

# Evaluation of tooth bleaching effectiveness through an experimental biological model at *Gallus spp* eggshell

Avaliação da eficácia do clareamento dentário num modelo biológico experimental em casca de ovo de *Gallus spp*.

Larissa Padilha CS SILVA<sup>1</sup>, Vanessa Ávila Sarmento SILVEIRA<sup>2</sup>, Karen Cristina Kazue YUI<sup>3</sup>, Miguel Christian Castillo MARIN<sup>4</sup>, Marianne SPALDING<sup>2</sup>

1 – Fundação Universitária Vida-Cristã – Dentistry School – FAPI-FUNVIC – Pindamonhangaba – SP – Brazil.

2 – Institute of Science and Technology – UNESP – Univ Estadual Paulista – School of Dentistry – Department of Bioscience and Oral Diagnosis – São José dos Campos – SP – Brazil.

3 – Institute of Science and Technology – UNESP – Univ Estadual Paulista – School of Dentistry – Department of Restorative Dentistry – São José dos Campos – SP – Brazil.

4 – Univ do Vale do Paraíba – UNIVAP – Department of Dentistry – São José dos Campos – SP – Brazil.

## ABSTRACT

Tooth darkening compromised oral aesthetics and bleaching is a conservative and efficient method to reestablish it. The aim of this study was to assess the action of 35% hydrogen peroxide in specimens pigmented with different substances. Twenty fragments obtained from chicken eggshell measuring 0.5 x 0.5 cm were randomly divided into 5 groups (G1, G2, G3, G4 and G5) with 4 specimens each. These were previously photographed and pigmented respectively with coffee, cola-based soft drink, wine, beetroot and tobacco for a 48-hour period. In groups 1, 2 and 3, the specimens were directly immersed respectively into the first three substances. In group 4, beetroot was mixed with water to obtain a solution. In group 5, the specimens were submitted to tobacco smoke by the same period. The groups were again photographed and then submitted to bleaching with 35% hydrogen peroxide and photo-activated by laser for 20 min. After the final photographs, the images were analyzed through Adobe Photoshop CS5 software. It was observed that wine most pigmented the specimens (33.34%), followed by coffee (10.35%), beetroot (9.56%), cola-based soft drink (8.85%) and tobacco (0.61%). The bleaching action was more effective on the specimens of G1 (93.9%) and G2 (91.55%), followed by G3 (71.09%), G4 (62.74%) and G5 (57.38%). It was concluded that the methodology was efficient in comparing the effectiveness of the bleaching agent on the pigments tested. The specimens pigmented with tobacco were those which exhibited the greater resistance to bleaching. Taking into consideration the use of this biological material, further studies are necessary to simulate the effects of many other substances onto tooth enamel.

## KEYWORDS

Bleaching; Discoloration; Hydrogen peroxide; Egg shell.

## RESUMO

O escurecimento dos dentes compromete a estética bucal. O clareamento é um método conservador e eficaz para restabelecê-la. Neste trabalho foi avaliada a ação do peróxido de hidrogênio 35% em espécimes pigmentados com diferentes substâncias. Vinte fragmentos obtidos da casca do ovo, medindo 0,5 x 0,5 cm, foram divididos aleatoriamente em 5 grupos (G1, G2, G3, G4 e G5) com 4 espécimes em cada. Estes foram previamente fotografados e pigmentados respectivamente com café, refrigerante a base de cola, vinho, beterraba e cigarro, por um período de 48 horas. Nos grupos 1, 2 e 3 os espécimes foram imersos diretamente nas respectivas substâncias. No grupo 4, a beterraba foi batida com água para obtenção da solução. No grupo 5 os espécimes ficaram acondicionados na fumaça do cigarro pelo mesmo período. Os grupos foram novamente fotografados e posteriormente submetidos ao clareamento com peróxido de hidrogênio 35%, ativado com laser, por 20 min. Após as fotografias finais, as imagens foram analisadas no programa Adobe Photoshop CS5. Observou-se que o vinho foi o que mais pigmentou os espécimes (33,34%), seguido pelo café (10,35%), beterraba (9,56%), refrigerante a base de cola (8,85%) e cigarro (0,61%). A ação clareadora foi mais efetiva nos espécimes dos grupos G1 (93,9%) e G2 (91,55%), seguidos pelos grupos G3 (71,09%), G4 (62,74%) e por último o grupo G5 (57,38%). Concluiu-se que o método foi eficaz para comparar a efetividade do agente clareador nos pigmentos testados, sendo os espécimes impregnados com fumaça de cigarro os que apresentaram maior resistência ao clareamento.

