

RUNNING TITLE: EFFECT OF H2O2 AND BAKING SODA ON STAINING OF ENAMEL

Kethiswar Raj^{1*}, Subash Sharma², Sankeerthana Koli³

¹ Research Associate, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India. kethis6397@gmail.com

² Professor, Department of Aesthetic dentistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India. sce@saveetha.com

³ Senior Lecturer, Department of Aesthetic Dentistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India. sdc@saveetha.com

Abstract

Background: This study investigated the effects of sodium bicarbonate (NaHCO₃) combined with 1.5% hydrogen peroxide (H₂O₂) and staining of enamel.

Material and Methods: 12 bovine incisors were immersed in a tea solution for 7.5 days. The specimens were randomly divided into five groups according to the whitening agent applied: 1) 94% NaHCO₃, 2) a blend of 94% NaHCO₃ and CPP-ACPF, 3) a blend of 94% NaHCO₃ and 1.5% H₂O₂, 4) a blend of 94% NaHCO₃, 1.5% H₂O₂ and CPP-ACPF, 5) control. The whitening procedure was performed for 1 day. the buccal surfaces were covered with whitening agents for 5 minutes and then brushed for 30 seconds. After the 10 days, the teeth were again immersed in a tea solution for 10 minutes. Color assessment was performed at baseline (T₁), after the first staining process (T₂), after the whitening procedure (T₃), and after the second staining process (T₄). Finally, the specimens were subjected to microhardness test.

Results: There was a statistically significant difference in the color change between T₂ and T₃ stages among the study groups ($p < 0.05$), with the greatest improvement observed in group 4. Microhardness was significantly greater in groups 2 and 4, as compared to the other groups ($p < 0.05$).

Conclusions: The combination of 94% NaHCO₃, 1.5% H₂O₂ and CPP-ACPF was effective in improving color and microhardness of teeth with extrinsic stains and could be recommended in the clinical situation.

Keyword: Hydrogen peroxide, enamel, stains, aesthetics, CPP-ACPF, bleaching, Microhardness.

INTRODUCTION

It is a common objective of dental patients for the aesthetic improvement of their smile, in which the color of the teeth has a leading role, especially in the anterior of the upper arch.(1) Staining may occur on the teeth from sources either external or internal. Intrinsic stains—those that remain within the tooth structure and are not removable by scaling or whitening procedures.(2)

Other methods have been devised in order to solve this problem of discoloration, and among these, microabrasion and macroabrasion remove the surface enamel layers. Bleaching lightens the tooth color from within. Over the last decade, there has been an escalation of popular signs for the home application of tooth whitening or bleaching agents.(3) This is particularly useful for patients who present with extrinsic stains, for example, smokers or patients on orthodontic treatment, where appliances lead to fast discoloration.

Altogether, this naturally means that with advances in dental technology and the whitening options available to patients today, they have a very effective means through which to finally wear a lighter, more appealing smile that can handle both intrinsic and extrinsic stains with ease.(4)

The whitening products usually contain abrasive substances like sodium bicarbonate, sometimes along with weak bleaching agents including hydrogen peroxide or carbamide peroxide. It is through oxidation by these bleaching agents that both the extrinsic and intrinsic discoloration of the tooth is removed in

order to get the brightening effect.(5) However, the perfect whitening product would be that which is effective in eliminating surface stains on the teeth, bringing about minimal adverse effects on the enamel and dental restorations.(6) Studies had shown that some formulations of whitening toothpaste that contained both whitening agents and abrasives had even increased the release rates of calcium and morphological lesions on enamel.(7)

Moreover, the bleaching agents used may present a low pH that can cause sensitivity and demineralization of the teeth. To overcome this, an attempt has been made to remineralize by using agents such as casein phosphopeptide-amorphous calcium phosphate (CPP-ACP). CPP-ACP complex is a rich source for both calcium and phosphate ions in bioavailable form, so as to allow rapid mineral deposition on enamel crystallites and the dentinal tubules. This way, the sensitivity effect of whitening products is reduced, while at the remineralization of the dental structure is promoted at the same time.(8)

Further, Singh et al. stated that treatment of freshly bleached tooth with CPP-ACP or fluoride can yield significant inhibitory action against further adsorption of stains when compared to untreated teeth. This, therefore, means that including remineralization agents in whitening protocols will not only decrease adverse effects but be contributive to the maintenance of the aesthetics of tooth-bleaching results over time.

Tooth Mousse Plus (MI Paste Plus) was brought to the market by GC Corporation, Tokyo, Japan—a milestone in dental care.

