

# Evaluation of Charcoal and Sea Salt–Lemon-based Whitening Toothpastes on Color Change and Surface Roughness of Stained Teeth

Rawda H Abd ElAziz<sup>1</sup>, Lamia K Gadallah<sup>2</sup>, Reham S Saleh<sup>3</sup>

## ABSTRACT

**Aim:** To compare the efficacy of two naturally based commercially available whitening toothpastes charcoal and sea salt–lemon on stain removal of teeth in terms of color change and surface roughness.

**Materials and methods:** Twenty-seven intact bovine incisors were selected and randomly allocated into three main groups ( $n = 9$ ) according to the tested toothpaste [I: Signal Complete 8 Charcoal; II: sea salt–lemon essence Closeup natural smile; and III: Signal Complete 8 Original (control)]. Following 4 successive days of staining protocol, each specimen in its corresponding group was brushed with the toothpaste using toothbrush simulator apparatus for three brushing cycles. Color assessment using Vita Easyshade spectrophotometer and surface roughness (Ra) measurement using contact type profilometer were performed for each specimen at baseline, after staining, and after each tooth brushing cycle.

**Results:** Nonparametric color data and parametric surface roughness data were analyzed. The color difference ( $\Delta E$ ) from after-staining protocol to different tooth brushing cycles (1,2,3) showed no significant difference on each cycle between the tested groups ( $p > 0.05$ ). While for color difference ( $\Delta E$ ) from baseline to the last tooth brushing cycle 3, the difference between groups was statistically significant where group II, sea salt–lemon-based toothpaste, had a significantly lowest ( $\Delta E_{00}$ ) value ( $p < 0.001$ ) indicating more whitening effect in relation to others. However, a significant increase in surface roughness was present in all tested groups ( $p < 0.001$ ); meanwhile, there was no significant difference between tested groups ( $p > 0.05$ ).

**Conclusion:** After three tooth brushing cycles, none of the natural whitening toothpastes or conventional toothpastes produced had effective whitening results nor completely removed the stains back to the initial baseline tooth color. Sea salt–lemon-based whitening toothpaste had a whitening effect better than the charcoal-based toothpaste. All of the tested toothpastes increased the degree of surface roughness.

**Clinical relevance:** Charcoal and sea salt–lemon-based whitening toothpastes do not guarantee to whiten nor completely remove the stains back to normal and their effects on enamel surface roughness should be highly clinically considered and managed.

**Keywords:** Charcoal-based whitening toothpaste, Color change, Natural whitening toothpastes, Sea salt–lemon whitening toothpaste, Surface roughness.

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## INTRODUCTION

The demand for improved dental esthetics has increased in all age-groups even among adolescents and children. This led to the introduction of different teeth whitening products.<sup>1</sup>

Teeth staining can be due to intrinsic and extrinsic causes. Intrinsic causes can occur during tooth development before their eruption as fluorosis, or after eruption due to traumatic injuries. However, extrinsic causes can be due to poor oral hygiene, high consumption of tooth-staining drinks, foods, and smoking which lead to deposition of either organic chromophores present tea, coffee, soft drinks, and alcohol or inorganic chromophores such as metal ions in iron supplements.<sup>2</sup>

Teeth whitening treatments for extrinsic stain removal can be achieved either by professionally applied products or home-based products such as bleaching gels, whitening strips, toothpastes, brush-on agents, chewing gums, and mouth rinses.<sup>1</sup> However whitening toothpastes seem to be one of the most preferred over-the-counter methods by many people due to their affordable cost and simplicity of application.<sup>3</sup>

There is a global demand toward using natural alternative products. In the same context, whitening toothpastes with natural ingredients offer acceptable tooth whitening effect with minimal side effects.<sup>4</sup> Furthermore, whitening toothpastes displayed in the

