamel morphology and the surface irregularities of the sample. Surface roughness, enamel composition and water absorption rate are known to influence the staining susceptibility and pigment accumulation (Titley et al., 1988). The pH value of a staining solution is also correlated to the degree of discoloration. For this reason, the low pH of tea, coffee and red wine has an important staining capacity (Attin et al., 2003).

Staining technique

Enamel is vulnerable to red wine staining due to wine's acidity, high alcohol content and important concentration of pigments such as tannins (Berger et al., 2008). Tea and coffee both contain yellow pigments, but these pigments have different polarities. The discoloration caused by tea is due to a high polarity colorant (Um et al., 1991) (Berger et al., 2008). With coffee, the

discoloration is due to low polarity colorant. According to (Guler et al., 2005), the daily average consumption of coffee represents 3.2 cups and the consumption time for one cup is approximately 15 minutes. Ertas and colleagues also demonstrated that 24 hours in vitro storage correspond to 1-month in vivo exposition. Considering that, we decided to immerge and store our specimens in the staining solution for 28 days at 37°C, which may be considered equivalent to two years of daily consumption (Ertaş et al., 2006). After the staining procedure, we cleaned the samples with high pressure hot water airbrush in order to remove the superficially absorbed stain while preserving the internally absorbed coloration. According to Tables 10 and Figures 3, 4 red wine is the mostly staining liquid.

Color change assessment

Color assessment was evaluated with a quantitative approach using a spectrophotometer to avoid bias relative to subjective measurements and non-standardized illumination (Dietschi et al., 2006). The spectrophotometer allowed for a quantitative objective evaluation with the ability to detect even small color variations (Ardu et al., 2010). We took the spectrophotometric measurements on a black and on a white background, the two backgrounds aim to reflect different clinical situation. For instance, white background may represent posterior clinical situation where one wall is still present (e.g. Class I, II, III and veneers), while black background may mimic the situation in anterior teeth where no wall is remaining (e.g. Class IV) (Ardu et al., 2014). However, in a previous study, (Ardu et al., 2014) described the influence of different backgrounds on natural teeth while assessing their spectrophotometric color values. They concluded that when performing in vitro studies, black background should be preferred over a white background as it characterizes best the clinical situation. In order to represent all different clinical situation, we decided to perform spectrophotometric measurements on both white and black background. For the purpose of this discussion, we will

consider only the color variation perceived as disturbing (ΔE00 higher than 1.8), therefore clinically relevant.

The color change ΔE00 is represented within the three-dimensional CIELAB color space, developed by the Commission Internationale de l'Eclairage (CIE). In this color space, L* axis corresponds to the lightness of a sample, varying from black (0) to white (100), a* axis ranges from green (-) to red (+) and b* axis ranges from blue (-) to yellow (+) (Commission Internationale de l'Eclairage (CIE), 2004). After an effective bleaching procedure, we expect an increase in the luminosity (ΔL*) and a decrease of the yellow tone (Δb*). In order to come closer to the perception of the human eye on color differences, we decided to use ΔE00 instead of ΔE for comparison of our results. It was also described in the literature that ΔE00 is able to discriminate smaller color difference than ΔE (Commission Internationale de l'Eclairage (CIE), 2004) (Um et al., 1991). The human eye can only perceive ΔE00 values above 0.8 as different and ΔE00 above 1.8 is considered to be disturbing and clinically not acceptable (Paravina et al., 2015). The value 1.8 represents in fact the point where 50% of the observers consider the color difference either disturbing or detectable (Paravina et al., 2015). In our study, all ΔE00 values were higher than 1.8. This means that all bleaching agents induced some degree of color variation. We even observed that the negative control group (GLY) presented an ΔE00 higher than 1.8. This outcome can be explained by the assumption that freshly stained samples stored in distilled water lost some inherent pigments, thus appearing lighter (Ardu et al., 2018).

Bleaching effect

Considering the results of this research, OPL showed the highest ΔE00 thus having the best bleaching capacity. This bleaching agent was used as the positive control. Its high performance was thus expected and may be explained by its content of 16% carbamide peroxide. The good

efficiency and bleaching effect of product composed of 16% carbamide peroxide was previously demonstrated (Dietschi et al., 2006).

EXP1 also showed high bleaching performance, with the second highest ΔE00 value in this study. EXP1 is an experimental solution in which the active ingredient is composed of a low concentration of hydrogen peroxide (0.1%), mixed with a doping agent. More details about the exact composition of this new solution cannot be revealed at this time as the patenting process is currently underway. Given the promising outcomes with such low concentration of hydrogen peroxide, we may speculate that the aim of the doping agents is to boost the oxidation-reduction reaction. Further research on EXP1 will be necessary in a later stage to obtain more information on the product efficacity.

HST showed good results in terms of the absolute numbers. However, if we take the detailed L*a*b* values (Figure 5 and Table 11) we can conclude that the ΔL* and Δb* did not change favorably with stains from tea and red wine. As mentioned previously, after an effective bleaching procedure, we expect an increase in the luminosity (ΔL*) and a decrease of the yellow tone (Δb*). However, for those two cases, after the application of the bleaching agent, the ΔL* values went down, which represents a decrease in brightness and Δb* increased, which represents an increase in the yellowness. When it comes to stains from coffee and curry, ΔL* and Δb* were positively impacted by the bleaching procedure. One assumption to explain these results may be the relation between the chemical affinity and molecular polarity, suggesting that HST has a low affinity to staining agent with high polarity (Gregor et al., 2016), as coffee has low polarity, while tea and red wine have high polarity (Waterhouse et al., 2016) (Um & Ruyter, 1991) (Fujita et al., 2006). HST not only showed a lack of whitening effect on the high polarity yellow staining agent and on red wine, but even had a negative effect, considering that teeth of these two groups appeared yellower and less bright after the bleaching procedure. Greenwall-Cohen and colleagues have raised a public health concern regarding OTC whitening

products presenting a lack of effectiveness. Due to the lack of efficiency, consumers will tend to overused them with the aim of obtaining a favorable outcome. This trend has been described as a "catch up mentality" (Greenwall-Cohen et al., 2019). HST is composed of Phthalimidoperoxycaproic acid (PAP) as the main bleaching active ingredient. Unlike Hydrogen peroxide, PAP has another method of oxidation action that does not come from the oxidation-reduction reaction but comes from an epoxidation reaction which as a result will form epoxide (oxirane) product (Denmark et al., 1995). The concentration of every active ingredient has to be considered when analyzing the effectiveness of a bleaching solution, however HST manufacturer's does not reveal any information regarding Phthalimidoperoxycaproic acid (PAP) concentration. Without further knowledge we can hypothesize that the HST's poor bleaching efficiency may be linked to a sub-optimal concentration of the active agent. Phthalimidoperoxycaproic acid (PAP) has been widely used among several industries besides dental bleaching. It is used as bleaching agents for textiles, in cleaning and laundry products as well as in personal care cosmetics including make-up, fragrance and shampoo. Surprisingly, Phthalimidoperoxycaproic acid (PAP) is also used in the agricultural sector including active agent for pesticides (PubChem Compound Summary for CID 9860421, Phthalimidoperoxycaproic acid).

HST displayed overall poor results and even worsen the appearance of teeth stained with tea and red wine. To better understand these results, we need to further investigate into HST composition. For example, Punica granatum seed (pomegranate) extract is one of its components and the manufacturer declares to use this ingredient for its anti-inflammatory proprieties. Indeed, in the literature, the pomegranate waste extracts have been described for its ability to "scavenger free radical and its potent antioxidant capacity" as well as its "antibacterial, antiviral, hypolipidemic and anti-inflammatory" abilities (Sorrenti et al., 2019). In addition to this properties, another research studied the "staining effect of pomegranate

flower extract on human blood cells" and highlighted pomegranate flower extract staining capacity (Nilgun Guler Kusculuo et al., 2017). It is described as a "deep orange-brown neutral dye", as pomegranate flower extract is able to stain human blood cells (which are pH neutral). One assumption to explain the unfavorable results obtains when HST is used with teeth stained with red wine and tea may be related to pomegranate extract staining capability on pH neutral substrates. Malir and al. display black tea beverages range around pH 6.68 which may be consistent with the "neutral dye" pomegranate staining ability (Malir et al., 2014). When it comes to red wine, clear data about the pH are not available, however the assumption is that it is acidic and its pH range bellow the neutral pH. Moreover, pomegranate deep orange-brown staining might explain the decrease in brightness (ΔL*) observe with tea and red wine subtract. The increase of the yellowness (Δb*) could be explained by the Chamomilla recutita flower (chamomile) extract, which is also part of HST composition. According to the manufacturer description this ingredient is used as a soothing agent and also for its anti-inflammatory properties. Chamomilla recutita flower (chamomile) extract is composed by a chemical compound called apigenin, which is part of the flavone class. Apigenin has a solid yellow crystalline appearance and is known for its anti-inflammatory, antioxidant and others properties. Moreover, due to its yellow appearance, apigenin has been used to dye wool (Ali et al., 2017). Even though HST manufacturer do not reveal the concentration of Punica granatum seed (pomegranate) extract and Chamomilla recutita flower (chamomile) extract, it is reasonable to assume that these two components play a role in the ΔL* and Δb* variations. However, more research is needed to better explain this phenomenon.