## PALAVRAS-CHAVE

Clareamento; Descoloração; Peróxido de Hidrogênio; Casca de Ovo.

## INTRODUCTION

Patients have now searched by a harmonious aesthetically smile comprising clearer and healthier teeth. This has increasingly aroused interest of dentists who have sought for new products and restorative techniques. Tooth bleaching is an effective and non-invasive method being the first choice for teeth presenting color alteration. Tooth darkening may occur because of either intrinsic or extrinsic pigments due to a complex physical and chemical interaction between the chromogenous agent and the tooth therefore compromising the oral aesthetics [1-4].

In vital teeth, many substances are capable of jeopardizing the tooth color. The extrinsic tooth pigmentation is essentially limited to the tooth enamel and generally results from: 1) the impregnation of pigments and dyes coming from either the diet or tobacco products onto the bacterial plaque or acquired pellicle during the tooth exposure to the environment; 2) the chemical interaction of the pigment with the tooth surface [1-3,5]. Black extrinsic stains may still be a side effect of the administration of ferrous sulphate for the treatment of iron-deficiency anemia [6] and occur because of the presence of chromogenic bacteria, therapeutic agents or metallic compounds [7].

Among the main substances with pigmentation potential, coffee, tobacco and red wine have been reported [5,8-10]. Lima et al. [9] evaluated the bleaching effect onto specimens obtained from human pre-molars, stained by coffee, black tea, cola-based soft drink, wine and tobacco solution. Téó et al. [5] assessed the color alteration of bovine teeth submitted to bleaching and posteriorly immersed into coffee, black tea, red wine, and cola-based soft drink and observed that all substances were capable of promoting tooth darkening. Magalhães [11] tested the color alteration provoked by acai berry extract and coffee in human teeth did or did not submit to bleaching and verified that teeth undergoing bleaching were more susceptible to

staining when exposed to these dyes. Carvalho et al. [10] reviewed the literature on the etiology, consequences and preventive measures of staining of resin composite restorations and concluded that the most common cause of staining is the penetration of pigments coming from: 1) food ingestion, such as coffee, soy sauce, cola-based soft drink, among others; 2) use of products, such as tobacco, nicotine and mouthrinses.

Tooth bleaching has been largely employed nowadays in response of the demanding for aesthetic dental treatments. It can be considered as a conservative alternative to remove stains when compared with other treatments as microabrasion and invasive restorative procedures.

The bleaching agents most used in Dentistry are based on peroxide [12]. Bleaching technique can be classified according to tooth vitality presence or absence and the application mode of the product. For vital teeth, depending on the type and concentration of the bleaching agent, the following techniques can be employed: in-office technique with or without light activation; night-guard bleaching applied by the patient; and an association of both. Currently, some products have also been sold directly to the patients, the so-called “over-the-counter” agents, but the effectiveness and safety of this technique is questionable [12].

The knowledge of the color alteration etiology is mandatory to treat and choose both the technique and the bleaching agents properly, thus constituting a determining factor for bleaching treatment success [13].

Studies aiming to assess the effectiveness of the bleaching agent have been conducted both “in vivo” [14] and “in vitro” and in this latter specimens from either human or bovine teeth have been used [9,15].

The chicken eggshell has been used as an educational tool in experiments aiming to simulate the effect of differences substances onto

tooth enamel [16-18]. It is basically composed of minerals, mainly calcium carbonate (93%), in addition to calcium phosphate, magnesium phosphate, magnesium carbonate, among others [19]. It is a mineralized matrix with high calcium content formed by mineral precipitation similar to the composition of tooth enamel, which is composed of 96% of mineral content, mainly calcium phosphate, hydroxyapatite and other ions such magnesium and fluoride, for example [20] Although the eggshell has chemically similarity tooth enamel, one should consider that these materials are structurally very different. As far as we are concerned, eggshell has not been employed in the studies searched in the literature. Therefore, the aim of this study was two-fold: 1) to assess in vitro the pigmentation capacity of five different substances in fragments from chicken eggshell; 2) to verify the effectiveness of 35% hydrogen peroxide on the bleaching of the specimens undergoing darkening.

## MATERIAL AND METHODS

Twenty fragments from chicken eggshell measuring 0.5 x 0.5 cm were used. They were randomly divided into five groups (n = 4): G1- coffee, G2- cola-based soft drink, G3- red wine, G4- beetroot and G5- tobacco smoke.