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market which contain hydrogen peroxide or carbamide peroxide in their formulation can cause serious damage of the organic matrix of dental structure due to the release of free oxygen radicals.<sup>4–6</sup> They also contain a large quantity of abrasives such as hydrated silica, alumina, and perlite that not only increase their

**Table 1:** Brand names, manufacturers, and composition of the used toothpastes

Group	Material (brand names and manufacturer)	Composition
I	Signal Complete 8 Charcoal (Unilever Mashreq, Egypt)	Charcoal powder, Hydrogenated starch hydrolysate, Water, Hydrated silica, PEG-32, Zinc citrate, Sodium lauryl sulfate, Aroma, Cellulose gum, Sodium fluoride, Sodium saccharin, Glycerin.
II	Sea salt and lemon essence. Closeup natural smile (Unilever Mashreq, Egypt)	Citrus lemon juice, Sodium chloride, Sorbitol, Water, Hydrated silica, Sodium lauryl sulfate, PEG-32, Flavor, Cellulose gum, Sodium fluoride, Sodium saccharin, Mica, Propylene glycol, Sodium sulfate, Sodium benzoate, Potassium sorbate, PEG-60, Hydrogenated castor oil.
III	Signal Complete 8 Original. (Unilever Mashreq, Egypt)	Water, Hydrated silica, PEG-32, Zinc citrate, Sodium lauryl sulfate, Aroma, Cellulose gum, Sodium fluoride, Sodium saccharin, Glycerin.

ability to remove the stains in a mechanical way but also increase the degree of surface roughness.<sup>5,6</sup>

Recently charcoal-based toothpastes which contain fine powder of activated charcoal-carbon have attracted the attention due to its ability to remove stain and whiten the tooth structure. This ability is attributed to the high porosity of activated charcoal which enables it to exchange ions through nanopores and adsorb pigments, stains, and chromophores from the tooth surface. Thus, charcoal-based toothpaste can produce an acceptable tooth whitening effect.<sup>6-8</sup>

Another naturally based whitening toothpastes that contain sea salt and lemon are introduced in the market nowadays.<sup>4,9</sup> Their whitening effect can be attributed to the presence of calcium carbonate salt which in turn remove the extrinsic stains through mechanical abrasive action.<sup>4</sup>

However, still there is no strong evidence regarding the efficacy of such newly introduced whitening toothpastes especially sea salt-lemon toothpaste, besides that color change and surface roughness are not widely studied together. In addition, only very few researches studied their efficacy of whitening on the previously heavily stained tooth structure.<sup>6-8</sup>

Thus, the aim of this *in vitro* study was to study the effect of charcoal- and sea salt-lemon-based whitening toothpaste on stain removal in terms of color change and teeth surface roughness compared to conventional toothpaste.

The null hypothesis assumed that (1) both charcoal- and sea salt-lemon-based whitening toothpastes would not lead to effective tooth whitening and have the same capability of stain removal after continuous cycles of tooth brushing. (2) They would not have a significant effect on the enamel surface roughness.

## MATERIALS AND METHODS

We followed the ethical regulations of the National Research Centre (NRC) for conducting experiments on extracted bovine teeth, Approval number: 1433042022.

### Study Design

This was a randomized, double-blinded, controlled, *in vitro* study. The sample size was calculated based on the primary outcome; the mean difference in the color change in a previous study<sup>4</sup> resulted in that the present study; it was needed to study nine experimental samples to be able to reject the null hypothesis with probability (power) 0.8 and type I error probability was 0.05 using PS software version 3.1.6.