MEA and IWH showed overall similar behavior. Both of them contain citric acid as active agent, and additionally IWH contains Phthalimidoperoxycaproic acid (PAP). Citric acid is mainly found in fruit drinks or juices and is known for its erosive action (Lussi et al., 1995). Citric acid contained in these bleaching agents main action results in etching the tooth surface.

It has a favorable action only with the pigments located in the external layer of the tooth rather than removing the staining in the deep surface. In some studies, citric acid is also described as an accelerator for bleaching (Ablal et al., 2013). When it comes to IWH, manufacturer do not reveal any details regarding Phthalimidoperoxycaproic acid (PAP) concentration, which limit deep analysis. In addition to the previous active agent, hydrated Silica is also present in IWH composition. Hydrated silica are abrasive particles which remove extrinsic stain by superficial abrasion and therefore result in a lightening effect (Jurema et al., 2018). It is mainly found in whitening toothpaste and Mosquim and al. widely described its action and highlighted that these particle "enhanced the enamel erosive wear (Mosquim et al., 2017).

Phthalimidoperoxycaproic acid (PAP) is a non-hydrogen peroxide active agent, increasingly used in OTC bleaching agent. In order to assess and compare its whitening potential, this study selected three bleaching products namely IWH, HST and PAP pure, all containing this active agent. Each product displayed different outcomes. PAP pure, with a concentration of 10-15% of active agent showed the lowest whitening potential in this study. Two assumptions can be made to explain these discrepancies, one related to the different concentration of the active ingredient present in each product and a second one related to the variations in the other ingredients constituting each product. Indeed, in addition to PAP as main active ingredient, MEA and IWH also contain abrasive agent such as citric acid, hydrated silica and sodium bicarbonate. The conclusion based on these observations is that PAP combined with abrasive agent present a more favorable overall bleaching outcome.

Finally, OZG demonstrate a very low whitening potential, similar to the negative control (GLY). Ozonized sunflower seed oil is OZG main active agent. Due to its various biological properties such as antimicrobial effects (bactericidal action), angiogenesis stimulation and high oxidative capacity, ozone is considered as a promising molecule (Guinesi et al., 2011) (AL-Omiri et al., 2018). Ozone has been used widely and successfully in dentistry, it is an instable

and very reactive gas with a short half-life. For this particular reason, it cannot be stored (Suh et al., 2019). Elements such as air, water, pH and temperature will have an impact on its decomposition. To explain OZG poor whitening effectiveness, it can be assumed that ozone does not display a favorable result when used in a form of paste due to the presence of oxygen. The oxidative potential depends on ozone concentration, however when it comes to OZG the manufacturer does not provide any information in this regard. It can be assumed that ozone's concentration and the radical's formation may be to insufficient in OZG. Lastly, the short exposition time between the tooth and OZG paste might be unfavorable for a deep action of the oxidative agent.

According to Tables 8, 9 and Figures 1, 2 the bleaching exposure time has a positive impact on the final color variation (ΔE00). Moreover, when exposed to a bleaching agent, red wine represents the staining substrate providing the highest color variation (ΔE00) over time.

Conclusion

The comparison between commonly used over-the-counter whitening kits and "traditional" products based on hydrogen peroxide resulted in three key takeaways:

1) All over-the-counter whitening kits tested in this study, except one, exhibited positive color variation. However, the individual performance differed vastly from one brand to the other and the overall performance was less compared to conventional carbamide peroxide-based positive control.
2) One product; Hismile teeth showed partial negative performance with two specific staining agents. Further research might be needed to understand and investigate the disparity in performance driven by the underlying staining agent.
3) The experimental bleaching agent showed the best results of all OCT products tested. These results were close to the positive control with carbamide peroxide.

Tables and Figures

Table 1 – Details of staining solution

Group	Staining agent	Manufacturer	Batch number	Proportion
Group A	Coffee	Ristretto, Nespresso, Nestlé, Switzerland	0272378606	60 ml
Group B	Tea	Twining Earl Gray tea, London, England	0000579251	3 tea bags in 60 ml of water
Group C	Red Wine	Côte du Rhône (DOC), Les arènes, Vacqueyras	1306471D	60 ml
Group D	Curry	Curry Bio Natura plan Coop	1291177	5g curry in 60 ml water
Group E	Distilled Water	N/A	N/A	60 ml

Table 2 – Details of bleaching agents

Group	Product	Manufacturer	Ingredients	Active agent
N° 1	MeaWhite kit teeth withening	Plastimea SA (Brussel, Belgium)	Glycerin, Propylene glycol, Purified water, Hazel extract, Sodium phytate, Citric acid, carboxymethyl	Citric Acid
N°2	iWhite instant teeth whitening	Sylphar NV (Deurle, Belgium)	Aqua, Hydrated Silica, Glycerin, Sorbitol, Chondrus Crispus Powder, PEG-40 Hydrogenated Castor Oil, Aroma, Phthalimidoperoxycaproic Acid, Citric Acid, Methylparaben, Acrylates/Acrylamide Copolymer, Paraffinum Liquidum, Xylitol, Calcium Lactate, Calcium Gluconate, Potassium Acesulfame, Polysorbate 85, BHT	Phthalimidoperoxycaproic Acid, Citric Acid
N°3	PAP pure	Cosmolab (Zurich, Switzerland)	Glycerin, propylene glycol, maltodextrin, phthalimidoperoxycaproic acid, acrylates/C10-30 alkyl acrylate cross polymer, menthe arvensps leaf oil, mica, CI 77891, menthe piperita oil sodium saccharin	Phthalimidoperoxycaproic Acid (10-15%)
N°4	Opalescence PF 16% regular	Ultradent (Dardilly, France)	Carbamide peroxide 16%, Glycerin, Water, Urea, Xylitol, Carbomer, PEG-6, Sodium Hydroxide, EDTA, Potassium Nitrate, Sodium Fluoride	Carbamide Peroxyde 16%
N°5	EXP1	CUMD (Geneva, Switzerland)	0,1% H2O2, Doping agent	0,1% H2O2

N°6	HiSmile teeth whitening kit	HiSmile Pty Ltd (Goldcoast, Australia)	Sorbitol, Water, Phthalimidoperoxycaproic Acid, Propylene Glycol, Glycerin, Potassium Nitrate, Polyethylene Glycol-8, Hydroxyapatite, Sodium Carboxymethyl Cellulose, Hydroxyethyl Cellulose, Xanthan Gum, Peppermint Essence, Saccharin Sodium, Methylparaben, Sodium Bicarbonate, Aloe Leaf Extract, Chamomile Extract, Pomegranate Seed Extract, Propylparaben	Phthalimidoperoxycaproic Acid
N°7	Lubricating Gel	K-Y Johnson & Johnson	Water, Glycerine, Propylene Glycol, Hydroxyethylcellulose, Methylparaben, Sodium phosphate, Disodium phosphate, Propylparaben, Tetrasodium EDTA	N/A
N°8	oZoral Gel oral	Innovares Srl (Sant'Ilario d'Enza, Italy)	Water, Ozonized Sunflower Seed Oil, Aroma, Glycerin, Carbomer, Polycarbophil, Sodium Hydroxide, Sodium Saccharin, Glyceryl Caprylate, Tocopherol, Ascorbyl Palmitate, Disodium EDTA, Limonene, Linalool	Ozonized Sunflower Seed Oil

Table 3 – Bleaching agent

Group	Product	Code	Batch number	Instruction for use	Experimental application	Light activation
N° 1	MeaWhite kit teeth withening	MEA	93/42/EEC 2007/47/EC	20 x 20 min	20 x 20 min	Yes
N°2	iWhite instant teeth whitening	IWH	AAA156 05-2020	5 x 20 min	10 x 20 min	No
N°3	PAP pure	PAP pure	No batch number as is has been freshly produced in the manufacturer's laboratory	10 x 20 min	10 x 20 min	Yes
N°4	Opalescence PF 16% regular	OPL	BGX34	7 x 5 hours	10 x 20 min	No
N°5	EXP1	EXP1	No batch number as is has been freshly produced in the CUMD laboratory	10 x 20min	10 x 20 min	No
N°6	HiSmile teeth whitening kit	HST	111042019	6 x 10 min	20 x 10 min	Yes
N°7	Gylcerin	GLY	8351914	N/A	10 x 20 min	No
N°8	oZoral Gel oral	OZG	30318	7 x 20 min	10 x 20 min	No

Table 4 - Repeated measure ANOVA table for white background. All the main effects are statistically significant. All the interactions are statistically significant with the interaction among TIME and Staining weakly significant. This means that the before/after staining applies differently according to the groups and, less evidently, according to staining liquids.