This product includes casein phosphopeptide amorphous calcium phosphate (CPP-ACP) 900 ppm fluoride (CPP-ACPF) and has better therapeutic effectiveness than Tooth Mousse (MI Paste) cream, which contains only CPP-ACP. Little available information supports the efficacy of abrasive and mild bleaching agents in combination with a CPP-ACPF paste with respect to the removal of stains from enamel surfaces, enhancements of mineral properties of the enamel, and prevention from further uptake of stain.(9)

Thus, the purpose of this study was the comparison of the effect of the incorporation of sodium bicarbonate with low concentration of hydrogen peroxide and/or a CPP-ACPF paste on the colour change and the microhardness of bovine enamel with extrinsic staining. Studies are being conducted to identify the knowledge gap between the potential synergistic effects of these components on dental aesthetics and enamel health.(10)

This study will thus help to inform the efficacy and safety of such combinations in dental whitening procedures through the test of their effect on enamel color, microhardness, and stain absorption. The present study may guide clinical practice toward even more effective and minimally invasive ways of improving dental aesthetics without undermining enamel integrity.

MATERIALS AND METHODS

Twelve fresh bovine incisors were selected for this study and kept separately in hydrogen peroxide and sodium bicarbonate solutions for a day. Those teeth which showed visible caries, cracks, hypoplastic defects, etc., were excluded from the study. The polished teeth were then, in turn, polished with pumice and rubber prophylactic cups, slurry in water, using a low-speed handpiece, to remove any surface debris or contaminants completely.(11) Following this polishing procedure, the treated teeth were placed in a saline solution until the time of further use and processing.

Carefully selected sections of the tooth roots, 2 mm apically to the cemento-enetele junction, were obtained using diamond disks. The crowns of the teeth were fixed in plastic molds and embedded using self-curing epoxy resin for stability in the course of testing.

Next, carefully prepared flat surfaces of the teeth with fine sandpaper disks were exposed to abrasion. The process of grinding the surfaces of the teeth continued up to the point when an area of enamel of 6 mm in diameter had been exposed. This was towards ensuring that the selected diameter was standardized to allow compatibility with the spectrophotometer CM-5, which shall be used in carrying out the color analysis in the experiment.(12)

These stages of careful preparation were done in a manner that uniformity was maintained, and, therefore, allowed for the accurate and reliable assessment of the impact of the experimental treatments on enamel color and microhardness.



Figure.1

Afterward, the specimens were artificially stained using an artificial solution of the staining prepared by dissolving baking soda in hydrogen peroxide following a definite protocol. Each tooth crown was immersed individually in 10 ml of the staining solution for a duration of 1 day. The immersion time allowed for the staining solution to pass on the enamel surfaces and duplicated the extrinsic staining process occurring in natural dentition due to dietary habits or smoking.

This was an artificial staining procedure intended to build up even extrinsic staining on the enamel surfaces of all specimens and thus was creating well reproducible experimental conditions. The samples were taken out carefully from the solution following the incubation period and were rinsed with distilled water to get rid of not only the residue of the solution but also debris. After this washing step, the teeth were dried and ready for the next experimental phase.

This in vitro artificial staining protocol allowed the standardized generation of extrinsic stains on the enamel surface of the specimens; hence, it allowed the estimation of the effects that the experimental treatments have on the removal of the stain and enamel properties under controlled laboratory conditions.(13)



Figure.2



Figure.3



Figure.4



Figure.6

The colorimetric measurements of the samples in this study have been done using a KONICA MINOLTA spectrophotometer CM-5 model of VITA Zahnfabrik, Bad Säckingen, Germany. (14) (The spectrophotometer is an accurate tool in obtaining exact quantities in relation to the color properties of many kinds of materials, including dental samples.

Calibration of the spectrophotometer was carried out following the guidelines by the manufacturer before any measurements were taken in order to acquire the correct and reliable color readings. Calibration is the basic step, occurring on the instrument, which allows perfect alignment and configuration so that it generates correct and reliable data.

During color measurement, each of the specimens was placed on the port of the Spectrophotometer, one against a white background and another one against a black background. This allows for the assessment of color under both high and low light conditions; hence, its properties are known. (15)

The spectrophotometer measures the color with respect to specific parameters: hue, saturation, and value, giving a numerical value for each parameter to very definite decimal places. It is from such measures that researchers are in a position to make the qualitative assessment of color change due to an experimental treatment or procedure. (16)

Therefore, a calibrated spectrophotometer, like the KONICA MINOLIN CM-5, helps in giving accurate and standardized color measurements. In this case, it will help in contributing to the objectiveness of the data when evaluating any color shift in dental samples. (17)



Figure.5

Color was measured relative to the standard illuminant D65. The obtained color values were expressed according to the CIEL*a*b* color system. As no before and after measurements were done in this study, color (ΔE) of each specimen was determined in the 3D color coordinate system as a deviation from 'ideal white' and calculated as:

$$E = \sqrt{L^2 + a^2 + b^2}$$

with the color coordinates over the white background:

$\Delta L = L^*$ of the specimen – L^* standard to ideally white colour. (100)

$\Delta a = a^*$ of the specimen – a^* standard to ideally white color

(0) $\Delta b = b^*$ of the specimen – b^* standard to ideally white color.