### Specimens' Preparation

Twenty-seven intact bovine incisors were selected for the study. The teeth were thoroughly washed under running tap water, scaled, and stored in distilled water until required. The root of each tooth was sectioned at the level of the cemento-enamel junction, and the crown was embedded in a mold of transparent acrylic resin with the labial surface exposed which was wet polished with both 600 and 1,200 grit silicon papers.<sup>7</sup>

### Staining Protocol

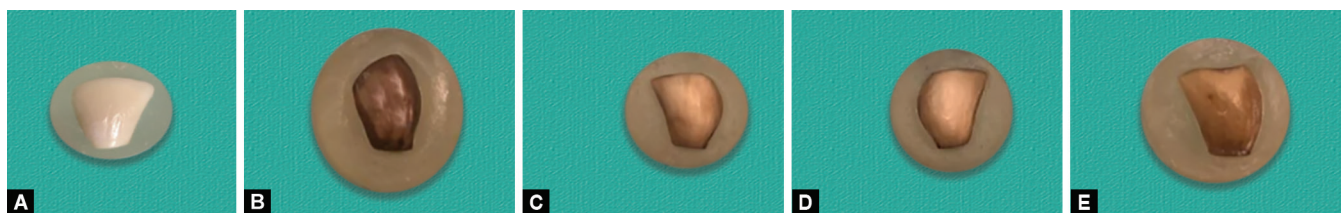
The staining solution was prepared by immersion of 10 packets of black tea (Lipton yellow label tea bag, Unilever Mashreq Co., Alexandria, Egypt) in 500 mL of boiling water. The specimens were immersed in this solution for about 18 hours per day then they were left dry for 6 hours. Then this procedure was repeated for four successive days. A new staining solution was prepared each day.<sup>7</sup>

### Experimental Design

The stained specimens were randomly allocated using simple randomization method into three groups ( $n = 9$  each) according to the three tested commercially available toothpastes from Unilever Mashreq Co., Alexandria, Egypt. Group I: Signal Complete 8 Charcoal; Group II: Sea salt-lemon essence Closeup natural smile; and Group III, the control group, Signal Complete 8 Original with 10  $\mu\text{m}$  mean particle size of the abrasive fillers. The ingredients of the three tested toothpastes are nearly similar except major difference from the control Signal Complete 8 Original toothpaste that Signal Complete 8 Charcoal is based on the addition of active ingredients: charcoal powder and hydrogenated starch hydrolysate to the tooth paste while citrus lemon juice and sodium chloride are added to sea salt-lemon essence Closeup natural smile. The full ingredients of all used toothpastes are represented in Table 1. The examiners who recorded both outcomes as well as the statisticians were blinded to groups' allocation (Fig. 1).

### Tooth Brushing Protocol (Stain Removal)

Each specimen in its corresponding group was brushed using a custom-made tooth brushing simulator. The simulated tooth brushing process was achieved according to ISO/TS 14569-1 specifications which guide testing of wear by toothbrushing.<sup>10</sup> The apparatus composed of a powered soft toothbrush head (Oral B Classic – Procter & Gamble, USA) mounted to a fixed apparatus accomplishing with horizontal movements and applied load 250  $\text{gm}/\text{cm}^2$ .<sup>11</sup> Each specimen was subjected to three brushing cycles. First, 1,120 cycles which were equivalent to 4 weeks of tooth brushing while the second round was 2,240 cycles equivalent to



**Figs 1A to E:** Representative photo showing (A) Baseline normal color after polishing; (B) After staining protocol; (C) After the third cycle of tooth brushing with charcoal; (D) After the third cycle of tooth brushing with sea salt-lemon; (E) After the third cycle of tooth brushing with Signal Complete 8 Original (control)

