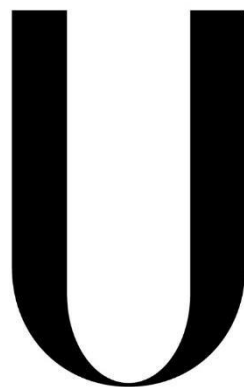


UNIVERSIDADE DE LISBOA
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**Effect of thermal treatments on optical properties
of bis-acryl resins**

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Mestrado Integrado em Medicina Dentária

2018

“Persistence is the shortest path to success!”

Charles Chaplin

ACKNOWLEDGEMENTS

Ao Prof. Doutor Jaime Portugal, por toda a disponibilidade que demonstrou, pela orientação, paciência e dedicação. Com a sua ajuda, este trabalho tornou-se mais fácil e interessante.

A todos os responsáveis e colaboradores do laboratório da GIBBO, pela ajuda e cedência dos equipamentos necessários à realização desta dissertação, e ao Prof. Doutor Pires Lopes, pela cedência do espaço e equipamentos do laboratório de Biomateriais.

À VOCO e à 3M, pela disponibilização dos materiais necessários para a realização deste protocolo experimental.

Aos meus pais, pelo apoio em momentos mais difíceis, deste e de todos os percursos.

Ao meu irmão, Bernardo, por estar sempre pronto a ajudar em tudo o que estivesse ao seu alcance.

Aos meus primos, Catarina e Tiago, por me animarem mesmo sem se aperceberem e estarem sempre disponíveis para mim. Ao Matias, que ainda é pequenino, mas que me traz vida e cor todos os dias com a inocência própria de uma criança.

A toda a minha família, pelo apoio e carinho em todas as horas.

Ao André, por me ouvir em todas as horas difíceis e nunca me deixar desistir. Por acreditar em mim mesmo quando eu mesma não o fiz. Por me marcar para sempre e mesmo que a vida nos separe.

À família que encontrei e que escolhi quando cheguei a Lisboa perdida e com medo, André, Dayana, Filipe, Karina, Luís, Margarida, Maria, Mónica Lourenço, Mónica Moreira e Sara, obrigada por me fazerem sentir sempre em casa. Sem vocês, estes 5 anos não teriam sido tão bons. Obrigada por me ouvirem sempre nos momentos bons e maus, sempre com a opinião mais sincera possível, por nunca se esquecerem de mim e estarem sempre de braços abertos e sorriso no rosto. A vida é melhor convosco.

A todas as pessoas que me continuam a receber com um sorriso mesmo com a ausência que a vida proporciona.

ABSTRACT

Aim: The purpose of this study was to evaluate the effect of various thermal treatments on the chromatic stability of two bis-acrylic resins, after 24h and 7d.

Materials and Methods: Ten experimental groups (n=10) were set according to the several possible combinations between the bis-acrylic resin (Protemp 4, P4; Structur 3, S3) and post-polymerization thermal treatment (without any post-polymerization treatment, nt; post-polymerization in a water bath at 60°C for 5 minutes, wb5; microwave at 750W for 1 minute, mw1; water bath at 60°C for 1 minute, wb1; conventional hairdryer for 1 minute, hd1). Thirty minutes after the polymerization, the baseline color values were measured, and all the specimens were immersed in the staining solution (coffee). After 24h and 7d new measurements were taken and ΔE calculated. A ΔE higher than 3.7 was considered clinically unacceptable. After confirming the normality and homogeneity of the data with Shapiro-Wilk and Levene's testes ($p > 0.05$), the ΔE data were submitted to a 2-way ANOVA, followed by 1-way ANOVA and Tukey post-hoc tests for each material ($\alpha = 0.05$).

Results: The ΔE ranged between 5.9 and 13.0, at 24h, and between 9.1 and 24.7, after 7d. The ΔE was statistically ($p < 0.001$) influenced by both bis-acrylic resin and post-polymerization heat treatment, at 24h and 7d. A statistically significant ($p < 0.001$) interaction was observed between the two factors. At 24h, regarding S3 specimens, all post-polymerization treatments showed a statistically ($p < 0.05$) higher ΔE than the nt group, but for the P4 specimens, the mw1 and wb5 groups showed similar ($p > 0.05$) ΔE than nt group. At 7d, no differences were found between S3 groups. In the P4 specimens, the wb5 showed a lower ΔE than nt, and only the wb1 and the hd1 obtained a ΔE higher than nt.

Conclusion: Both, bis-acrylic resin and post-polymerization treatments, influence the ΔE , at 24h and 7d.

Key-words: bis-acrylic resin; colour, stability; post-polymerization treatments; coffee.

RESUMO

Introdução: Restaurações provisórias são um passo essencial no plano de tratamento em prótese fixa. Os seus principais objetivos são proteger tanto o complexo polpa-dentina como o periodonto e estabilizar e restaurar a função e a estética do dente durante a reabilitação, desde a preparação até a cimentação da restauração final. Além disso, são também usados com ferramenta de diagnóstico para avaliar a eficácia estética, funcional e terapêutica do plano de tratamento, desempenhando um papel essencial no seu prognóstico. Considerando estes objetivos, as restaurações devem obedecer a certos requisitos estéticos, mecânicos e biológicos, como uma boa adaptação marginal, retenção e resistência adequadas, baixa condutividade térmica, sem causar irritação pulpar ou do tecido gengival, estabilidade cromática e permitir uma fácil higiene, pois podem ser utilizados durante longos períodos de tempo. Assim, os materiais que os constituem devem ser biocompatíveis, fáceis de manusear e não-tóxicos, determinando o seu sucesso ou insucesso. Atualmente, existem dois grandes grupos de materiais, tendo em conta a composição química, para a confecção destas restaurações temporárias. Resinas de polimetilmetacrilato e resinas compostas bis-acrílicas. Existem também resinas de dimetacrilato de uretano fotopolimerizáveis. As resinas de polimetilmetacrilato têm um bom acabamento marginal, bom polimento e alta resistência. Mas têm uma elevada contração de polimerização, induzem a irritação pulpar associada ao monómero livre, têm um odor forte e têm uma reação de polimerização exotérmica. Como a mistura é realizada manualmente, essas propriedades físicas podem eventualmente ser influenciadas, inadvertidamente, por diferenças na proporção de monómero e pó recomendada pelo fabricante.