Effect	SS	Degr. Of Freedom	MS	F	p-value
Intercept	16180,9	1	16180,9	1214,338	<0.001
Staining	2026,83	4	506,71	38,027	<0.001
Group	1272,94	7	181,85	13,647	<0.001
Staining*Group	1673,81	28	59,78	4,486	<0.001
Error	5862,95	440	13,32		
TIME	74,57	2	37,29	16,552	<0.001
TIME*Staining	40,84	8	5,1	2,266	0.02
TIME*Group	102,04	14	7,29	3,235	<0.001
TIME*Staining*Group	229,81	56	4,1	1,822	<0.001
Error	1982,42	880	2,25		

Table 5 - Repeated measure ANOVA table for black background. All the main effects are statistically significant. All the interactions are statistically significant but the interaction among TIME and Staining liquid. This means that the before/after staining effect applies differently according to the groups but not according to the staining liquids.

Effect	SS	Degr. Of Freedom	MS	F	p-value
Intercept	19410,77	1	19410,77	1130,28	<0.001
Staining	1459,265	4	364,8163	21,24308	<0.001
Group	886,1379	7	126,5911	7,371343	<0.001
Staining*Group	1724,966	28	61,60592	3,587284	<0.001
Error	7556,303	440	17,17342		
TIME	61,13151	2	30,56576	16,64183	<0.001
TIME*Staining	10,55795	8	1,319744	0,718548	0,675
TIME*Group	151,7928	14	10,84234	5,90322	<0.001
TIME*Staining*Group	252,3942	56	4,507039	2,453903	<0.001
Error	1616,28	880	1,836682		

Table 6 - Average CIEDE00 color changes over time and standard deviations (in parentheses) per group analyzed over a white background and corresponding grouping (A=best, D= worst). Results with the same capital letter are not significantly different according to Fisher's LSD test; p value < 0.01.

White BG		Distilled water			Coffee			Curry + oil		
Group	**Description**	**t1-t2**	**t1-t3**	**t1-t4**	**t1-t2**	**t1-t3**	**t1-t4**	**t1-t2**	**t1-t3**	**t1-t4**
G_1	**MeaWhite**	1.67^{A} (1.05)	2.44^{A} (1.87)	2.97^{A} (2.00)	1.84^{B} (0.92)	1.97^{A} (1.89)	2.89^{A} (3.18)	2.74^{A} (1.55)	2.64^{A} (1.12)	2.66^{A} (1.31)
G_2	**Iwhite**	1.21^{A} (0.79)	1.97^{A} (1.9)	2.88^{A} (3.33)	3.26^{B} (2.42)	3.18^{A} (2.2)	3.56^{A} (2.61)	2.81^{A} (0.93)	3.34^{A} (1.22)	3.4^{A} (0.69)
G_3	**PAP pure**	1.4^{A} (0.50)	1.34^{A} (0.61)	1.78^{A} (0.58)	2.52^{B} (1.55)	2.34^{A} (1.2)	2.13^{A} (1.14)	2.33^{A} (0.8)	2.24^{A} (0.62)	2.13^{A} (0.6)
G_4	**Opalescence PF**	2.56^{A} (1.11)	2.55^{A} (1.59)	3.26^{A} (1.53)	2.47^{B} (1.28)	2.15^{A} (1.32)	3.51^{A} (1.95)	2.93^{A} (0.75)	2.63^{A} (0.95)	3.45^{A} (1.02)
G_5	**EXP1**	1.36^{A} (0.73)	2.01^{A} (1.12)	3.04^{A} (0.88)	4.24^{A} (1.64)	3.02^{A} (1.07)	3.69^{A} (1.68)	3.3^{A} (1.66)	3.41^{A} (0.89)	$5.07^{A/B}$ (1.71)
G_6	**Hismileteeth**	1.32^{A} (0.7)	1.35^{A} (0.75)	1.90^{A} (0.55)	1.64^{B} (0.95)	2.1^{A} (0.82)	2.13^{A} (0.94)	2.76^{A} (1.7)	3.00^{A} (1.03)	3.67^{A} (1.73)
G_7	**Placebo**	1.74^{A} (1.05)	1.42^{A} (0.51)	1.56^{A} (0.64)	3.54^{B} (5.87)	1.69^{A} (0.86)	1.54^{A} (0.78)	2.07^{A} (0.85)	2.63^{A} (0.83)	2.53^{A} (1.01)
G_8	**oZoral gel**	1.93^{A} (1.07)	1.42^{A} (0.77)	1.04^{A} (0.7)	2.89^{B} (2.38)	2.83^{A} (2.45)	3.05^{A} (2.23)	3.05^{A} (1.05)	3.53^{A} (1.45)	3.62^{A} (1.04)

White BG		Red Wine			Tea			Overall		
Group	**Description**	**t1-t2**	**t1-t3**	**t1-t4**	**t1-t2**	**t1-t3**	**t1-t4**	**t1-t2**	**t1-t3**	**t1-t4**
G_1	**MeaWhite**	5.43^{B} (6.79)	4.67^{B} (2.29)	5.49^{C} (3.15)	3.7^{B} (3.51)	2.89^{C} (2.77)	2.59^{C} (2.82)	3.08^{B} (3.69)	2.92^{C} (2.2)	3.32^{C} (2.74)
G_2	**Iwhite**	5.27^{B} (2.63)	5.02^{B} (3.02)	6.45^{B} (2.35)	3.24^{B} (4.08)	2.14^{C} (1.08)	2.42^{C} (1.6)	3.16^{B} (2.73)	3.13^{B} (2.23)	3.74^{C} (2.64)
G_3	**PAP pure**	3.83^{C} (2.34)	4.57^{B} (2.59)	5.12^{C} (2.56)	2.08^{C} (0.8)	1.94^{C} (0.97)	2.21^{C} (0.9)	2.43^{B} (1.55)	2.49^{C} (1.75)	2.67^{D} (1.81)
G_4	**Opalescence PF**	7.81^{A} (3.77)	9.43^{A} (4.6)	11.2^{A} (4.39)	8.37^{A} (6.69)	8.54^{A} (7.08)	10.17^{A} (6.51)	4.83^{A} (4.35)	5.06^{A} (4.98)	6.32^{A} (5.09)
G_5	**EXP1**	4.07^{C} (2.26)	3.68^{B} (2.46)	5.86^{C} (2.24)	3.02^{B} (1.41)	2.89^{C} (0.97)	4.27^{B} (1.31)	3.2^{B} (1.87)	3.00^{B} (1.49)	4.38^{B} (1.86)
G_6	**Hismileteeth**	6.44^{A} (2.97)	7.58^{A} (2.67)	7.7^{B} (2.45)	7.99^{A} (8.27)	5.28^{B} (4.61)	5.31^{B} (3.69)	4.03^{A} (4.75)	3.86^{B} (3.32)	4.14^{B} (3.03)
G_7	**Placebo**	3.53^{C} (1.99)	3.75^{B} (3.03)	2.97^{D} (1.94)	1.82^{C} (0.74)	1.91^{C} (1.13)	2.09^{C} (1.08)	2.54^{B} (2.88)	2.28^{C} (1.73)	2.14^{D} (1.27)
G_8	**oZoral gel**	4.28^{C} (3.44)	2.89^{B} (1.27)	2.86^{D} (1.63)	1.48^{C} (1.45)	1.19^{C} (1.63)	2.56^{C} (2.31)	2.73^{B} (2.25)	2.37^{C} (1.8)	2.62^{D} (1.86)

Table 7 - Average CIEDE00 color changes over time and standard deviations (in parentheses) per group analyzed over a black background and corresponding grouping (A=best, D= worst). Results with the same capital letter are not significantly different according to Fisher's LSD test; p value < 0.01.