**Table 2:** Inter- and intragroup comparisons of color change ( $\Delta E$ )

Difference	Color change ( $\Delta E$ ) (Mean $\pm$ SD)			
	Group I (charcoal)	Group II (sea salt-lemon)	Group III (conventional)	p value
After staining—after brushing cycle 1	9.96 $\pm$ 4.83 <sup>Ab</sup>	12.09 $\pm$ 5.55 <sup>Abc</sup>	12.26 $\pm$ 5.58 <sup>Aa</sup>	0.602
After staining—after brushing cycle 2	10.61 $\pm$ 5.79 <sup>Ab</sup>	13.46 $\pm$ 6.65 <sup>Ab</sup>	13.09 $\pm$ 5.36 <sup>Aa</sup>	0.389
After staining—after brushing cycle 3	14.90 $\pm$ 6.51 <sup>Aa</sup>	20.44 $\pm$ 7.11 <sup>Aa</sup>	14.40 $\pm$ 6.50 <sup>Aa</sup>	0.153
Baseline—after brushing cycle 3	17.92 $\pm$ 6.28 <sup>Aa</sup>	8.03 $\pm$ 4.50 <sup>Bc</sup>	17.87 $\pm$ 4.44 <sup>Aa</sup>	<0.001*
p value	<0.001*	<0.001*	0.359	

Means with different upper and lowercase superscript letters within the same horizontal row and vertical column respectively are significantly different, \*significant ( $p < 0.05$ )

8 weeks use and 3,360 cycles equivalent to 12 weeks. One new brush was used for every nine specimens with a slurry ratio of 2:1 (distilled water: toothpaste). After each brushing cycle, the specimens were washed under running water, and stored in artificial saliva, which was composed of 1.5 mM Ca, 0.9 mM P, 0.05  $\mu$ g F/mL, 150 mM KCL, and 0.1 M Tris buffer, pH of 7.0.<sup>12</sup> Then both color change and surface roughness were recorded for each specimen; before staining procedures (baseline), after staining procedures, and after continuous tooth brushing cycles (cycle 1,2,3).

### Color Change Determination

Vita Easyshade spectrophotometer (Vita-Zahnfabrik, Germany) was used to record color change under standardized measuring conditions against a white background.<sup>8</sup> It was automatically set to be frequently calibrated before measuring each specimen. Teeth were slightly wet before each measurement to avoid optical changes that may occur due to dehydration. Data were expressed in CIE (Commission Internationale de L'Eclairage)  $L^* a^* b^*$ . Three readings from the middle part of the middle one-third of the labial surface of each specimen were recorded and the mean measurement was calculated. Color difference ( $\Delta E_{00}^*$ ) values were calculated using the following formula CIEDE2000 for each specimen:<sup>13</sup>

$$\Delta E_{00}^*(L^*_1, a^*_1, b^*_1; L^*_2, a^*_2, b^*_2) = \Delta E_{00}^{12} = \Delta E_{00}^*$$

The  $L^*$  value represents the lightness of an object where the value of zero equals a perfect black while the value of 100 represents a perfect reflecting diffuser. The  $a^*$  value is a degree of redness of the color (positive  $a^*$ ; +80) or its greenness (negative  $a^*$ ; -80). Finally, the  $b^*$  value counts for the degree of yellowness (positive  $b^*$ ; +80) or its blueness (negative  $b^*$ ; -80). The smaller the  $\Delta E_{00}^*$ , the lower the color change between the initial color measurement and the final color of the tooth. The perceptibility threshold ( $\Delta E_{00}^*$ ) was 0.8 whereas the 50:50% acceptability threshold ( $\Delta E_{00}^*$ ) was 1.8.<sup>14</sup>

### Surface Roughness (Ra) Measurement

The surface roughness (Ra) of each specimen was measured using a contact-type profilometer with a stylus (SJ-210 surface roughness tester, Multiyoyo, Japan).<sup>6,8</sup> Each specimen was fitted inside the specimen holder with the surface to be measured placed in a horizontal direction, then the specimen holder moved in a vertical direction up to the specimen surface just touching the stylus. Device calibration was done using the standard calibration specimen before use.

Testing parameters were as follows: measuring distance was 8 mm at speed 0.5 mm/s, returning 1 mm/s, and force 0.75 mN. The Stylus profile tip radius was 2 microns, tip angle 60°, and evaluation parameter (Ra) values were expressed in microns. Three readings were recorded for each specimen at a distance of 500 microns each.