Mais recentemente, as resinas compostas, nomeadamente a resina composta bis-acrílica, ganhou alguma popularidade e mais estudos foram feitos. Esta resina tem uma fácil manipulação, o tempo clínico necessário para a fabricação de uma coroa temporária é reduzido, bom ajuste marginal e facilidade de reparação. Como vantagem em relação às resinas de polimetilmetacrilato, apresenta baixa contração de polimerização e reduzida exotermia da reação de presa. No entanto, tem algumas desvantagens, como a dificuldade em polir e a controversa estabilidade de cor.

O facto de não haver estudos suficientes para comprovar a estabilidade cromática da resina composta bis-acrítica pode resultar num problema estético. Alterações de cor perceptíveis podem comprometer a aceitabilidade do tratamento provisório, o que pode levar à insatisfação do paciente e a um custo económico e tempo extra para a sua substituição.

A estabilidade cromática pode ser afetada por 2 tipos de fatores: 1. pigmentação intrínseca, como descoloração do próprio material, grau de conversão, alteração da matriz da resina ou interface matriz/carga e através dos diferentes procedimentos de polimento do material, levando a diferentes rugosidades da superfície; 2. pigmentação extrínseca, que inclui manchas por adsorção ou absorção de corantes resultantes da contaminação da dieta, hábitos do paciente, nicotina e algumas bebidas. Alguns autores estudaram formas de aumentar a estabilidade cromática desses materiais por meio de tratamentos pós-polimerização, com microondas ou banho de água quente, por exemplo, para aumentar o grau de conversão do material. Assim, aumentar a resistência mecânica, dureza superficial, capacidade de polimento, redução de poros e proporcionar maior estabilidade cromática. A diferença cromática (ΔE) é determinada comparando as respetivas coordenadas obtidas em dois momentos, inicial e final, pela seguinte equação $\Delta E^* = [(L^*_{final} - L^*_{inicial})^2 + (a^*_{final} - a^*_{inicial})^2 + (b^*_{final} - b^*_{inicial})^2]^{1/2}$ (Sakaguchi R & Powers JM, 2012). Neste estudo uma $\Delta E > 3,7$ foi considerada como clinicamente perceptível.

Objetivos: o objetivo deste estudo foi avaliar o efeito de vários tratamentos de pós-polimerização de duas resinas bis-acríticas imersas em café durante 24 horas e 7 dias, de acordo com as seguintes hipóteses nulas: 1) não existem diferenças nas propriedades óticas entre os dois materiais; 2) a estabilidade cromática é influenciada pelos tratamentos de pós-polimerização.

Materiais e Métodos: foram preparados 100 espécimes cilíndricos com 12mm de diâmetro e $2 \pm 0,15$ mm de espessura, cinquenta com *Protemp 4*, cor A3 (Lote: 628352, Ref.70201132621 data de validade: 2018-05, 3M ESPE, Seefeld, Alemanha) e cinquenta com *Structur 3*, cor A3, (Lote: 1807226, Ref. 2505, data de validade: 2020-02, VOCO, Cuxhaven, Alemanha). Imediatamente após o tempo polimerização, indicado pelo fabricante, os espécimes de cada material foram submetidos a um dos tratamentos térmicos estudados: sem tratamento, nt; microondas (Samsung, GW73B, South Korea) com a potência de 750W durante 1 minuto, mw1; banho-maria (J.P. Selecta, Precisterm,

Spain) a 60°C durante 1 minuto, wb1, ou 5 minutos, wb5; e secador de cabelo convencional (Philips, SalonDry Control Hairdryer, HP 8182/23, Netherlands) de 1800W a 20cm provocando uma temperatura de 60°C, hd1. Os espécimes foram limpos, secos e mantidos num meio seco a 22°C durante 30 minutos antes da medição inicial da cor com o espectrofotómetro *VITA Easyshade®* (Vident Brea, CA, EUA). A cor foi analisada de acordo com o espaço de cor CIE L* a* b. As medições foram feitas sobre um fundo cinzento neutro, sempre numa câmara escura. De seguida, foram colocados numa solução de 10mL, em que se diluiu 2g de café em 120mL de água, a uma temperatura de 37°C. Após 24 horas, os espécimes foram escovados e foi efetuada uma nova medição. Os espécimes foram, então, novamente imersos na solução de café e nos 6 dias seguintes, até se atingir uma semana. A solução foi todos os dias renovada. A diferença cromática (ΔE) foi, quer para as 24 quer para os 7 dias, calculada através da equação $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. Os valores ΔL^* , Δa^* e Δb^* foram calculados através da equação $\Delta L = L \text{ final} - L \text{ inicial}$, consoante a coordenada da cor em questão. Os dados foram analisados estatisticamente com o *software* SPSS (IBM SPSS Statistics 20; SPSS Inc). Após confirmar a normalidade e homogeneidade dos dados com os testes de *Shapiro-Wilk* e *Levene* ($p > 0,05$), os dados de ΔE foram submetidos a uma ANOVA de duas dimensões ($\alpha = 0,05$). Finalmente, foi feito um ANOVA de uma dimensão ($\alpha = 0,05$) foi feito para cada material. Quando diferenças significativas entre os grupos foram verificadas, os dados foram analisados pelo teste post-hoc *Tukey*. Para todos os testes, foi fixada uma significância estatística de 5% ($\alpha = 0,05$).

Resultados: os valores da ΔE variaram entre 5,9 e 13,0, após 24 horas, e entre 9,1 e 24,7, após 7 dias. A ΔE foi influenciada estatisticamente ($p < 0,001$) tanto pela resina bis-acrílica como pelo tratamento térmico pós-polimerização, às 24 horas e 7 dias. Foi observada uma interação estatisticamente significativa ($p < 0,001$) entre estes dois fatores. Às 24 horas, em relação às amostras S3, todos os tratamentos de pós-polimerização apresentaram valores de ΔE estatisticamente superiores ($p < 0,05$) ao grupo nt, mas para os espécimes P4, os grupos mw1 e wb5 apresentaram valores de ΔE estatisticamente semelhantes ($p > 0,05$) ao grupo nt. Aos 7 dias, não foram encontradas diferenças ($p = 0,098$) entre os grupos S3. Nos espécimes de P4, o wb5 apresentou valores de ΔE menores que nt, e apenas wb1 e hd1 obtiveram uma ΔE maior que nt.

Conclusões: existiram diferenças entre a estabilidade cromática dos materiais estudados, com o *Structur 3* a apresentar valores de ΔE mais elevados. A estabilidade

cromática foi influenciada também pelo tipo de tratamento de pós-polimerização, ou a falta deste. Apenas os espécimes do grupo mw5, microondas 5 minutos, conseguiram obter uma ΔE estatisticamente inferior ao controlo. Todos os espécimes apresentaram uma alteração de cor considerada clinicamente inaceitável após um período de 24 horas de imersão em café.