Black BG		Distilled water			Coffee			Curry + oil		
Group	**Description**	**t1-t2**	**t1-t3**	**t1-t4**	**t1-t2**	**t1-t3**	**t1-t4**	**t1-t2**	**t1-t3**	**t1-t4**
G_1	**MeaWhite**	2.93^{A} (2.33)	2.87^{A} (2.57)	3.11^{A} (2.34)	1.72^{A} (1.19)	1.38^{B} (1.05)	3.71^{A} (4.26)	3.84^{A} (2.4)	3.25^{A} (1.55)	3.8^{B} (1.91)
G_2	**Iwhite**	2.31^{A} (2.29)	2.97^{A} (2.55)	3.56^{A} (3.26)	3.13^{A} (1.25)	4.59^{A} (1.83)	4^{A} (2.81)	2.92^{A} (1.69)	3.66^{A} (1.8)	3.67^{B} (1.05)
G_3	**PAP pure**	2.33^{A} (1.91)	2.79^{A} (2.12)	2.3^{A} (1.94)	2.51^{A} (1.62)	2.29^{B} (1.76)	2.5^{A} (1.61)	2.27^{A} (1.14)	2.4^{A} (1.4)	2.36^{B} (1.18)
G_4	**Opalescence PF**	4.47^{A} (4.27)	4.27^{A} (4.44)	4.83^{A} (4.41)	3.84^{A} (3.37)	3.16$^{A/B}$ (2.11)	3.4^{A} (1.61)	3.1^{A} (1.64)	3.16^{A} (1.59)	3.57^{B} (1.79)
G_5	**EXP1**	2.03^{A} (1.09)	2.62^{A} (1.89)	3.65^{A} (1.24)	3.06^{A} (1.21)	2.25^{B} (0.94)	3.05^{A} (1.64)	3.89^{A} (1.58)	3.27^{A} (0.85)	6.02^{A} (1.91)
G_6	**Hismileteeth**	1.52^{A} (1.01)	1.29^{A} (0.59)	2.08^{A} (1.29)	1.93^{A} (1.47)	2.17^{B} (1.29)	2.11^{A} (1.37)	2.81^{A} (1.34)	3.19^{A} (1.00)	4.17^{B} (1.27)
G_7	**Placebo**	2.88^{A} (1.63)	1.79^{A} (1.57)	1.91^{A} (1.71)	2.71^{A} (1.81)	1.4^{B} (0.88)	1.73^{A} (1.05)	2.85^{A} (1.27)	3.19^{A} (1.39)	3.13^{B} (1.49)
G_8	**oZoral gel**	2.14^{A} (1.11)	1.18^{A} (0.83)	1.25^{A} (0.94)	2.00^{A} (1.5)	2.4^{B} (1.44)	2.43^{A} (1.08)	4.69^{A} (1.74)	5.04^{A} (2.39)	5.86^{A} (1.68)

Black BG		Red Wine			Tea			Overall		
Group	**Description**	**t1-t2**	**t1-t3**	**t1-t4**	**t1-t2**	**t1-t3**	**t1-t4**	**t1-t2**	**t1-t3**	**t1-t4**
G_1	**MeaWhite**	4.77^{A} (2.45)	5.55^{B} (2.73)	6.28^{B} (2.80)	3.92^{C} (3.52)	4.53^{C} (3.46)	4.37^{B} (3.26)	3.44^{B} (2.62)	3.51^{B} (2.75)	4.25^{B} (3.12)
G_2	**Iwhite**	4.34^{A} (2.04)	5.19^{B} (2.51)	5.83^{B} (3.10)	3.77^{C} (2.92)	3.77^{C} (1.84)	4.06^{B} (2.13)	3.29^{B} (2.16)	4.04^{B} (2.20)	4.23^{B} (2.64)
G_3	**PAP pure**	6.27^{A} (3.22)	6.09^{B} (3.59)	5.92^{B} (4.02)	2.71^{C} (2.73)	2.81^{C} (2.86)	2.98^{C} (2.86)	3.22^{B} (2.67)	3.28^{B} (2.79)	3.21^{C} (2.81)
G_4	**Opalescence PF**	6.83^{A} (2.57)	8.16^{A} (2.94)	9.39^{A} (2.59)	6.05^{B} (4.11)	6.14^{B} (3.6)	7.73^{A} (5.1)	4.86^{A} (3.51)	4.98^{A} (3.57)	5.78^{A} (4.07)
G_5	**EXP1**	4.78^{A} (2.17)	3.77^{C} (2.56)	5.28^{B} (2.44)	2.72^{C} (1.64)	3.10^{C} (1.16)	4.47^{B} (1.46)	3.30^{B} (1.81)	3.00$^{B/C}$ (1.65)	4.49^{B} (2.04)
G_6	**Hismileteeth**	5.40^{A} (2.64)	6.34^{B} (2.52)	5.43^{B} (2.77)	9.97^{A} (10.33)	9.17^{A} (9.82)	7.50^{A} (6.77)	4.33^{A} (5.66)	4.43^{A} (5.33)	4.26^{B} (3.91)
G_7	**Placebo**	6.37^{A} (3.45)	3.13^{C} (1.80)	3.03^{C} (1.63)	1.59^{D} (0.76)	1.62^{D} (0.99)	1.77^{C} (1.2)	3.28^{B} (2.53)	2.23^{C} (1.53)	2.31^{C} (1.53)
G_8	**oZoral gel**	2.64^{B} (1.34)	2.65^{C} (0.92)	3.68^{C} (1.51)	1.43^{D} (0.82)	2.02^{D} (1.58)	2.58^{C} (2.53)	2.58^{B} (1.72)	2.66^{C} (1.98)	3.16^{C} (2.23)

Figure 1 – Average color difference in terms of CIEDE00 over time according to different staining liquids analysed over a white background. Vertical bars denote 95% confidence intervals.

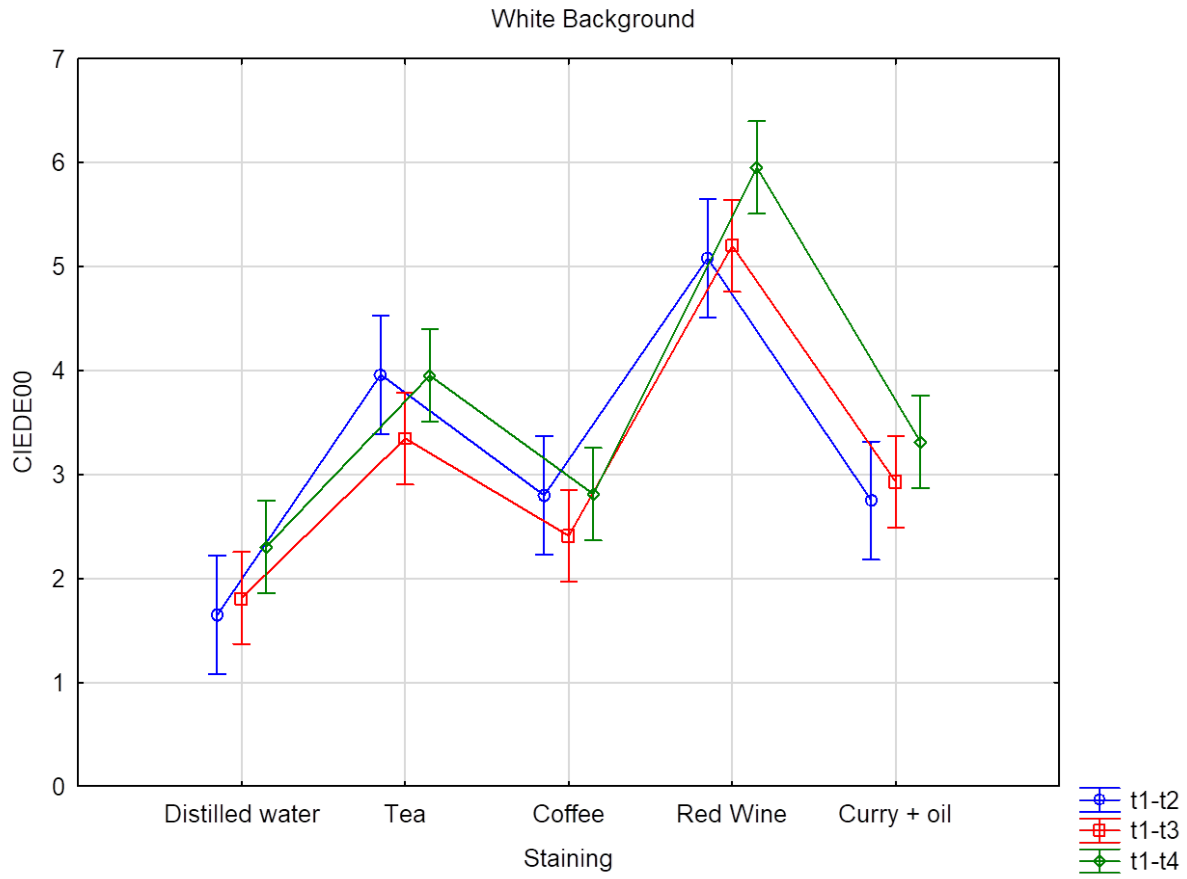


Table 8 – Mean and Standard Deviations of CIEDE00 color differences over time according to different staining liquids analysed over a white background. ABC rankings are from the highest (A) to the lowest staining results using Fisher's LSD post-hoc test at a p-value <0.01.