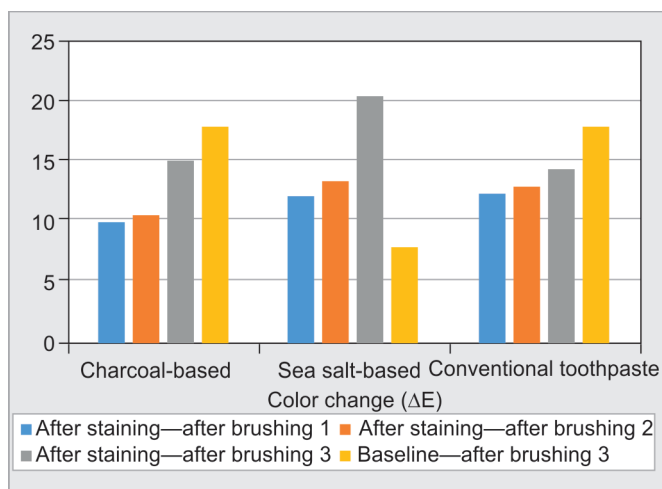
### Statistical Analysis

Numerical data were presented as mean and standard deviation (SD) values. Shapiro-Wilk's test was used to test for normality. The significance level was set at  $p < 0.05$  within all tests. Statistical analysis was performed with R statistical analysis software version 4.1.2 for Windows.<sup>15</sup> Color change data showed nonparametric distribution, so they were analyzed using Kruskal-Wallis test followed by Dunn's *post-hoc* test with Bonferroni correction for intergroup comparisons and Friedman's test followed by Nemenyi *post-hoc* test for intragroup comparisons. Surface roughness data were normally distributed so they were analyzed using one-way ANOVA followed by Tukey's *post-hoc* test for intergroup comparisons and repeated measures ANOVA followed by Bonferroni *post-hoc* test for intragroup comparisons.

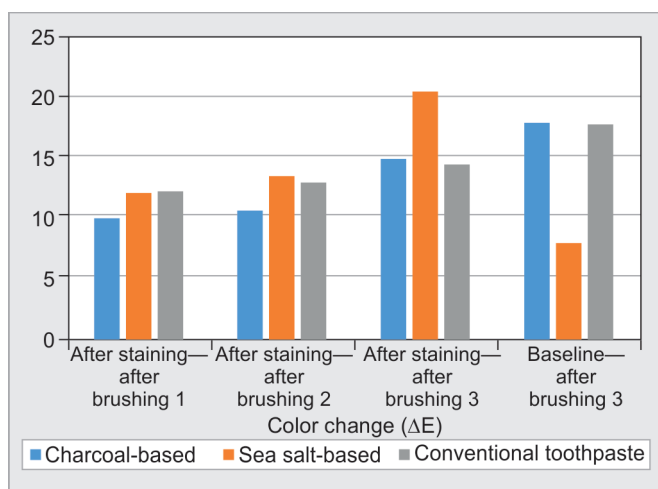
## RESULTS

### Color Change ( $\Delta E$ )

Results of inter- and intragroup comparisons of color change, presented in Table 2 and in Figures 2 and 3, showed that the color



**Fig 2:** Bar chart showing average color change in different intervals for each group



**Fig. 3:** Bar chart showing average color change in different groups

difference ( $\Delta E$ ) from after-staining protocol to different tooth brushing cycles (1,2,3) had no significant difference on each cycle between the tested groups ( $p > 0.05$ ). While for the color difference ( $\Delta E$ ) from baseline to the last tooth brushing cycle 3, the difference between groups was statistically significant ( $p < 0.001$ ) where group II sea salt-lemon toothpaste had a significantly lowest ( $\Delta E_{00}$ ) value among the other groups ( $p < 0.001$ ) indicating more whitening effect in relation to the others.