These fragments were previously identified and photographed before any treatment. The digitized images were analyzed through Adobe Photoshop C5S software. Percentages of the following pigments were assessed: cyan, magenta, yellow and black at four equidistant points of all fragments. Next, fragments from G1, G2 and G3 were placed into test tubes containing coffee, cola-based soft drink, and red wine, respectively. In G4, the beetroot was mixed with water to obtain a solution prior to the immersion of the fragments. In G5, the specimens were placed into a rubber bulb in which a cigarette was inserted through its opening. The cigarette was totally sucked and the bulb was closed so that the smoking was in contact with the fragments (Figure 1).



**Figure 1** – Method of pigmentation of the specimens used in this study.

After elapsed the period of 48h, all specimens were removed, washed in running water for 30 s, dried in absorbent paper, photographed, and analyzed again. To calculate the darkening degree of the specimens (DD), the percentages of the amount of pigments were obtained at baseline (Ab) and after the experiment (Aa). Therefore, the following formula was used:  $DD = Aa - Ab$ .

After that, the fragments were submitted to bleaching. 35% hydrogen peroxide (Whiteness HP) was employed according to the manufacturer's instructions, photo-activated through a LED device (Three Light, Clean Line,

Taubaté - SP, Brazil) for 20 s. Following, the specimens were again washed in running water and photographed for analysis thus obtaining the amount of pigments from the specimens submitted to bleaching (Abl). To assess the amount of remaining pigments (Arp) the following formula were used:  $Arp = Abl - Ab$ . By considering that DD is the maximum degree of darkening obtained in each group, to verify the bleaching agent effectiveness (BAE) the following formula was applied:

$$BAE = 100 - \frac{(Arp \times 100)}{DD}$$

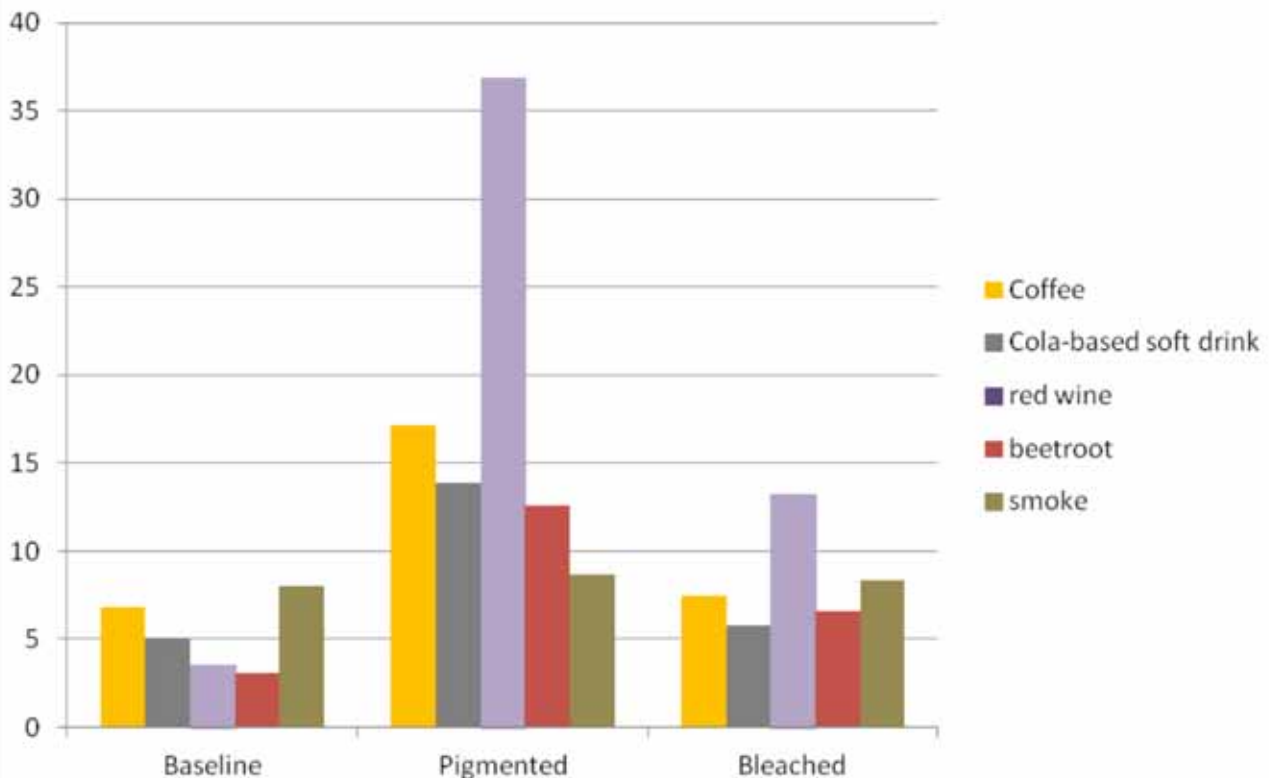
## RESULTS

The pigment amount found in the specimens at the beginning of the experiment and after the impregnation with the chromogenous substance and the bleaching procedure can be seen in Graph 1. By using the formulas proposed

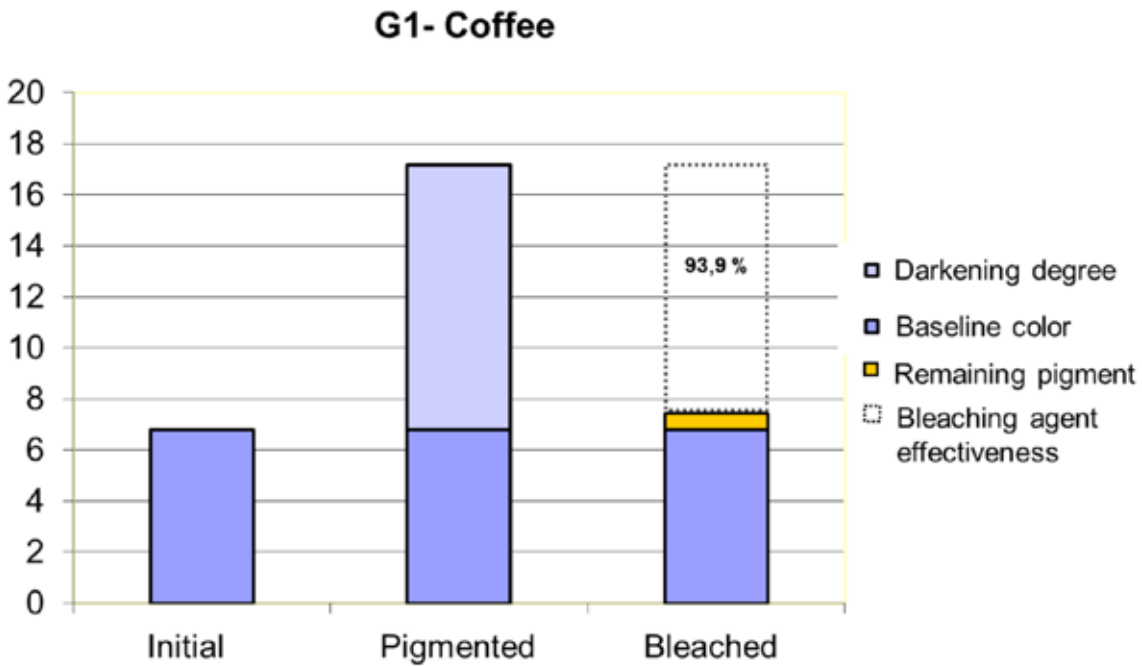
in the methodology of this study, the analysis of the darkening degree of the specimens showed that the fragments from G3 (red wine) were the most pigmented with 33.34% of pigments, followed by G1 (coffee) with 10.35%, G4 (beetroot) with 9.56%, G2 (cola-based soft drink) with 8.85% and G5 (smoke) with 0.61%.

Hydrogen peroxide promoted the bleaching of all groups, but none group returned to its original color. The amount of remaining pigment (Arp) after bleaching was also higher in G3 (9.64%), followed by G4, with 3.56% of pigments. G1, G2, and G5 exhibited a color very closer to that of the beginning of the study with Arp of 0.75, 0.64 and 0.26%, respectively.