	White Background					
	Mean t1-t2	**SD t1-t2**	**Mean t1-t3**	**SD t1-t3**	**Mean t1-t4**	**SD t1-t4**
Distilled water	1,65^{D}	0,96	1,81^{D}	1,30	2,30^{C}	1,70
Coffee	2,80^{C}	2,64	2,41^{C}	1,61	2,81^{C}	2,05
Curry + oil	2,75^{C}	1,23	2,93^{C}	1,09	3,31^{B}	1,44
Red Wine	5,08^{A}	3,71	5,20^{A}	3,46	5,96^{A}	3,63
Tea	3,96^{B}	4,85	3,35^{B}	3,89	3,95^{B}	3,93
Total value	**3,25**	**3,26**	**3,14**	**2,79**	**3,67**	**3,02**

Figure 2 – Average color difference in terms of CIEDE00 over time according to different staining liquids analysed over a black background. Vertical bars denote 95% confidence intervals.

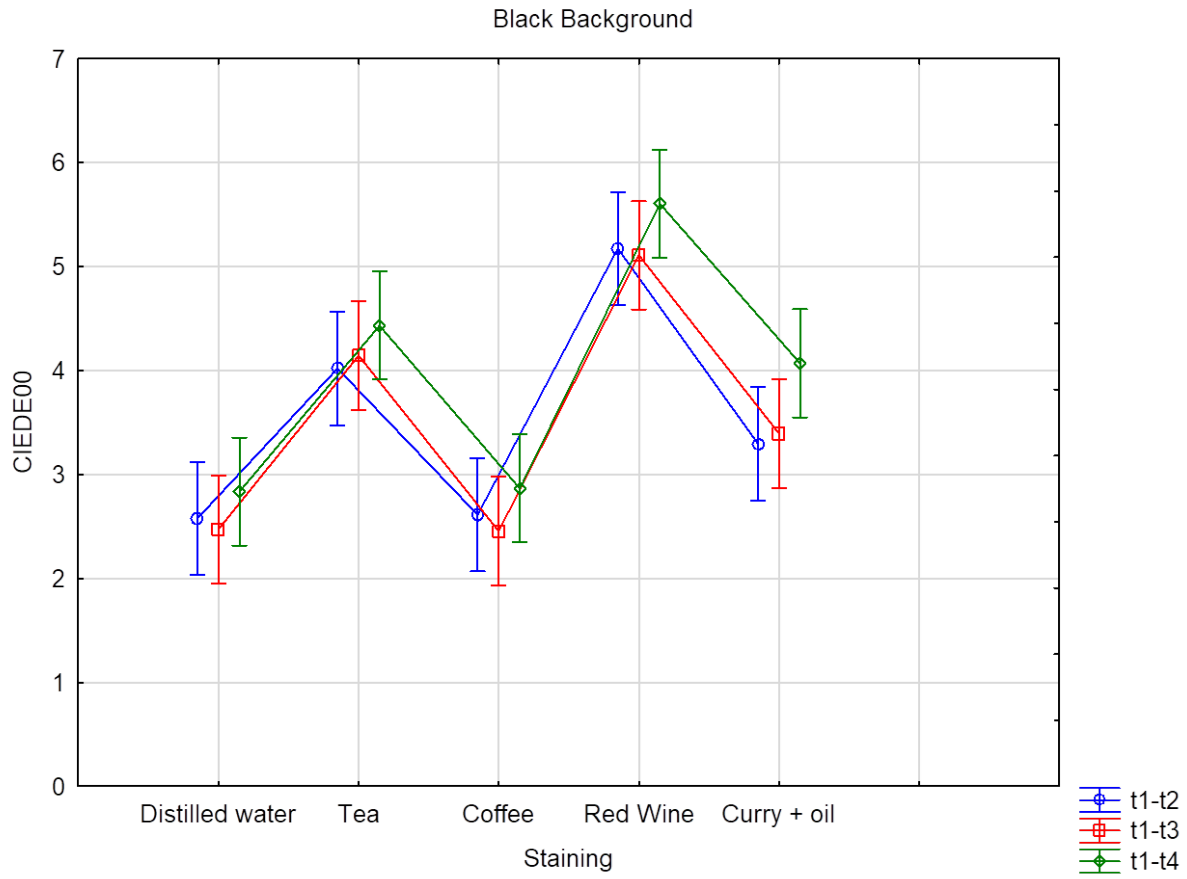


Table 9 – Mean and Standard Deviations of CIEDE00 color differences over time according to different staining liquids analysed over a black background. ABC rankings are from the highest (A) to the lowest staining results using Fisher's LSD post-hoc test at a p-value <0.01.

	Black Background					
	Mean t1-t2	**SD t1-t2**	**Mean t1-t3**	**SD t1-t3**	**Mean t1-t4**	**SD t1-t4**
Distilled water	2,58^{C}	2,27	2,47^{C}	2,46	2,84^{C}	2,56
Coffee	2,61^{C}	1,86	2,46^{C}	1,72	2,87^{C}	2,23
Curry + oil	3,30^{C}	1,74	3,40^{B}	1,66	4,07^{B}	1,92
Red Wine	5,17^{A}	2,78	5,11^{A}	3,01	5,61^{A}	3,16
Tea	4,02^{B}	5,01	4,14^{B}	4,63	4,43^{B}	4,04
Total value	**3,54**	**3,13**	**3,52**	**3,07**	**3,96**	**3,05**

Figure 3 – Average color difference in terms of CIEDE00 (t0-t1) according to different staining liquids analysed over a white background. Vertical bars denote 95% confidence intervals.

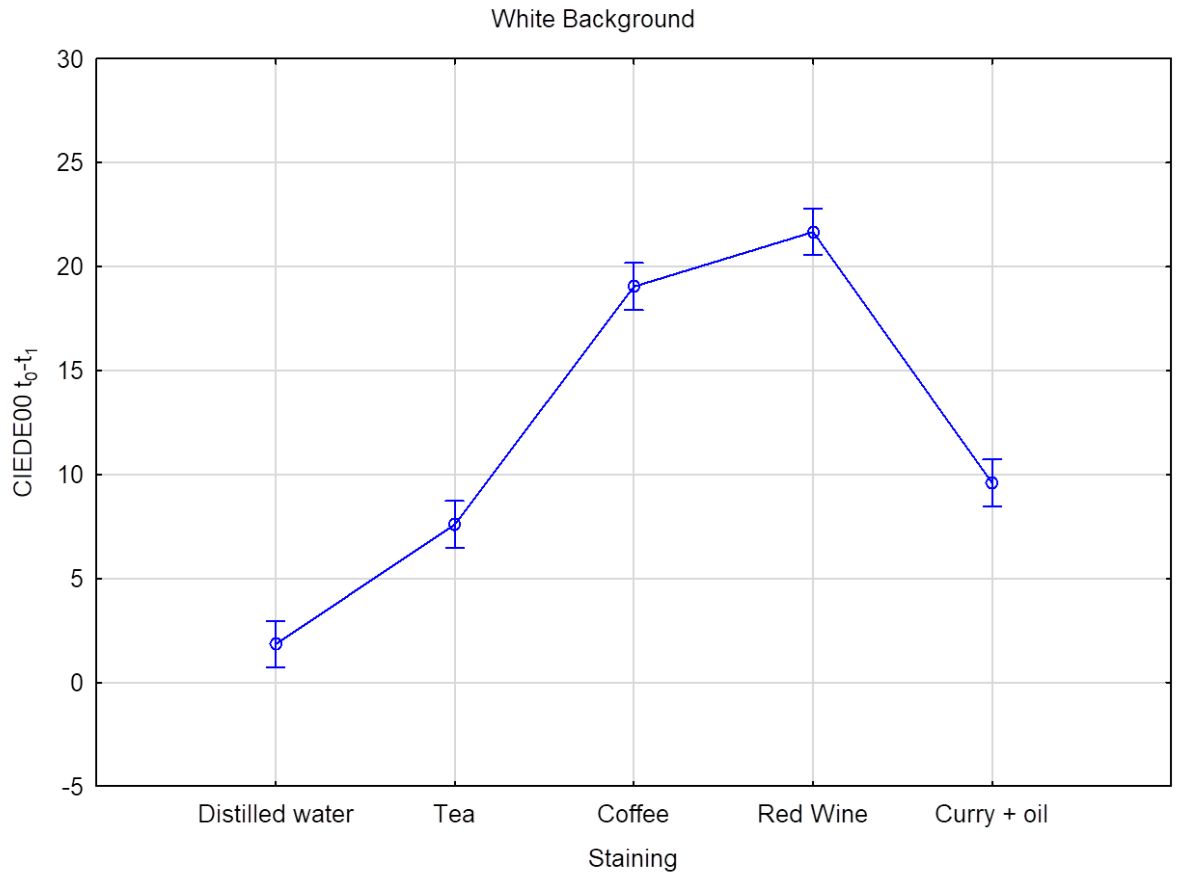


Table 10 – Mean and Standard Deviations of CIEDE00 color differences (t0-t1) according to different staining liquids analysed over a white and black background. ABC rankings are from the highest (A) to the lowest staining results using Fisher's LSD post-hoc test at a p-value <0.01.