For group I charcoal-based toothpaste, there was a significant difference in color change between different intervals with values measured from baseline to the third tooth brushing cycle and from after staining to the third tooth brushing cycle being significantly higher than values measured at other intervals ( $p < 0.001$ ). While for group II, sea salt-lemon toothpaste, the difference in color change was statistically significant with values measured from after-staining to the third tooth brushing cycle were significantly higher than values measured at other intervals ( $p < 0.001$ ). In addition, the showed values measured from after-staining to the second tooth brushing cycle were significantly higher than values measured from baseline to the third tooth brushing cycle ( $p < 0.001$ ). For group III, the control, Signal Complete 8 original toothpaste, there was no

significant difference in color change between values measured at different intervals ( $p = 0.359$ ).

### Surface Roughness (Ra)

Results of inter- and intragroup comparisons of surface roughness presented in Table 3 and in Figures 4 and 5 showed that for different intervals, there was no significant difference between tested groups ( $p > 0.05$ ). While each of the tested groups showed a significant difference in surface roughness between different intervals.

For group I charcoal-based toothpaste, there was a significant difference between different intervals with values measured at different intervals being significantly different from each other ( $p < 0.001$ ). While group II, sea salt-lemon-based toothpaste, the difference was statistically significant with the values measured after the third tooth brushing cycle that were significantly higher than values measured at the baseline and after the first tooth brushing cycle ( $p < 0.001$ ). For group III, the control, Signal Complete 8 conventional toothpaste, the difference was statistically significant with values measured at the baseline, after the second and third tooth brushing cycles being significantly different from each other ( $p < 0.001$ ). Based on these results, all the tested toothpastes increased the degree of surface roughness by increasing the number of tooth brushing cycles.

### DISCUSSION

The current study aimed to detect the whitening effect of two different natural whitening toothpastes after intense staining protocol and to evaluate the alterations of enamel surface after different tooth brushing cycles in comparison to regular toothpaste.

A bovine teeth model was utilized as they were more available than human teeth with larger and flatter surfaces meanwhile it was stated that the chemical structure and physical properties of the bovine teeth are comparable to those of human teeth.<sup>4,7</sup> In addition, a severe staining protocol was carried out by using hot black tea solution cycles followed by dryness periods. Black tea is consumed by a large population and has a marked staining effect on tooth structure due to the presence of tannic acid and its high temperature.<sup>7</sup>

Despite the availability of different whitening toothpastes in the market, only a limited number of research studies have been conducted to investigate the efficacy of stain removal of such commercially available natural-based whitening toothpastes and their impact on the hard tooth structure.<sup>6-8</sup>