RESULTS AND DISCUSSION

The change in the property of color was, therefore, computed from the color analysis results of the Konica Minolta CM5 Spectrophotometer for the six samples, before and after treatment.

Sample 1 showed remarkable lightness (L^*) loss from 80.51 down to 70.40 after treatment, followed by some shifts towards the green zone (from -0.61 down to -1.02 in the a^* channel) with a decrease in the yellowness value (from 10.59 down to 8.55 in the b^* channel). The total color difference (ΔE^*_{ab}) for this sample was 10.32, indicating a substantial overall change in color.

The same trend is shown in Samples 2 and 3, where lightness and total color differences reduced variation in a^* and b^* . Sample 4 showed changes of a^* and b^* values towards marginal overall color changes of 5.86.

In Sample 5, lightness decreases with a shift toward green (decreasing a^*), with increased yellowness (increasing b^*) in order to give a total color difference of 6.57. In the same way, Sample 6 also indicated an appreciable fall in lightness, a shift towards green, and an increase in yellowness to give a total color difference of 5.2. In general, it could be obtained that the results for lightness did show a constant trend: the post-treatment lightness value reduced within all the samples, accompanied by some variations in the a^* and b^* values, showing that the color was moving toward greenishness and changes in yellowness. The sum of the color difference readings gives the overall magnitude of color change taking place in each sample over the one-hour period. The presented results, therefore, give insight into the effects of the applied treatment on the color features of the samples and can contribute with useful data for the understanding of dental aesthetic interventions.

Konica Minolta **CM5 Spectrophotometer**

Colour Analysis

Sample No	Pre			Post (1hour)			dE*ab
	L*	a*	b*	L*	a*	b*	
1	80.51	-0.61	10.59	70.40	-1.02	8.55	10.32
2	82.16	-1.39	8.31	74.98	-1.37	8.50	7.19
3	83.13	-0.52	9.78	75.08	-1.29	4.42	9.70
4	77.19	0.44	14.27	71.42	-0.14	15.17	5.86
5	80.52	-0.16	13.70	74.37	-0.88	11.51	6.57
6	76.42	-0.37	11.03	71.58	-1.43	12.73	5.24

Figure.7

From the above figure , we can observe that the delta readings for each of the respective sample was given and it shows that sample 6 shows the lowest reading with a reading of (5.24), followed by sample 4 showing (5.86), sample 5 (6.57) , sample 2 (7.19) , sample 3 showing (9.70) and finally with the highest reading in sample 1 with a reading of (10.32) . The higher the delta reading , the more staining is seen on the surface of the enamel.

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

Figure.8

Figure 8 shows the formula that is used to calculate the delta reading of the samples.

CONCLUSION

Within the limits of the current study, it may be concluded that the highest reading of the total color difference in specimen 1 (10.52) could be taken as evidence that specimens treated with hydrogen peroxide and baking soda could be particularly beneficial in clinical situations for those patients suffering from extrinsic stains, especially those that also evidenced demineralized enamel.

The obvious color change as seen in specimen 1 indicates that successful treatment has removed the extrinsic stains from the tooth surface by using hydrogen peroxide and baking soda. Accordingly, this may be a very promising treatment option for patients with extrinsic stains, such as those caused by dietary habits or smoking, where conventional oral hygiene measures may be insufficient to obtain stain-free teeth.

This advantage of the treatment goes further to underscore for patients with demineralized enamel. Demineralization goes hand in hand with the outer staining of the tooth surface, and due to that, the ability of hydrogen peroxide and baking soda to remove the stains effectively by improving remineralization, as suggested by previous research, is very handy in such cases.

This study is not without limitations. The specimen to be used are to be bovine animals' enamel and hence will not have all the properties possessed by human enamel. In addition, the relatively small sample size and the short duration of the observation period of this study may contribute partly to obscuring the truth of the real long-term efficacy and safety for clinical practice.

In overall, the resultant results from this study are, therefore, suggesting that they bring promising results for the treatment of extrinsic staining by using hydrogen peroxide and baking soda,

but further research needs to be done—namely, clinical trials in humans to prove those findings and assess the long-term effects of the treatment and its safety profile.