Palavras-chave: bis-acrílico; estabilidade cromática; tratamento de pós-polimerização; café.

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INTRODUCTION

Temporary crown and bridge materials are essential for treatment procedures with fixed prosthodontic (Haselton DR *et al.*, 2004). The main goals are to protect both the pulp-dentin complex and the periodontium, to stabilize and restore function and esthetics on the tooth in rehabilitation, from the preparation to the cementation of the final restoration (Burns, DR *et al.*, 2003; Bayindir F *et al.*, 2012; Mei, ML.; 2015). In addition, they are also used as a diagnostic tool to evaluate the aesthetic, functional and therapeutic efficacy of a treatment plan, playing an essential role in its prognosis (Gujjari AK *et al.*, 2013; Sham AS *et al.*, 2004;).

Considering these goals, restorations must comply with certain aesthetic, mechanical and biological requirements (Haselton DR *et al.*, 2004; Rutkunas V *et al.*, 2010) such as good marginal adaptation, adequate retention and resistance, low thermal conductivity, without causing pulp or gingival tissue irritation, chromatic stability and ease of hygiene, once they can be used between several days to half a year or more. Therefore, the materials that embody them should be biocompatible, easy to handle and non-toxic, having a key role in their success or failure (Burns, DR *et al.*, 2003; Patras *et al.*, 2012).

Currently, there are two major groups of materials according to the chemical composition available for the preparation of these temporary restorations (Haselton DR *et al.*, 2004; Givens EJ *et al.*, 2008; Gujjari AK *et al.*, 2013). Methacrylate resins that are available in a powder / liquid, hand mixed formulation such as polymethyl methacrylate (PMMA) resin, polyethyl methacrylate resin or polyvinyl methacrylate resin; and composite-based materials that have a paste / paste formulation, mainly automixed, such as bis-acryl composite resin. There are also photopolymerized urethane dimethacrylate resins. (Michalakis *et al.*, 2006; Patras *et al.*, 2012; Prasad *et al.*, 2014).

Methacrylate resins have a good marginal finish, good polishing and high strength. Nonetheless, their polymerization contraction is high, they induce pulp irritation associated with free monomer, they have a strong odor and their polymerization reaction is exothermic. As the blending is performed manually, these physical properties may eventually be influenced inadvertently by differences in the monomer and powder ratio recommended by the manufacturer.

Recently, this composite-based, namely the bis-acryl composite resin, has gained some popularity and more studies have been done regarding this resin, which composition consists of two or more types of materials (Young HM *et al.*, 2001; Balkenhol M *et al.*, 2007; Strassler HE *et al.*, 2007; Givens EJ *et al.*, 2008; Patras M *et al.*, 2012).

Usually in its constitution has three structural components:

1. Organic matrix: A plastic resin material that forms a continuous phase and binds the charge particles. Commonly, it is a dimethacrylate such as Bis-GMA or UDMA that crosslinks upon polymerization and provides strength to the material (Carneiro, JT. 2017). These monomers have a high molecular weight and are very viscous at room temperature, making it difficult to incorporate filler into the resin matrix. Therefore, it is necessary to associate lower molecular weight diluent co-monomers such as TEGDMA (triethylene glycol dimethacrylate) and EDMA (ethylene glycol dimethacrylate) (Carneiro, JT. 2017);

2. Inorganic filler particles: reinforcing particles and / or fibers dispersed in the matrix. They are usually glass, quartz and / or silica particles and a bonding agent that promotes adhesion between the filler particles and the organic resin matrix, such as silane (Carneiro, JT. 2017).

3. Silane: a bifunctional molecule capable of binding both the inorganic filler and the matrix polymer. These charge particles increase hardness, mechanical resistance to fracture and abrasion (Carneiro, JT. 2017).

This resin can be easily handled, has a good marginal fit, is easy to repair and the clinical time required for the manufacture of a temporary crown with this material is lower. In advantage to Methacrylate resins, a lower polymerization contraction as well as a lower exothermic reaction (Patras *et al.*, 2012, Carneiro, JT. 2017). However, it has some drawbacks such as being difficult to polish (Sen D *et al.*, 2002; Rutkunas V *et al.*, 2010; Turgut *et al.*, 2013) and its controversial colour stability (Yannikakis SA *et al.*, 1998; Haselton DR *et al.*, 2004; Guler AU *et al.*, 2005a; Haselton DR *et al.*, 2005; Givens EJ *et al.*, 2008; Rutkunas V *et al.*, 2010; Gupta G & Gupta T, 2011; Bayindir F *et al.*, 2012; Gujjari AK *et al.*, 2013; Turgut *et al.*, 2013.)

The fact that there are insufficient studies to prove the chromatic stability of bis-acryl composite resin may result in an aesthetic problem. Perceptible colour changes may compromise the acceptability of the provisional treatment, which may lead to patient

dissatisfaction, consequentially costing more money and time to substitute the temporary crown (Guler AU *et al.* 2005; Gupta G & Gupta T, 2011).

Chromatic stability can be affected by 2 types of factors: 1. intrinsic, such as discoloration of the material itself, degree of conversion, alteration of the resin matrix or the interface matrix / load and related to different polishing procedures of the material leading to different roughness of the surface; 2. Extrinsic inclusions, for instance, that include spotting by adsorption or absorption of dyes resulting from contamination from exogenous sources from the patients diet and habits such as smoking and certain beverages (Szesz, AL *et al.*, 2011); (Turgut *et al.*, 2013). Water accumulation and photo-oxidation have been reported to be responsible for internal colour change. Water plays an important role in the chemical degradation processes such as oxidation and hydrolysis and the subsequent change of the optical properties of the restorative materials (Bayindir F *et al.*, 2012). Studies have demonstrated that water absorption in composite materials can be affected by filler content, degree of cross-linking of resin molecules, and the amount of air inclusion or unreacted monomers. Automixing of bis-acryl methacrylate instead of hand-spatulation reduces the amount of air entrapment, but it is unclear how colour stability is affected. Automixing of bis-acryl methacrylate instead of hand-spatulation reduces the amount of air entrapment, but it is unclear how colour stability is affected (Sham AS *et al.*, 2004).

The pigment agent is usually derived from food products and oral hygiene and its effect is dependent upon the time of contact with the restoration. (Yannikakis *et al.*, 1998; Haselton DR *et al.*, 2005; Gujjari AK *et al.*, 2013). According to the literature, red wine (Rutkunas V *et al.*, 2010; Gupta G & Gupta T, 2011), coffee, tea and coca cola (Gupta G & Gupta T, 2011; Bayindir F *et al.* 2012; Gujjari AK *et al.*, 2013) have been identified as foods that are associated with more significant colour changes.