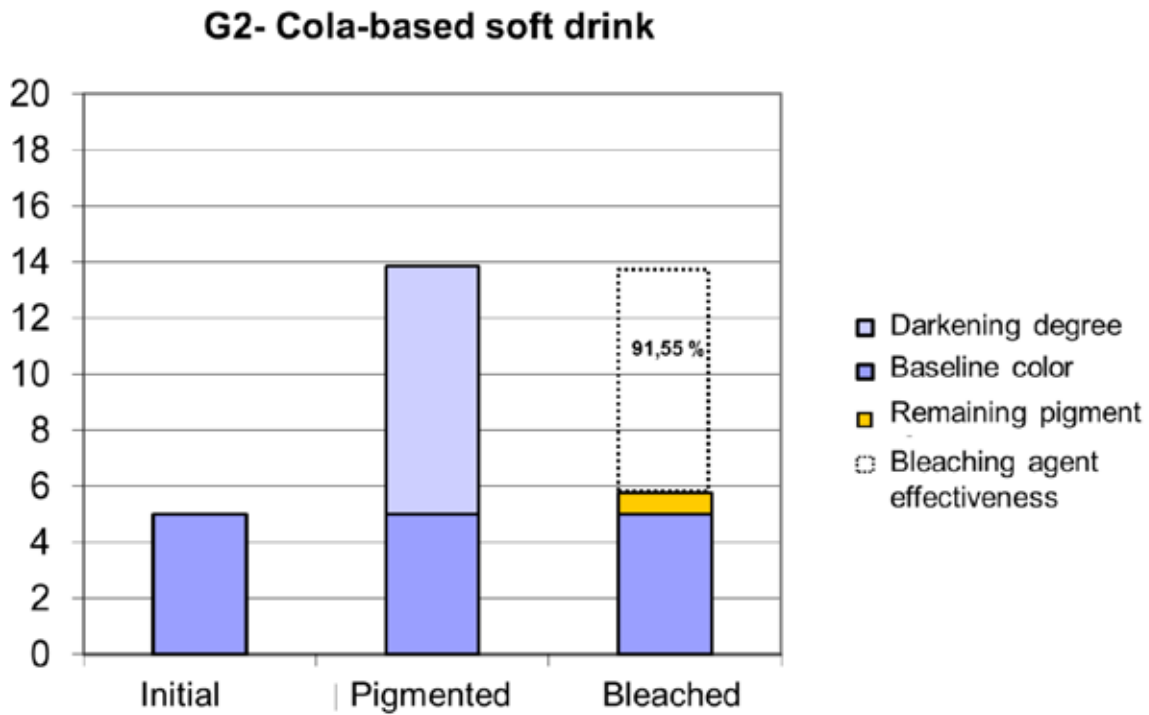
The highest bleaching effectiveness was observed in the specimens from G1 (93.9%) and G2 (91.55%), followed by G3 (71.09%), G4 (62.74%) and G5 (57.38%) (Graphs 2, 3, 4, 5 and 6).



**Graph 1** – Comparative analysis of the percentage of pigments observed in the groups at the beginning of the study, after pigmentation and bleaching procedures.