	White Background		Black Background	
	Mean t0-t1	**SD**	**Mean t0-t1**	**SD**
Distilled water	1,85^{E}	1,34	2,42^{E}	2,64
Coffee	19,05^{B}	8,74	18,88^{B}	9,09
Curry + oil	9,60^{C}	3,44	10,48^{C}	4,32
Red Wine	21,67^{A}	6,39	21,30^{A}	6,45
Tea	7,60^{D}	5,24	7,25^{D}	4,42

Figure 4 – Average color difference in terms of CIEDE00 (t0-t1) according to different staining liquids analysed over a black background. Vertical bars denote 95% confidence intervals.

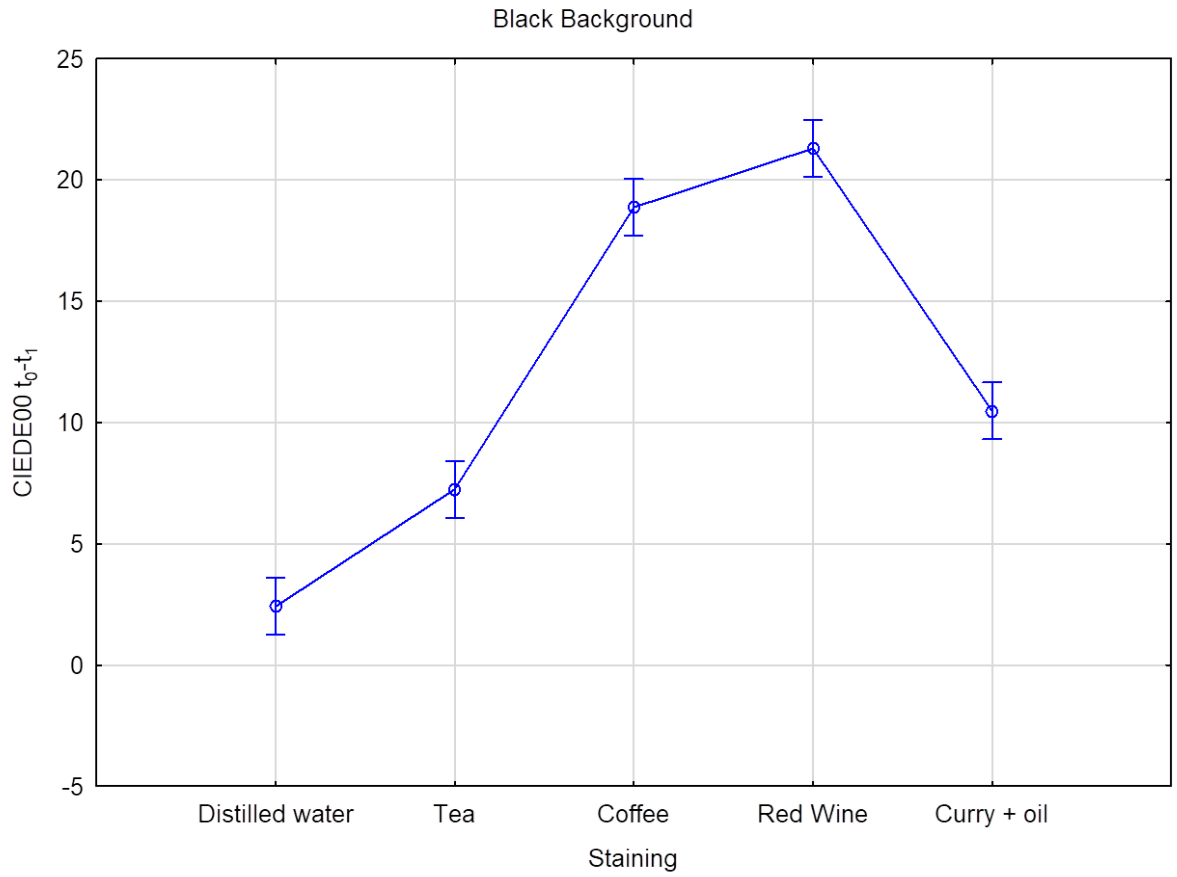
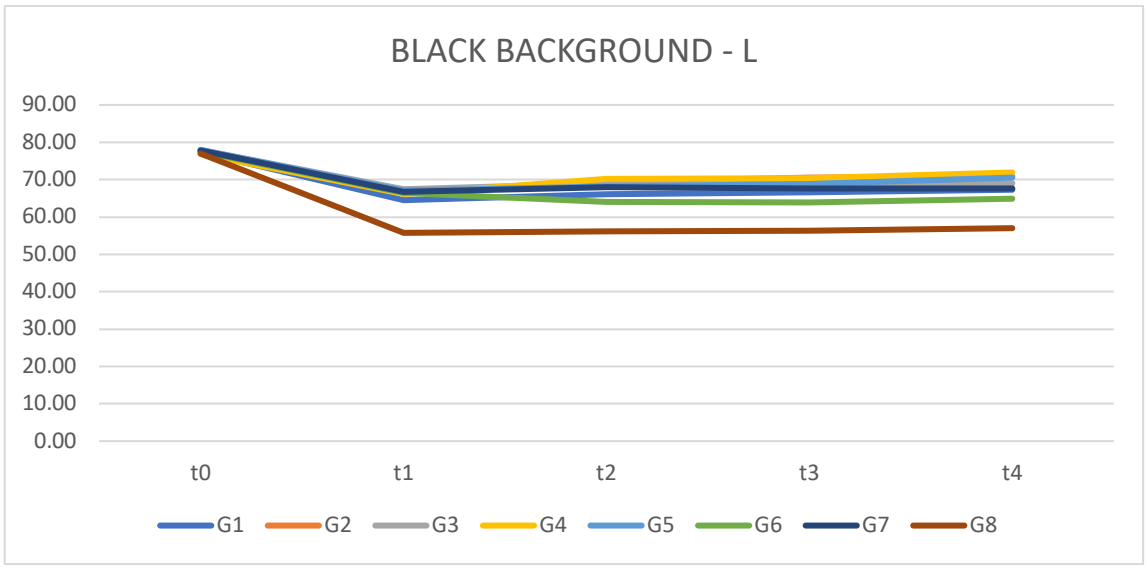
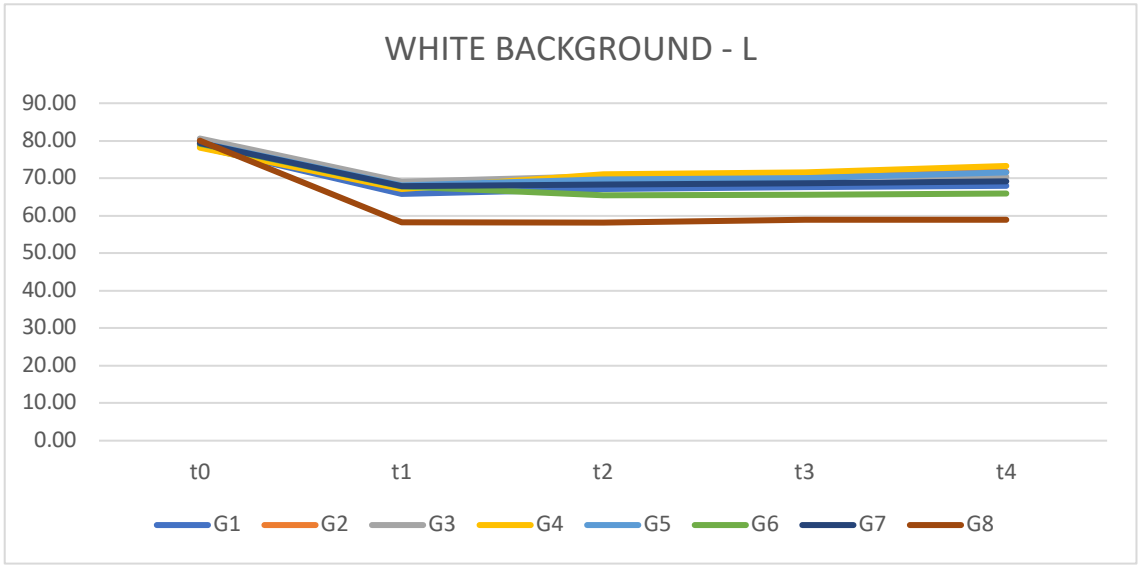
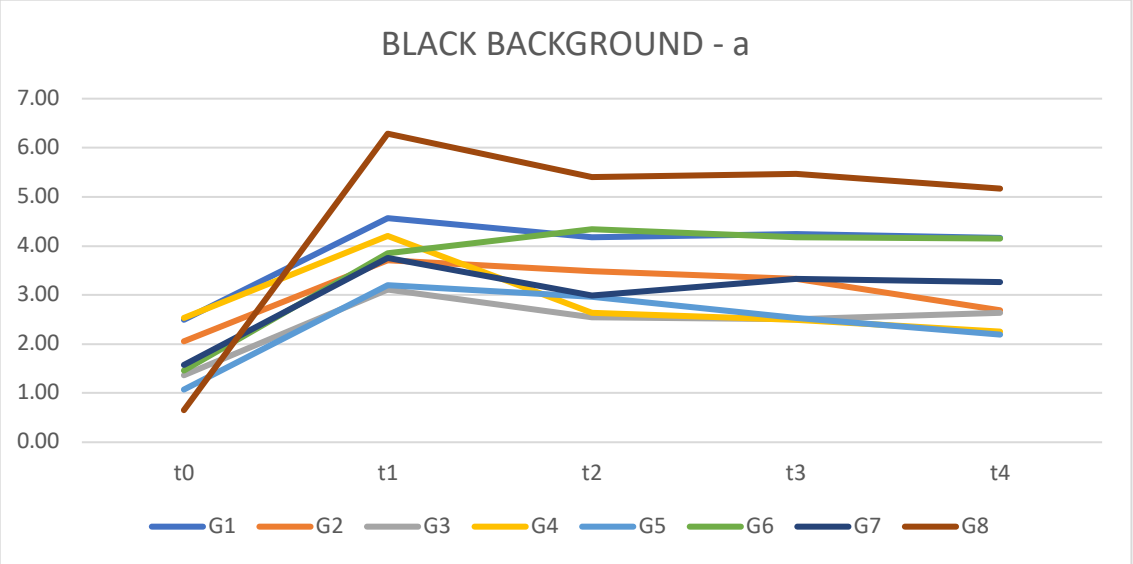
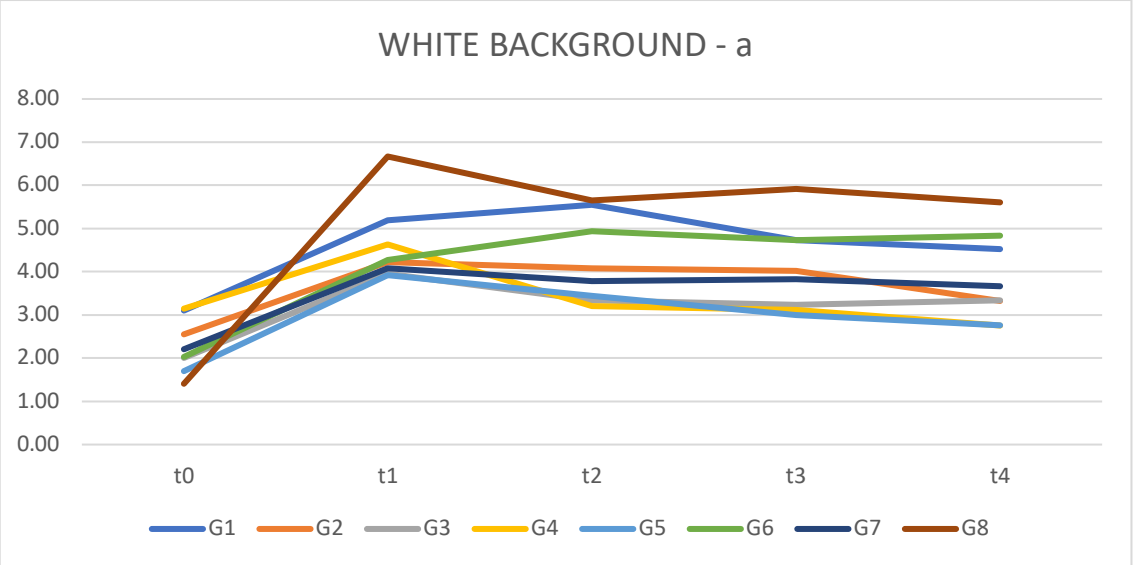