Some authors have studied ways of increasing the chromatic stability of these materials through post-polymerization treatments, with microwaves or hot water baths for example, to increase the degree of conversion of the material. Thus, increasing mechanical strength, surface hardness, polishing ability as well as better pore reduction and greater chromatic stability.

To evaluate the colour stability of a material, in-vitro artificial aging techniques can be used (Sham AS *et al.*, 2004; Haselton DR *et al.*, 2005).

Colour assessment can be achieved in two ways: qualitatively by visual (subjective) comparison or quantitatively by electronic (objective) measurement equipment (Jarad *et al.*, 2007).

Based on the parameters of the system CIELab (1971) (Commission International de l'Eclairage, L = Luminosity, a = chroma along the red-green axis, b = chroma along the azure axis), data obtained from colorimeters or spectrophotometers allow a mathematical comparison (Seghi *et al.*, 1990). The CIELab colour space is a numerical area where the colour of an object can be expressed and referenced by Cartesian coordinates L * a * b *. L * is vertical coordinate and a * and b * are horizontal coordinates. This is the most widely used system for instrumental colour measurement (Stevenson *et al.*, 2010). In another system, called CIELCh the colors with the same colour space of the CIELab system are described, but through cylindrical coordinates. L * expresses the brightness of the sample between black (0) and white (100). C * is measure of the saturation of a chroma and represents the distance of the neutral axis. Finally, h * is the hue measure and is represented by an angle between 0 ° and 360 ° (VITA Easyshade Manual). Colour notation in the CIELCh * system offers an advantage over the CIELab * system because it is easier to relate to the systems used initially, namely the Munsell system, which gave rise to the traditional colour scales used by dental professionals. The communication between these professionals is traditionally done with reference to hue, chroma and value. The conversion of the values from CIELab * to CIELCh * is purely mathematical, being translated by the formulas: $C^* = (a^2 + b^2)^{1/2}$ and $h^* = \text{tang}^{-1}(b/a)$. The values of L * are the same in both systems.

The chromatic difference (ΔE) is determined by comparing the respective coordinates obtained in two moments, initial and final, by the following equation $\Delta E^* = [(L^*_{\text{final}} - L^*_{\text{initial}})^2 + (a^*_{\text{final}} - a^*_{\text{initial}})^2 + (b^*_{\text{final}} - b^*_{\text{initial}})^2]^{1/2}$ (Sakaguchi R & Powers JM, 2012). L * represents the value while a * and b * represent the hue. Positive values of a * indicate the prevalence of red tint, while negative values indicate a prevalence of green tint. Positive values of b * indicate a tendency for yellow hue while negative values indicate a more bluish hue (Givens EJ *et al.*, 2008).

Although there is no consensus among authors, values of $\Delta E < 1$ are considered as not perceptible by the human eye, values of ΔE between 1 and 3.3 are perceptible by people with training (ex: dentists / dental technicians / dental assistants) but clinically

acceptable and values of $\Delta E > 3.3$ are perceptible by untrained people (eg patients) and therefore clinically unacceptable (Vichi *et al.*, 2004, 2011).

OBJECTIVES

The aim of this study was to investigate the effects of post-polymerization treatments on the optical properties of two bis-acrylic resins, at 24 hours and 7 days, according to the following hypotheses:

H0: There are no differences between the optical properties of the studied materials.

H1: There are differences between the optical properties of the studied materials.

H0: Chromatic stability is not influenced by the post-polymerization treatment.

H1: Chromatic stability is influenced by the post-polymerization treatment.

MATERIALS AND METHODS

1. Specimens preparation

The influence of three post-polymerization methods on the colour stability using two commercial bis-acrylic resins after 24 hours and 7 days in a staining solution (coffee) was studied. One hundred (N=100) cylindrical specimens of bis-acrylic with a diameter of 12 mm diameter and a thickness of 2 ± 0.15 mm thickness were prepared, fifty using Protemp 4, shade A3 (Lote: 628352, Ref.70201132621 expiring date: 2018-05, 3M ESPE, Seefeld, Alemanha) and fifty using Structur 3, shade A3, (Lot:1807226, Ref. 2505, expiring date: 2020-02, VOCO, Cuxhaven, Germany) (Figure 1) (Table 1).

Material	Abbreviation	Batch No./Manufacturer	Composition*
Structur 3	S3	1807226/VOCO GmbH, Cuxhaven, Germany	Catalyst (Urethanedimethacrylate, Bis GMA, benzoyl peroxyde); Base (Urethanedimethacrylate)
Protemp 4	P4	628352/3M ESPE, St. Paul Minnesota, USA	Catalyst (2,2'-[(1-methylethylidene) bis (4,1-phenyleneoxy)] bisethyldiacetate, benzyl-phenyl-barbituric acid, silane treated silica, Tert-butyl 3,5,5-trimethylperoxyhexanoate); Base (Dimethacrylate (bisema6), silane treated amorphous sílica, reaction products of 1,6-diisocyanatohexane with 2-[(2-metacryloyl)ethyl]6-hydroxyhexanoate and 2-hydroxyethyl methacrylate (desma), silane treated silica)

*According to the information provided by the manufacturer.

Table 1: Characteristics of the bis-acrylic resin used.



Figure 1: Bis-acrylic resins (Structur 3 and Protemp 4)

A sheet of acetate was placed on a glass slab and on this was a rubber ring with an internal cavity with 12 mm of diameter and 2 mm high, which served as a mold. Bis-acrylic resin was inserted into the mold in a single increment, keeping the tip of the dispenser always immersed in the material and making a slow and continuous movement around the mold to minimize the incorporation of air bubbles. After filling the mold with the bis-acrylic resin, a second sheet of acetate and another glass slab were placed thereon. Constant digital pressure was applied for 10 seconds for the flow of excess material to ensure the production of discs with a uniform thickness of 2 mm and smooth and parallel surfaces. After 5 minutes (Structur 3) and 4 minutes (Protemp 4), where the entire system was held stationary to ensure complete polymerization of the material and avoid any distortion, the glass slabs and acetate sheets were removed, the specimens were removed from the mold and carefully inspected on both sides in backlight (figure 2).