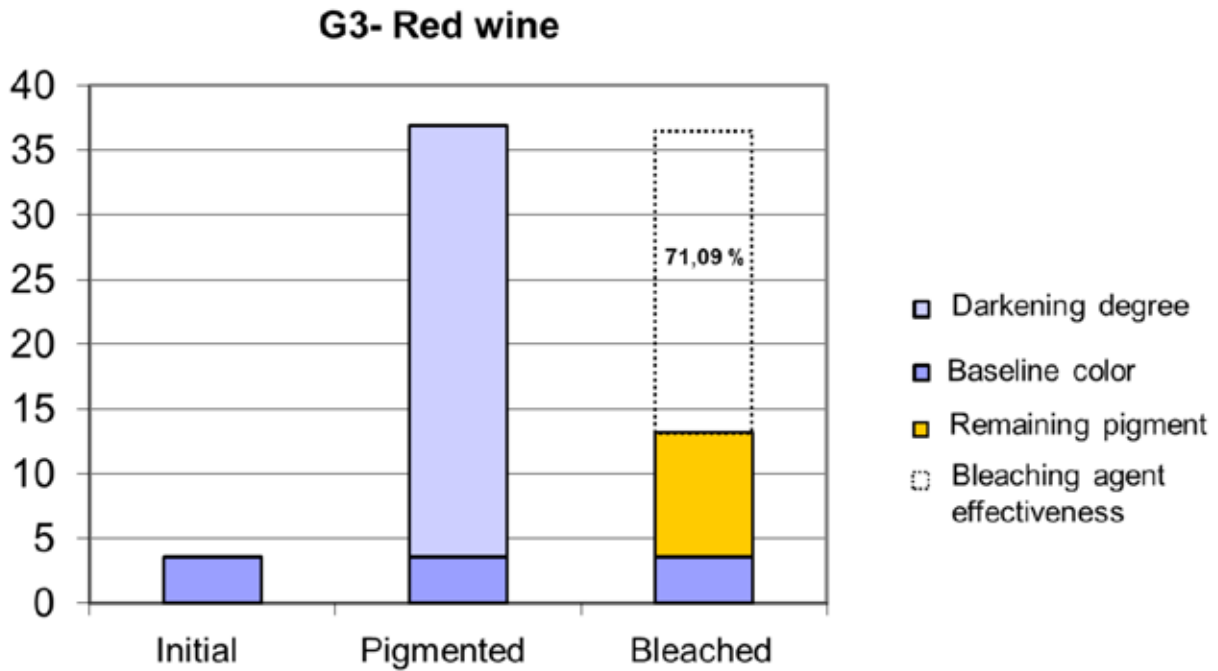


Graph 2 – Darkening degree and bleaching agent effectiveness of G1 (coffee) specimens.

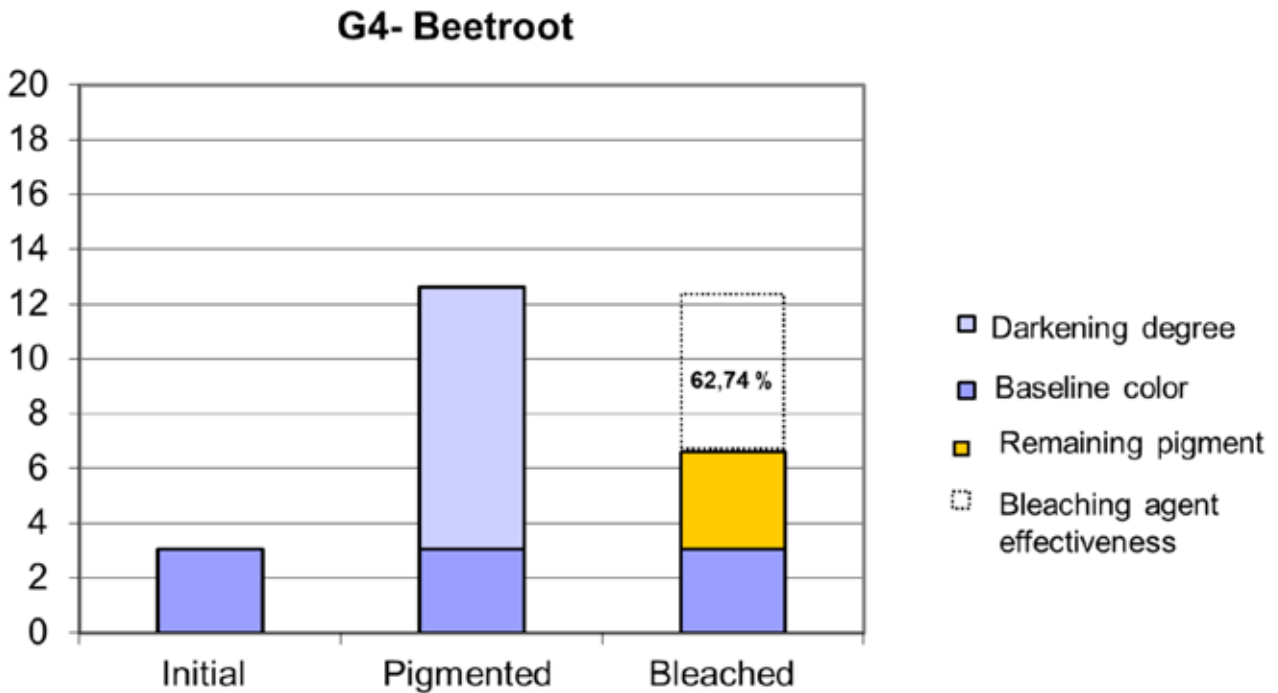


Graph 3 – Darkening degree and bleaching agent effectiveness of G2 (cola-based soft drink) specimens.