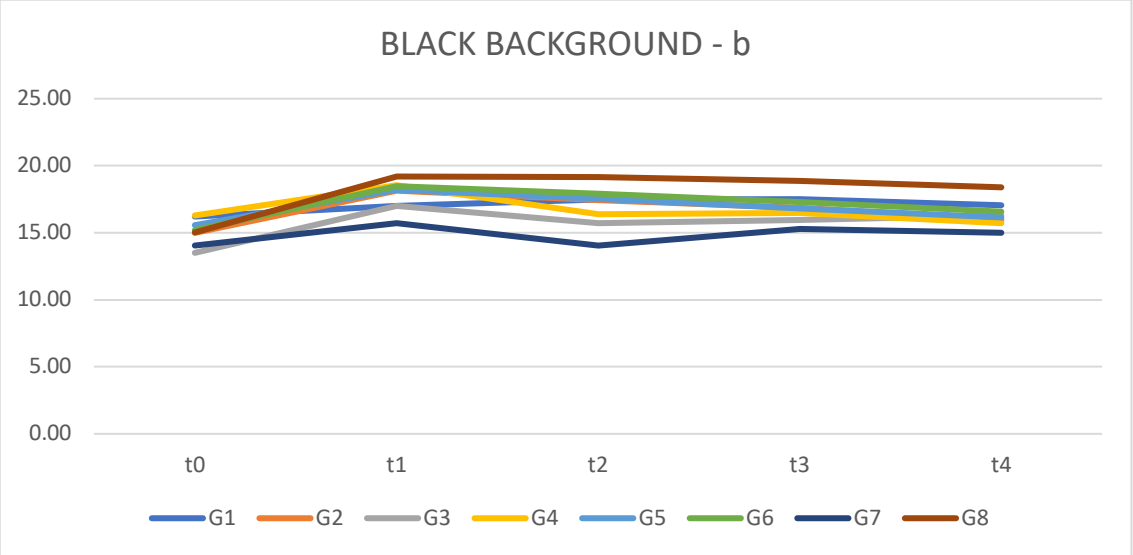
Figure 5– L*a*b* change over time for every bleaching agent over a black and white backgrounds.






BLACK BACKGROUND - a
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
t0
t1
t2
t3
t4
G1
G2
G3
G4
G5
G6
G7
G8


WHITE BACKGROUND - a
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
t0
t1
t2
t3
t4
G1
G2
G3
G4
G5
G6
G7
G8


BLACK BACKGROUND - b
25.00
20.00
15.00
10.00
5.00
0.00
t0
t1
t2
t3
t4
G1
G2
G3
G4
G5
G6
G7
G8

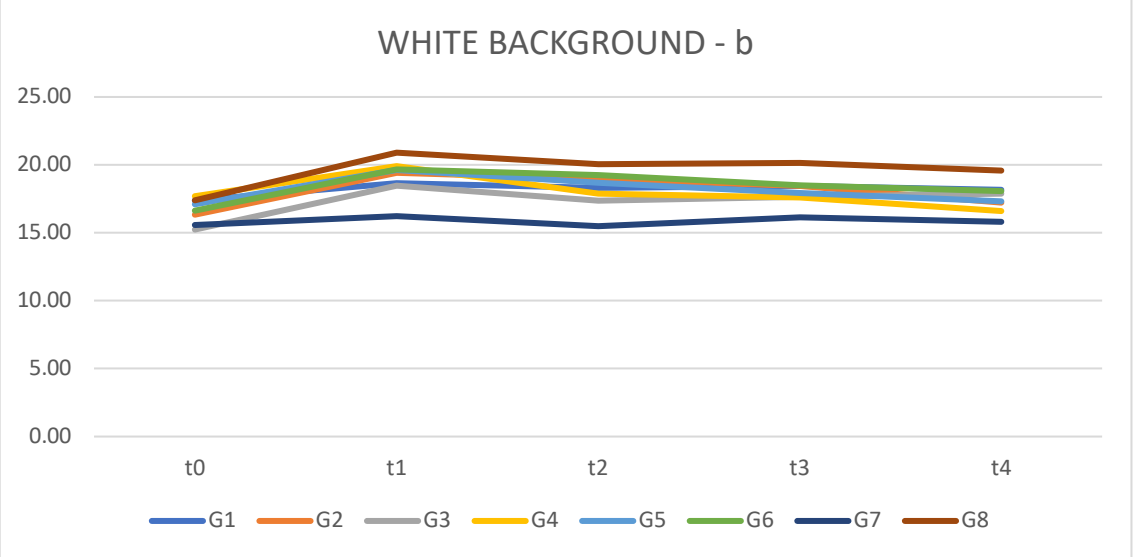

WHITE BACKGROUND - b
25.00
20.00
15.00
10.00
5.00
0.00
t0
t1
t2
t3
t4
G1
G2
G3
G4
G5
G6
G7
G8

Table 11– L*a*b* change over time for every bleaching agent over a black and white backgrounds.