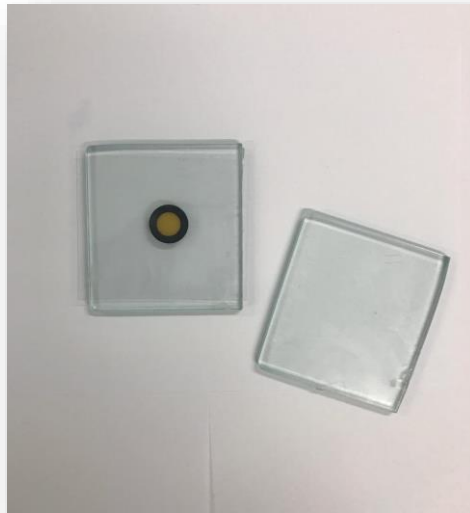
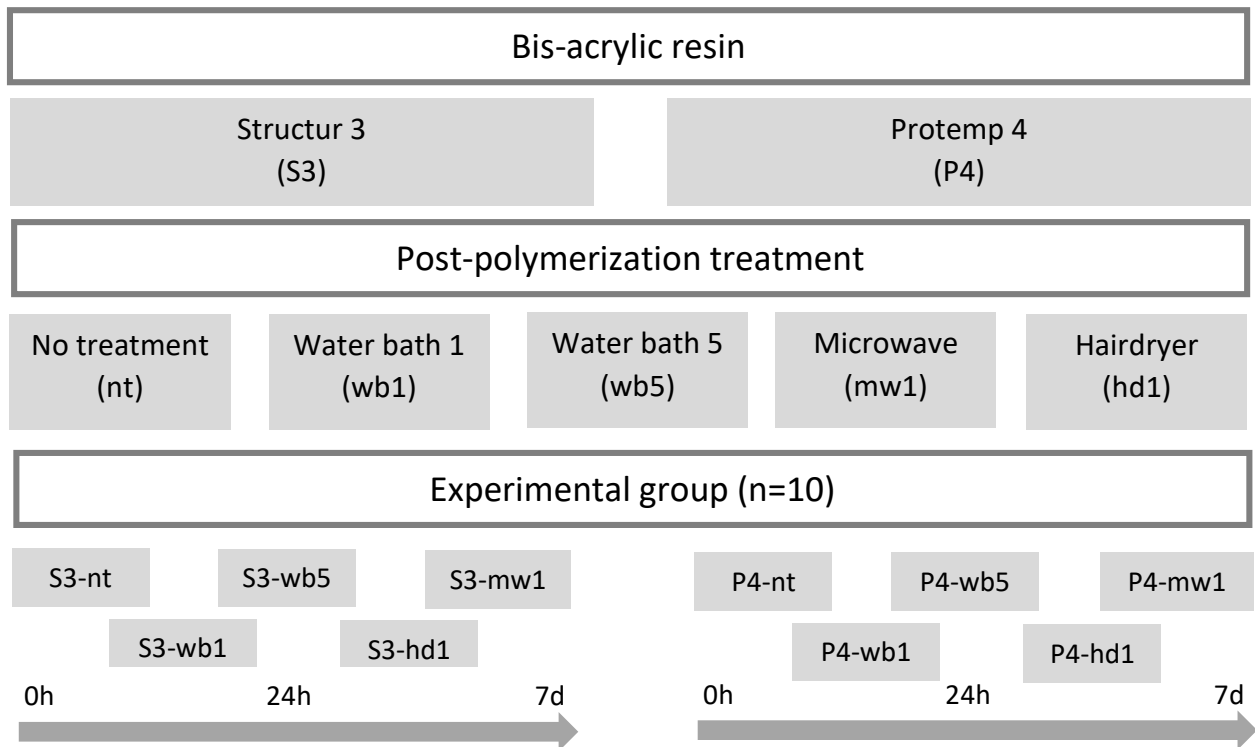


Figure 2: Specimen representative of the sample.

All discs that showed pores or irregularities were discarded and replaced. The specimens were finished by removing the excess material with a spatula and then cleaned with a cotton ball soaked in alcohol. The cotton ball was ruffled on both surfaces to remove the surface layer of material which could exhibit polymerization inhibited by possible contact with oxygen according to the instructions of the respective manufacturers.

2. Post-polymerization treatment

Ten experimental groups were formed according to the various possible combinations between the bis-acrylic resin [Protemp 4 (P4) and Structur 3 (S3)] and the post-polymerization method used ($n = 10$). All procedures will be performed in a standardized manner by the same operator



Immediately after fabrication, specimens were submitted to post-polymerization treatment according to the experimental group:

- a) No treatment (nt): specimens without any post-polymerization treatment and following the manufacturer's instructions;

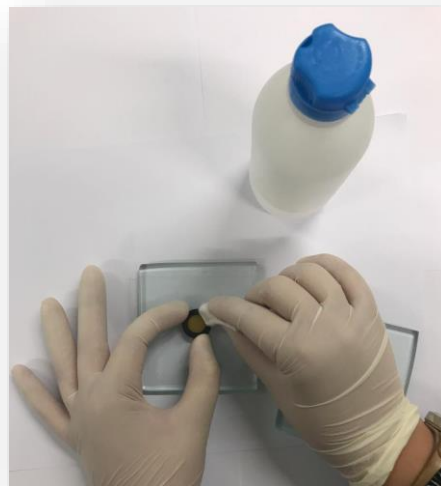


Figure 3: No treatment group

- b) Water bath (J.P. Selecta, Precisterm, Spain) post-polymerization (wb5): specimens subjected to post-polymerization in a water bath at 60 ° C for 5 minutes;

- c) Water bath post-polymerization (wb1): specimens subjected to post-polymerization in a water bath at 60 ° C for 1 minute;



Figure 4: Water bath post-polymerization group

- d) Microwave post-polymerization (mw1): Specimens subjected to post-polymerization in a microwave at 750w for 1 minute;



Figure 5: Microwave post-polymerization group

e) hairdryer post-polymerization (hd1): specimens subjected to post-polymerization with a conventional hairdryer of 1800w, at 60°C and 20 cm;