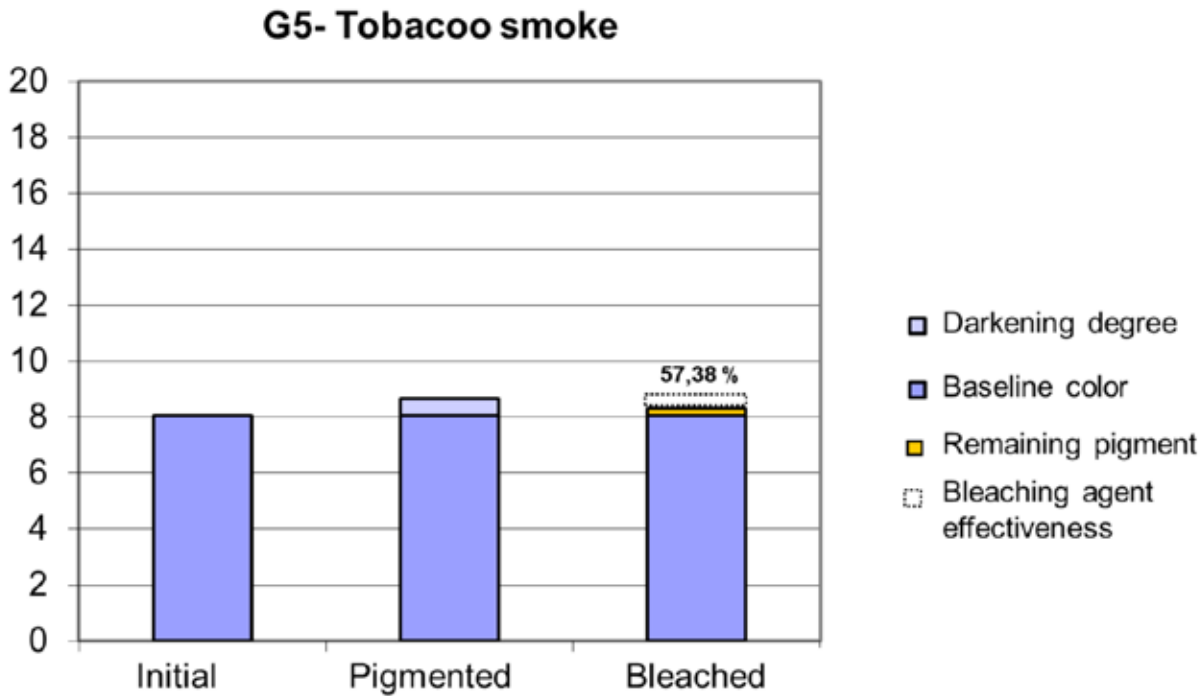




**Graph 4** – Darkening degree and bleaching agent effectiveness of G3 (red wine) specimens.



**Graph 5** – Darkening degree and bleaching agent effectiveness of G4 (beetroot) specimens.



**Graph 6** – Darkening degree and bleaching agent effectiveness of G5 (tobacco smoke) specimens.

## DISCUSSION

The methodology proposed in this study enabled to compare the degree of pigmentation of five chromogenous substances in a highly mineralized matrix – chicken eggshell – as well as the effectiveness of 35% hydrogen peroxide for the bleaching of these specimens.

Chicken eggshell was employed because it has a high content of calcium, is of easy access and low cost, and has been used as educational tool to simulate the effect of many substances onto the tooth enamel [16-18]. However, as far as we are concerned, the literature has not reported any studies with similar methodology, so that our results were compared with those of studies conducted in either bovine or human teeth.

The 48-hour period was enough for promoting the pigmentation of the specimens in all groups, although Lima et al. [9] used a 96-hour period of specimen immersion into cola-

based soft drink, red wine, and tobacco solution. Araújo et al. [15] employed a 2-week period for the impregnation of coffee in bovine teeth.

Of the five groups, red wine immersion showed the highest darkening degree. Red wine has been cited in the literature as a substance with high potential of pigmentation [5, 21, 22]. The polyphenols coming from grape within the wine may adhere to hydroxyapatite and this adhesion is increased by salivary proteins, such as proline-rich protein [23]. Almeida et al. [24] analyzed the color alteration of a resin submitted to different coloring solution. These authors tested the effect of immersion of a nanoparticulate resin composite into grape juice, coffee, and mate and observed that only grape juice provoked a significant color alteration. Téó et al. [5] evaluated the effectiveness of 35% hydrogen peroxide gel in the bleaching of 50 bovine teeth pigmented with coffee, black tea, red wine, cola-based soft drink solution and verified that all solution exhibited high

pigmentation potential; however, black tea, red wine and the soft drink caused the highest darkening.

In this present study, the group submitted to coffee showed the second highest darkening degree. The influence of coffee in both tooth darkening and bleaching degree has also been studied [5,9,11,15,25]. Silva and Lewgoy [25] investigated whether coffee associated or not with a propolis-based mouthrinse was capable of altering the color of two resin composites. These authors concluded that coffee and mouthrinse associated or not were capable of provoking the pigmentation of the resin composites tested.