L

BLACK					
	t0	t1	t2	t3	t4
G1	77.36	64.51	66.05	66.67	67.25
G2	77.70	66.68	69.29	70.56	70.87
G3	77.93	67.44	69.05	68.97	69.02
G4	76.85	66.16	70.16	70.45	71.85
G5	77.96	67.09	68.18	68.85	70.55
G6	77.55	66.47	64.02	63.90	64.96
G7	77.79	66.73	67.91	67.72	67.65
G8	76.97	55.76	56.10	56.41	57.05
WHITE					
	t0	t1	t2	t3	t4
G1	78.42	65.81	67.04	67.55	68.01
G2	79.14	68.19	70.52	71.06	71.78
G3	80.58	69.08	70.47	70.81	70.41
G4	78.14	67.12	71.12	71.55	73.22
G5	79.47	68.07	69.69	69.99	71.62
G6	79.18	67.80	65.45	65.63	65.84
G7	79.36	67.88	68.36	68.60	69.19
G8	80.01	58.31	58.16	58.93	58.96

a

BLACK					
	t0	t1	t2	t3	t4
G1	2.49	4.57	4.18	4.24	4.17
G2	2.06	3.71	3.49	3.33	2.68
G3	1.36	3.11	2.55	2.51	2.64
G4	2.54	4.20	2.64	2.49	2.25
G5	1.07	3.20	2.96	2.53	2.20
G6	1.46	3.85	4.34	4.18	4.14
G7	1.58	3.76	2.99	3.33	3.27
G8	0.65	6.29	5.40	5.46	5.17
WHITE					
	t0	t1	t2	t3	t4
G1	3.10	5.19	5.55	4.73	4.53
G2	2.55	4.22	4.08	4.02	3.33
G3	2.01	3.95	3.34	3.23	3.34
G4	3.15	4.63	3.21	3.12	2.76
G5	1.70	3.92	3.44	3.00	2.76
G6	2.03	4.27	4.94	4.73	4.84
G7	2.21	4.08	3.78	3.83	3.67
G8	1.41	6.67	5.65	5.92	5.61

b

BLACK					
	t0	t1	t2	t3	t4
G1	16.21	17.02	17.49	17.50	17.07
G2	14.99	18.14	17.44	16.88	15.96
G3	13.50	17.00	15.74	15.94	16.30
G4	16.29	18.54	16.38	16.46	15.71
G5	15.56	18.20	17.54	16.81	16.13
G6	15.20	18.47	17.91	17.28	16.59
G7	14.04	15.71	14.06	15.31	14.99
G8	15.02	19.19	19.15	18.88	18.39
WHITE					
	t0	t1	t2	t3	t4
G1	17.37	18.65	18.31	18.47	18.15
G2	16.33	19.42	18.96	18.44	17.23
G3	15.24	18.47	17.37	17.67	17.90
G4	17.69	19.91	17.87	17.58	16.60
G5	17.10	19.62	18.68	17.94	17.33
G6	16.63	19.67	19.24	18.51	18.09
G7	15.58	16.23	15.49	16.14	15.79
G8	17.39	20.90	20.07	20.16	19.56

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Appendix

Appendix 1: Sample size and power analysis

Considering the most conservative comparison done a T2, the sample size needed to achieve a standard 0.8 power level for a one-way ANOVA would be of 11 units per combination among liquids and bleaching agent for an overall number of 440 specimens. The used sample size of 480 units rises the power of the one-way ANOVA test in the most conservative condition to 0.8438 at the standard 0.05 significance level. Power analyses has been done using the "Power and sample-size tool" of STATA by StatSoft Inc.

Appendix 2 : CIEDE 2000 (ΔE00) formula. The formula has been described by (Sharma et al., 2005).

$$\Delta E_{00}(L_1^*,a_1^*,b_1^*;\ L_2^*,a_2^*,b_2^*) = \Delta E_{00}^{12} = \Delta E_{00}.$$

1. Calculate C′*i*, *h*′*i*:

$$C_{i,ab}^* = \sqrt{(a_i^*)^2+(b_i^*)^2} \qquad i=1,\ 2$$

$$\bar{C}_{ab}^* = \frac{C_{1,ab}^* + C_{2,ab}^*}{2}$$

$$G = 0.5\left(1-\sqrt{\frac{\bar{C}_{ab}^{*\,7}}{\bar{C}_{ab}^{*\,7}+25^7}}\right)$$

$$a_i' = (1+G)a_i^* \qquad i=1,\ 2$$

$$C_i' = \sqrt{(a_i')^2+(b_i^*)^2} \qquad i=1,\ 2$$

$$h_i' = \begin{cases} 0 & b_i^* = a_i' = 0 \\ \tan^{-1}(b_i^*, a_i') & \text{otherwise} \end{cases} \qquad i=1,\ 2$$

2. Calculate $\Delta L'$, $\Delta C'$, $\Delta H'$:

$$\Delta L' = L_2^* - L_1^*$$

$$\Delta C' = C_2' - C_1'$$

$$\Delta h' = \begin{cases} 0 & C_1'C_2' = 0 \\ h_2' - h_1' & C_1'C_2' \neq 0;\ |h_2' - h_1'| \leq 180^\circ \\ (h_2' - h_1') - 360 & C_1'C_2' \neq 0;\ (h_2' - h_1') > 180^\circ \\ (h_2' - h_1') + 360 & C_1'C_2' \neq 0;\ (h_2' - h_1') < -180^\circ \end{cases}$$

$$\Delta H' = 2\sqrt{C_1'C_2'}\sin\left(\frac{\Delta h'}{2}\right)$$

3. Calculate CIEDE2000 Color-Difference $\Delta E00$:

$$\bar{L}' = (L_1^* + L_2^*)/2$$

$$\bar{C}' = (C_1' + C_2')/2$$

$$\bar{h}' = \begin{cases} \dfrac{h_1' + h_2'}{2} & |h_1' - h_2'| \leq 180^\circ;\ C_1'C_2' \neq 0 \\ \dfrac{h_1' + h_2' + 360^\circ}{2} & |h_1' - h_2'| > 180^\circ;\ (h_1' + h_2') < 360^\circ;\ C_1'C_2' \neq 0 \\ \dfrac{h_1' + h_2' - 360^\circ}{2} & |h_1' - h_2'| > 180^\circ;\ (h_1' + h_2') \geq 360^\circ;\ C_1'C_2' \neq 0 \\ (h_1' + h_2') & C_1'C_2' = 0 \end{cases}$$

$$T = 1 - 0.17\cos(\bar{h}' - 30^\circ) + 0.24\cos(2\bar{h}') + 0.32\cos(3\bar{h}' + 6^\circ) - 0.20\cos(4\bar{h}' - 63^\circ)$$

$$\Delta\theta = 30\exp\left\{-\left[\frac{\bar{h}' - 275^\circ}{25}\right]^2\right\}$$

$$R_C = 2\sqrt{\frac{\bar{C}'^7}{\bar{C}'^7 + 25^7}}$$

$$S_L = 1 + \frac{0.015(\bar{L}' - 50)^2}{\sqrt{20 + (\bar{L}' - 50)^2}}$$

$$S_C = 1 + 0.045\bar{C}'$$

$$S_H = 1 + 0.015\bar{C}'T$$

$$R_T = -\sin(2\Delta\theta)R_C$$

$$\Delta E_{00}^{12} = \Delta E_{00}(L_1^*, a_1^*, b_1^*;\ L_2^*, a_2^*, b_2^*)$$

$$= \sqrt{\left(\frac{\Delta L'}{k_L S_L}\right)^2 + \left(\frac{\Delta C'}{k_C S_C}\right)^2 + \left(\frac{\Delta H'}{k_H S_H}\right)^2 + R_T\left(\frac{\Delta C'}{k_C S_C}\right)\left(\frac{\Delta H'}{k_H S_H}\right)}.$$