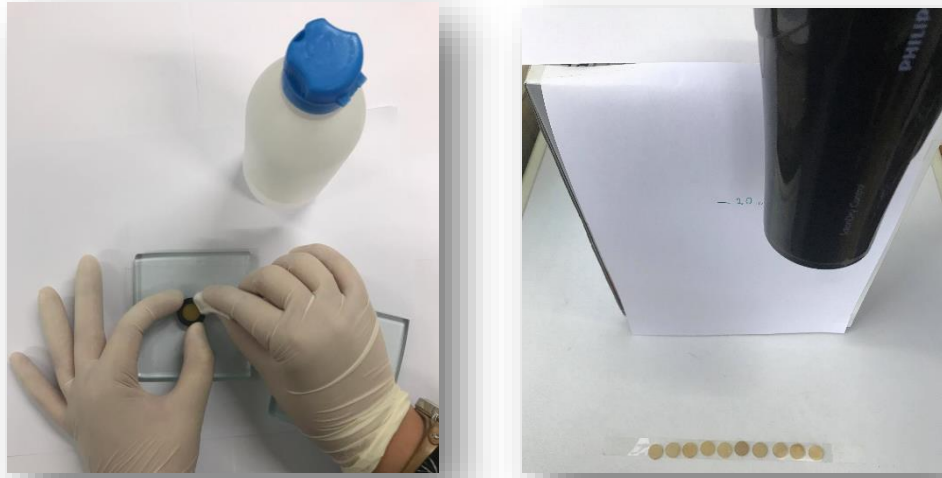


Figure 6: hairdryer post-polymerization group

Material	Post-polymerization treatment	Group's name
	Without any post-polymerization treatment;	S3-nt
	Post-polymerization in a water bath at 60 ° C for 5 minutes;	S3-wb5
Structur 3	Post-polymerization in a microwave at 750w for 1 minute;	S3-mw1
	Post-polymerization in a water bath at 60 ° C for 1 minute;	S3-wb1
	Post-polymerization with a conventional hairdryer;	S3-hd1
	Without any post-polymerization treatment;	P4-nt
	Post-polymerization in a water bath at 60 ° C for 5 minutes;	P4-wb5
Protemp 4	Post-polymerization in a microwave at 750w for 1 minute;	P4-mw1
	Post-polymerization in a water bath at 60 ° C for 1 minute;	P4-wb1
	Post-polymerization with a conventional hairdryer;	P4-hd1

Table 2: Representative table of thermal treatments.

3. Initial colour measurement - T0

The specimens were washed, dried and kept on a dry medium at ambient temperature of 22°C for 30 minutes before starting the initial colour measurement with the VITA Easyshade® contact spectrophotometer (Vident Brea, CA, USA).

The colour was analyzed according to the CIE colour space $L^* a^* b^*$. The measurements were made on a neutral gray background in a darkroom, and the parameters L^* , a^* and b^* were collected. Each measurement was performed three times at the center of the specimen for reproducible results. The three results were recorded, and the mean of the results was calculated, which was used as the initial colour of the specimen in question. Prior to the measurement of each experimental group, the spectrophotometer was calibrated according to the manufacturer's instructions.



Figure 7: A) Easyshade® VITA; B) Measuring the specimen

Then, the specimens were placed in reservoirs, properly identified, with 10 mL of coffee solution (Café solúvel, Pingo Doce) and kept in an oven at 37°C for 24 hours. This solution was prepared with a dilution of 2 g of coffee in 120 ml of water.



Figure 8: A) Reservoir for each Group B) Reservoir with the solution of aging and an experimental group C) Aging solution

4. Colour measurement after 24 hours - T1

After 24 hours of immersion in the coffee solution, the specimens were washed with water and brushed with a tooth brush for 10 seconds on each surface. They were dried with absorbent paper and kept on dry medium at room temperature for 30 minutes, before proceeding to the colour T1 measurement, according to the procedure described above. The specimens were then immersed in a new coffee solution, with the same dilution as previously used, and kept at 37 ° C for 6 days.



Figure 9: Dry stove at 37 ° C

5. Colour measurement after 7 days - T7

During this 6-day period, the coffee solution was renewed once a day and the specimens were brushed with a tooth brush for 10 seconds on each face.

After the total immersion period, which represents an aging of 7 days from its manufacture, the specimens were washed with water and brushed with a tooth brush for 10 seconds, on each surface. They were dried with absorbent paper and kept on dry media for 30 minutes at 22 ° C before the T7 colour was measured according to the procedure described for the initial measurement.

6. Analysis of the colour difference

The chromatic difference (ΔE) was calculated for the 24 hours and the 7 days by the equation $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. The values ΔL^* , Δa^* and Δb^* were calculated through the initial $\Delta L = L_{\text{final}} - L_{\text{initial}}$ equation, depending on the coordinate of the colour in question. ΔE higher than 3.7 was considered clinically unacceptable.

7. Statistical analysis

Data were analyzed statistically with SPSS software (IBM SPSS Statistics 20; SPSS Inc). After confirming the normality and homogeneity of the data with Shapiro-Wilk and Levene's testes ($p > 0.005$), the ΔE data calculated for each aging period were submitted to a 2-way ANOVA ($\alpha = 0.05$). Followed by a one-way ANOVA test for each material. When significant differences between the groups were verified, the data were analyzed through a Tukey post-hoc test ($\alpha = 0.05$).

RESULTS

The descriptive statistics of the samples are presented in table 3, according to the median values of ΔE_{24h} and ΔE_{7d} , with their respective standard deviation.

The ΔE ranged between 5.9 (P4-nt) and 13.0 (S3-wb1), at 24h, and between 9.1 (P4-wb5) and 24.7 (S3-wb1), after 7d.

Material	Post-polymerization treatment	ΔE_{24h} Median (standard deviation)	ΔE_{7d} Median (standard deviation)
Structur 3	No treatment	7.9 (1.99)	23.6 (1.84)
	Water bath 60°C for 5 minutes	11.0 (3.45)	22.5 (2.64)
	Microwave 750w 1 minute	12.8 (1.98)	24.2 (2.50)
	Water bath 60°C for 1 minute	13.0 (1.64)	24.7 (1.45)
	Hair dryer 60°C 1 minute	11.8 (2.43)	22.1 (3.11)
Protemp 4	No treatment	5.9 (3.02)	12.3 (2.17)
	Water bath 60°C for 5 minutes	8.4 (3.05)	9.1 (2.09)
	Microwave 750w 1 minute	5.2 (1.56)	15.2 (2.68)
	Water bath 60°C for 1 minute	10.9 (3.12)	15.6 (3.47)
	Hair dryer 60°C 1 minute	11.6 (0.99)	17.4 (1.33)

Table 3: Mean and standard deviation of the ΔE values for Protemp 4 and Structur 3 after 24 hours and after 7 days of artificial aging in coffee.

The ΔE was statistically ($p < 0.001$) influenced by both bis-acrylic resin and post-polymerization heat treatment, at 24h and 7d. A statistically significant ($p < 0.001$) interaction was observed between the two factors.

At 24h, S3 specimens showed higher ΔE values than the P4 specimens.