Cola-based soft drinks are beverages with pigmentation potential, which agrees with the data from literature [5,26].

Citric fruit juices have erosive potential onto the tooth enamel potential and the exposure to acid substances may cause enamel demineralization [27]. Despite of its high mineral content, the eggshell shows great porosity which together with its demineralization potential may have contributed to the greatest penetration of the coloring substances. The acidity of both coffee and cola-based soft drink, [28], for example, may have partially demineralized the fragment surface, increasing the porosity and contributing for the darkening of the specimens.

Costa et al. [29] reviewed the literature on the foods with staining capacity and reported that beetroot has the potential of staining composites. In our study, beetroot solution promoted a darkening degree greater than that of smoke.

The group submitted to tobacco smoke did not show an expressive darkening in this present study. Probably, this fact occurred because of the methodology employed. It has been well described in the literature the influence of tobacco on tooth darkening [1]. Notwithstanding, few studies described a well-defined methodology to achieve the impregnation from tobacco-derivate pigments into tooth structure in vitro [9,30]. Lima et al. [9] reported the use of a

tobacco solution to evaluate the color alteration in human pre-molars, but they did not describe the technique used to prepare such solution. In this present study, a rubber bulb was employed in which a cigarette was inserted through its orifice enabling the smoke suction and the contact of the specimens for the given period. This methodology employed a principle similar to that adopted by Takeuchi et al. [30] who used a device to aspirate the smoke and promoted the deposition of chemical products onto the specimens.

To measure the color alteration, specific devices such as Shade Eye NCC (Shfu Inc. Japan), Easyshade (Vita, Germany), among others have been also employed [14]. The assessment of the bleaching effectiveness has also been executed by the visual method and comparison with color ranges available in the market through spectrophotometry [9,15], photoreflectance [31], among others. To evaluate the color alteration of the specimens, we used the digital photograph analysis through software (Adobe Photoshop C5S), which allowed the detection of cyan, magenta, yellow and black pigments. The digital photograph is currently a very used tool and enables to evaluate the result of the bleaching procedure through image software [14]. According to Carnevali et al. (2010) the digital photograph is an excellent tool to evaluate the result of exogenous bleaching.

Thirty five hydrogen peroxide associated with LED photo-activation was very effective on the bleaching of pigmented specimens, although all groups showed a certain degree of residual pigment, that is, in none group the original color was achieved. Probably, this fact occurred because we had used one-appointment bleaching. Lima et al. [9] showed success in the removal of exogenous pigments after two bleaching appointments.

Hydrogen peroxide effectiveness was similar in G1-coffee (93.9%) and G2-cola-based soft drink (91.55%). In G3-red wine, G4-beetroot, and G5-tobacco smoke, bleaching effectiveness was 71.09%, 62.74%, 57.38%,



respectively. According to Watts and Addy [1] the extrinsic stains can occur because of either the incorporation of the chromogenous agents within the structure or chemical interaction with the tooth structure. These authors also affirmed that the first explains the action of pigments from both foods and tobacco. Thus, the resistance to the bleaching material could be attributed to the type and size of the molecule of the different substances tested; and the most acid pH of the cola-based soft drink, coffee and red wine that could have provoked an increasing of the porosity of the specimens consequently making easy both the pigment impregnation and the bleaching agent effectiveness. Moreover, the variation in the methodology used for tobacco could have played a role since in this group, the specimens were not immersed into a solution but in contact with the smoke, so that the impregnation of the chromogenous agents could have been different from that of the other groups, therefore enabling a greater adhesivity to the specimens.

Although the methodology of this study was efficient to compare both the darkening degree promoted by the exogenous substances and the effectiveness of 35% hydrogen peroxide on the specimens pigmented, further studies are necessary to support the use of this material in the simulation of the effect of many substances on tooth enamel, for example, to verify other variables acting on the chicken eggshell such as the evaluation of the microscopic aspects of eggshell surface, the permeability, and the biochemical interactions between eggshell and dental enamel.

## CONCLUSION

It can be concluded that the method was efficient to compare the bleaching agent effectiveness on the pigments tested. The specimens impregnated by tobacco smoke showed the highest resistance to the bleaching procedure. Considering the use of this biological material, further studies are necessary to simulate the effect of other substances on tooth enamel.

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**Marianne Spalding  
(Correspondent Author)**

Av. Eng. Francisco José Longo, nº 777,  
Jardim São Dimas – 12245-000 – São José dos Campos – SP  
Telefone: (12) 3947-9000

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