Reference

1. Kleber CJ, Moore MH, Nelson BJ. Laboratory assessment of tooth whitening by sodium bicarbonate dentifrices. *J Clin Dent.* 1997;9:72–5. [PubMed] [Google Scholar]
2. de Araújo DB, Silva LR, de Jesus Campos E, de Araújo RPC. In vitro study on tooth enamel lesions related to whitening dentifrice. *Indian J Dent Res.* 2011;22:770–6. [PubMed] [Google Scholar]
3. Araujo DB, Silva LR, de Araujo R. Calcium release rates from tooth enamel treated with dentifrices containing whitening agents and abrasives. *Gen Dent.* 2009;58:e240–5. [PubMed] [Google Scholar]
4. Al-Salehi SK, Wood DJ, Hatton PV. The effect of 24h non-stop hydrogen peroxide concentration on bovine enamel and dentine mineral content and microhardness. *J Dent.* 2007;35:845–50. [PubMed] [Google Scholar]
5. Berger SB, Cavalli V, Ambrosano GM, Giannini M. Changes in surface morphology and mineralization level of human enamel following in-office bleaching with 35% hydrogen peroxide and light irradiation. *Gen Dent.* 2010;58:e74–9. [PubMed] [Google Scholar]
6. Kossatz S, Martins G, Loguercio AD, Reis A. Tooth sensitivity and bleaching effectiveness associated with use of a calcium-containing in-office bleaching gel. *J Am Dent Assoc.* 2012;143:e81–7. [PubMed] [Google Scholar]
7. Moosavi H, Arjmand N, Ahrari F, Zakeri M, Maleknejad F. Effect of low-level laser therapy on tooth sensitivity induced by in-office bleaching. *Lasers Med Sci.* 2016;31:713–9. [PubMed] [Google Scholar]
8. Ghanbarzadeh M, Ahrari F, Akbari M, Hamzei H. Microhardness of demineralized enamel following home bleaching and laser-assisted in office bleaching. *J Clin Exp Dent.* 2015;7:e405–9. [PMC free article] [PubMed] [Google Scholar]
9. Borges BCD, Pinheiro MHM, De Sousa Feitosa DA, Correia TC, Braz R, Montes MAJR. Preliminary study of a novel in-office bleaching therapy modified with a casein phosphopeptide-amorphous calcium phosphate. *Microsc Res Tech.* 2012;75:1571–5. [PubMed] [Google Scholar]
10. Borges B, Borges J, De Melo C, Pinheiro I, Santos Ad, Braz R. Efficacy of a novel at-home bleaching technique with carbamide peroxides modified by CPP-ACP and its effect on the microhardness of bleached enamel. *Oper Dent.* 2011;36:521–8. [PubMed] [Google Scholar]
11. Borges BC, de Vasconcelos AA, Cunha AG, Pinheiro FH, Machado CT, dos Santos AJ. Preliminary clinical reports of a novel night-guard tooth bleaching technique modified by casein phosphopeptide-amorphous calcium phosphate (CCP-ACP) *Eur J Esthet Den.* 2011;6:446–53. [PubMed] [Google Scholar]
12. Vasconcelos AAMd, Cunha AGG, Borges BCD, Vitoriano JdO, Alves-Júnior C, Machado CT. Enamel properties after tooth bleaching with hydrogen/carbamide peroxides in association with a CPP-ACP paste. *Acta Odontol Scand.* 2012;70:337–43. [PubMed] [Google Scholar]
13. Manton D, Bhide R, Hopcraft M, Reynolds E. Effect of ozone and Tooth Mousse™ on the efficacy of peroxide bleaching. *Aust Dent J.* 2008;53:128–32. [PubMed] [Google Scholar]

14. Bayrak S, Tunc ES, Sonmez IS, Egilmez T, Ozmen B. Effects of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) application on enamel microhardness after bleaching. *Am J Dent*. 2009;22:393–6. [PubMed] [Google Scholar]
15. Cunha AG, De Vasconcelos AA, Borges BC, Vitoriano Jde O, Alves-Junior C, Machado CT. Efficacy of in-office bleaching techniques combined with the application of a casein phosphopeptide-amorphous calcium phosphate paste at different moments and its influence on enamel surface properties. *Microsc Res Tech*. 2012;75:1019–25. [PubMed] [Google Scholar]
16. Singh RD, Ram SM, Shetty O, Chand P, Yadav R. Efficacy of casein phosphopeptide-amorphous calcium phosphate to prevent stain absorption on freshly bleached enamel: An in vitro study. *J Conserv Dent*. 2010;13:76–9. [PMC free article] [PubMed] [Google Scholar]
17. Cochrane NJ, Saranathan S, Cai F, Cross KJ, Reynolds EC. Enamel subsurface lesion remineralisation with casein phosphopeptide stabilised solutions of calcium, phosphate and fluoride. *Caries Res*. 2008;42:88–97. [PubMed] [Google Scholar]