Regarding S3 specimens, all post-polymerization treatments showed a statistically ($p < 0.05$) higher ΔE than the nt group. All post-polymerization groups showed similar ΔE ($p > 0.05$) (figure 10).

In the P4 specimens, the mw1 and wb5 groups showed similar ($p > 0.05$) ΔE than nt group. Only the wb1 and the hd1 obtained a ΔE higher than nt ($p < 0.05$). The mw1 group showed a statistically ($p < 0.05$) lower ΔE than the other post-polymerization groups (figure 10).

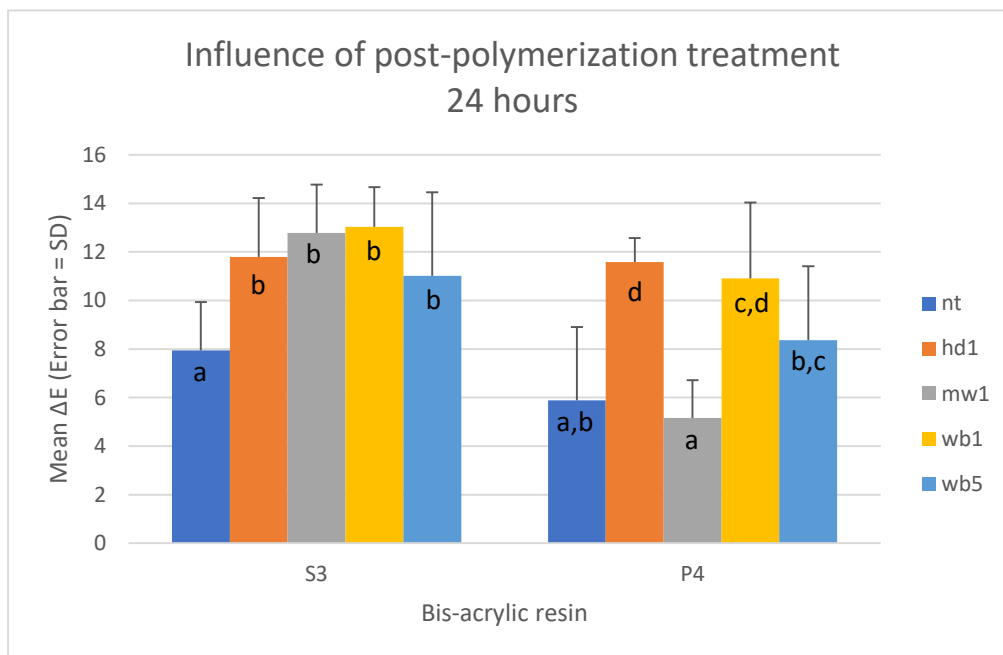


Figure 10: Mean and standard deviation of the ΔE values for Protemp 4 and Structur 3 after 24 hours.

Bars with equal letters are statistically similar ($p \geq 0.05$).

At 7d, as well as at 24h, S3 specimens showed higher ΔE values than P4 specimens.

At 7d, no differences ($p=0.098$) were found between S3 groups (figure 11).

In the P4 specimens, the wb5 showed a lower ΔE than nt, and only the wb1 and the hd1 obtained a ΔE higher than nt. The mw1 showed similar ($p>0.05$) ΔE than nt group (figure 11).

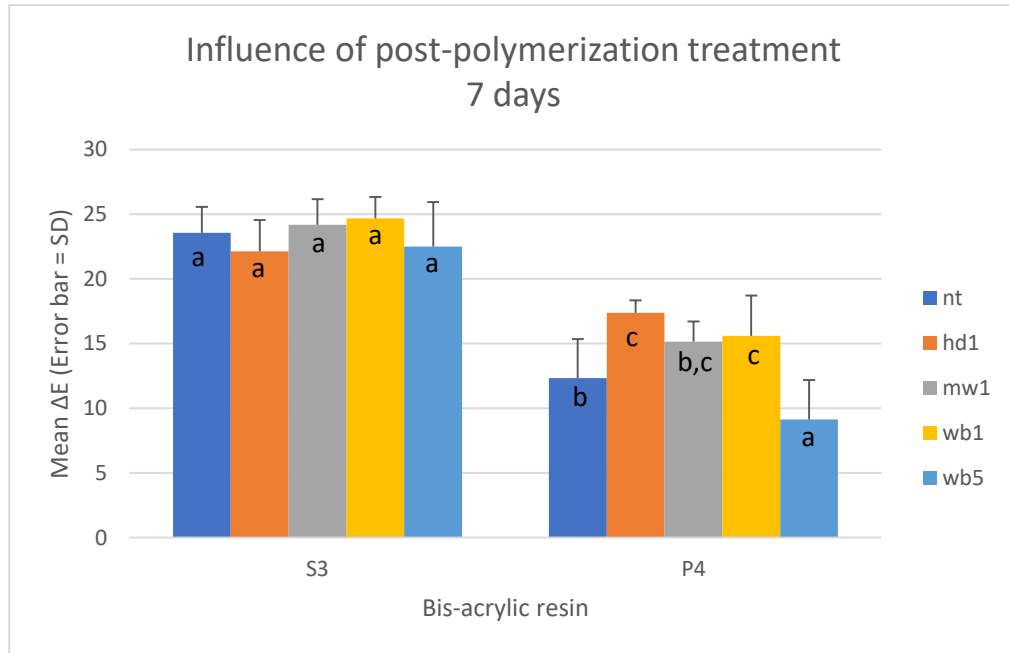


Figure 11: Mean and standard deviation of the ΔE values for Protemp 4 and Structur 3 after 7 days

Despite the observed differences, all experimental groups presented a $\Delta E > 3.3$, considered clinically unacceptable, at 24h and 7d.

DISCUSSION

In this study, coffee was used as staining solution since is a common drink in the Portuguese diet. According to some studies, this drink is capable of a higher and shorter in time staining in materials (Rutkunas V *et al.*, 2010; Gupta G & Gupta T, 2011; Bayindir F *et al.*, 2012; Gujjari AK *et al.*, 2013). Colour changes due to coffee can be explained by a mechanism of surface adsorption and absorption of coloring particles (Gupta G & Gupta T, 2011; Bayindir F *et al.*, 2012). The less polar dyes and the water-soluble polyphenols in the dyes appear to have penetrated deeply into these materials possibly because these are compatible with the polymer matrix (Bayindir F *et al.*, 2012; Sham AS *et al.*, 2004). On the other hand, it has been reported that the coloring ability of the coffee is also due to its smaller molecular size which can be deposited in the pores of the bis-acrylic resin (Sham AS *et al.*, 2004; Gujjari AK *et al.*, 2013). These pores may have been formed due to polymerization shrinkage between the matrix fillers. Brushing performed prior the colour measurement only removes partially particles that are adsorbed to the surface but does not remove the absorbed dye.

The specimens were immersed in the aging solution for 7 days and colour changes were measure at 24 hours and 7 days. The coffee manufacturer purports that the average time for consumption of 1 cup of a drink is 15 minutes. Among coffee drinkers, the average consumption of coffee is 3.2 cups per day. Therefore, the 24 hours' storage time simulated consumption of the drink over 1 month and the measurement after 7 days, a period of approximately one year (Guler AU *et al.*, 2005a).

Colour changes can be evaluated and measured with various instruments. Since these instruments eliminate the subjective interpretation of visual colour comparison, spectrophotometers and colorimeters have been used to measure colour change in dental materials (Szesz, AL *et al.*, 2011). Colour measurements were performed with a spectrophotometer and expressed in CIE (Commission Internationale de l'Eclairage) L*a*b* colour system once because it is well suited for determination of small colour differences.

The CIE-Lab colour system is a uniform colour scale that covers all the colours visible to the human eye and is hence suitable for perceptual studies of colour differences in dental materials (Roque, JS., 2015). Using the CIE L*a*b* colour system, different thresholds of colour change (ΔE) values that produce visually perceptible differences have been reported in several studies. This system provides exactness to the results

obtained, following numerous studies, which describe the agreement of the results obtained with the human visual perception (Szesz, AL *et al*, 2011). The main advantage of using this system is that it allows a summary value, ΔE , to be calculated and used as a tolerance for colour matching (Givens, EJ *et al*, 2008). In this study, the results were obtained through comparisons between colour differences denominated ΔE (Roque, JS., 2015). The value of ΔE represents the numerical distance between the coordinates L^* , a^* and b^* and the relative colour changes an observer can report for materials. Thus, ΔE is more significant than the individual values L^* , a^* and b^* . In the CIE colour system, L^* is defined as the value, or lightness / darkness of the specific colour, while a^* and b^* specify the actual hue or colour. Positive a^* values indicates predominance of red hue, while negative values indicate predominance of green. Positive values b^* indicates a tendency for yellow, while negative values show a bluish tone. (Givens, EJ *et al*, 2008). Some studies emphasize that L^* represents luminosity, and the human eye perceives this characteristic of a lighter colour, since the number of cells (rods) responsible for black and white vision is much larger than that of responsible cells by coloured vision (cones). Thus, any loss of brightness is critical to colour stability and clinical success (Szesz, AL *et al*, 2011).

The first null hypothesis, "there are no differences between the optical properties of the studied materials" was rejected.

The differences in the composition of the two bis-acrylic materials under test may be the justification for the differences observed at both 24h and 7 days.

The second null hypothesis, "chromatic stability is not influenced by the post-polymerization treatment." was also rejected.

Post-polymerization treatments aim to increase the degree of conversion and reduce surface pores of materials. Thus, to reduce both the intrinsic and extrinsic factors of discolouration through simple and practical procedures (Jorge, JH, 2007; Bonatti, MR, *et al*, 2009).

However, the untreated group of Structur at 24 hours showed better results. While at 7 days, there are no differences between all groups. However, with Protemp 4 at 24h, the microwave 1 minute and control groups showed the same results of ΔE . Other groups showed worse ΔE . At 7 days the water-bath group 5 minutes presented the best results, with significant differences for all the other groups.

It has been suggested that the colour change is related, in a first phase, to the extrinsic factors such as the type of pigment and the type of polishing of the restoration material (Turgut *et al.*, 2013). As these resinous materials undergo water absorption, intrinsic factors are the main factors responsible for their colour stability (Sham AS *et al.*, 2004; Rutkunas V *et al.*, 2010). According to some studies, when the degree of conversion is low there may be a chemical discolouration due to the oxidation of the polymer matrix or the oxidation of unreacted double bonds in the residual monomers and the subsequent formation of degradation products from water diffusion (Yannikakis SA *et al.*, 1998).

Therefore, at 24 hours the post-polymerization treatments may have led to an increase in surface pores, and then to a lower chromatic stability. Despite this, wb 5 may have increased the degree of conversion of Protemp 4, thereby causing the intrinsic pigmentation to be reduced.

Johnston and Kao studied the evaluation of the appearance by visual observation and clinical colorimetry and stated that the average colour difference between teeth classified as "match" in the oral environment was $\Delta E = 3.7$. A ΔE value equal to or less than 3.7 is considered visually imperceptible and clinically acceptable. In all groups, with or without post-polymerization treatment, and in the 2 materials, ΔE was > 3.7 , therefore, clinically unacceptable (Roque, JS., 2015).

The problem found in all *in vitro* studies is the correlation of properties measured in the laboratory with those measured under clinical conditions. In the intraoral environment, restorations are subject to several types of aggressors that may contribute to colour change, which were not simulated in this investigation. According to the literature, biofilm formation correlated positively with surface free energy, surface roughness and hydrophobicity (Rutkunas V *et al.*, 2010; Bayindir F *et al.*, 2012).

On the other hand, in the oral environment, a prosthetic restoration is submitted to several influences and conditions: saliva with various proteins and enzymes, a great variety of foods and beverages, temperature extremes caused by food and beverages, smoking, poor oral hygiene habits, functional and parafunctional load. These factors could lead to a higher colour change (Rutkunas V *et al.*, 2010).

In this study, surfaces of the specimens were flat, while clinically, provisional restorations would have an irregular shape with convex and concave surfaces. Therefore, pigments can remain in these irregularities (Gujjari AK *et al.*, 2013).

The specimens were immersed in test solutions that were static, unlike the oral cavity, where the solutions are in a dynamic state (Gujjari AK *et al.*, 2013). The solution used in this study did not constitute all the substances to which the provisional materials may be exposed, although it has been used in similar researches and represents a common environment for the oral environment. Together, all these factors contribute to colour changes. Therefore, a more comprehensive strategy should be developed in order to study influences of the oral environment on the colour stability of provisional prosthetic materials.

CONCLUSIONS

All specimens showed visibly detectable colour change and were considered clinically unacceptable after a 24-hour immersion in coffee.

There were differences between the chromatic stability of the studied materials, at 24h and 7d.

Chromatic stability was influenced by the type of post-polymerization treatment type. Only the treatment in water-bath 5 minutes, at 7 days and in Protemp 4, allowed a ΔE lower than the control.

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