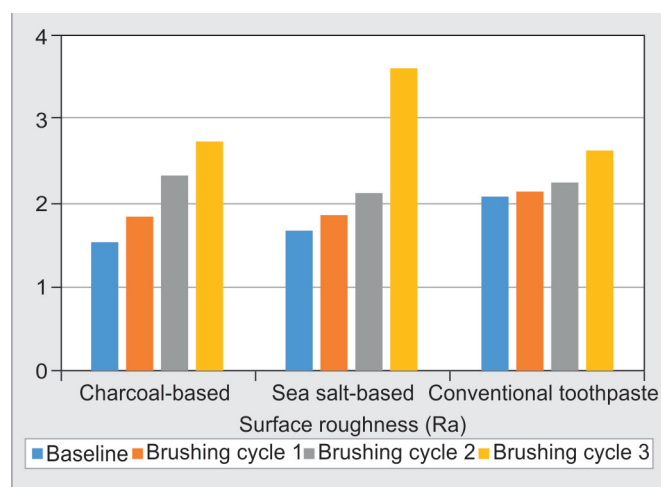
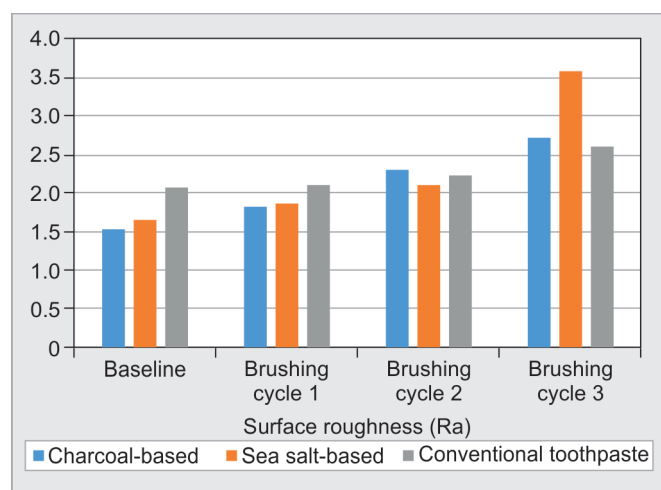
The Vita Easyshade spectrophotometer was selected for this study to detect color change due to its precision, strong data consistency, and repeatability.<sup>16,17</sup> Mehrgan et al.<sup>3</sup> stated that the spectrophotometer can detect small values of  $\Delta E$  which cannot be noticed by naked eye. In addition, the review article carried out by Basson et al.<sup>18</sup> stated that the numerical measurements of the spectrophotometer and the quantification of colors in a three-dimensional color space were the cause of its improved accuracy. The CIEDE 2000 color difference formula ( $\Delta E_{00}$ ) was applied in this study because it could create a single-number shade pass/fail calculation for evaluating minor to medium color discrepancies being more sensitive than the old CIE  $L^* a^* b$  formula.<sup>14</sup>

The abrasion of enamel or dentin by toothpastes was thought to be depending on two factors: the toothpaste's abrasive ingredients like silica particles and the toothbrush's hair hardness. To reduce the toothbrush abrasiveness, "soft" hair hardness was used in this study.<sup>8</sup> It is worth to mention that the amount, size,

**Table 3:** Inter- and intragroup comparisons of surface roughness (Ra)

Measurement	Surface roughness (Ra) (Mean $\pm$ SD)			p value
	Group I (charcoal)	Group II (sea salt-lemon)	Group III (conventional)	
Baseline	1.55 $\pm$ 0.69 <sup>Ad</sup>	1.66 $\pm$ 0.73 <sup>Ab</sup>	2.07 $\pm$ 0.87 <sup>Ac</sup>	0.331
Brushing cycle 1	1.83 $\pm$ 0.72 <sup>Ac</sup>	1.87 $\pm$ 0.77 <sup>Ab</sup>	2.13 $\pm$ 0.88 <sup>Abc</sup>	0.683
Brushing cycle 2	2.33 $\pm$ 0.80 <sup>Ab</sup>	2.13 $\pm$ 0.82 <sup>Aab</sup>	2.24 $\pm$ 0.88 <sup>Ab</sup>	0.869
Brushing cycle 3	2.75 $\pm$ 0.87 <sup>Aa</sup>	3.59 $\pm$ 3.05 <sup>Aa</sup>	2.60 $\pm$ 0.86 <sup>Aa</sup>	0.501
p value	<0.001*	0.038*	<0.001*	

Means with different upper and lowercase superscript letters within the same horizontal row and vertical column respectively are significantly different, \*significant ( $p < 0.05$ )

**Fig. 4:** Bar chart showing average surface roughness in different intervals in each group**Fig. 5:** Bar chart showing average surface roughness in different groups

and form of silica-based particles as well as their water content and agglomeration all influence the degree of tooth abrasion.<sup>19</sup>

On the other hand, from the clinical point of view, surface roughness exhibited great importance due to its impact on bacterial adhesion which in turn causes irreversible damage of both hard tooth structure and soft tissues.<sup>20</sup> Increased surface roughness can also cause dentin hypersensitivity, gingival recession, and the most

related here is the accumulation of oral stains which can affect the optical and color properties of enamel and restoration margins.<sup>8</sup>

Our study found that none of the tested whitening toothpastes was capable of complete removal of the tea stains after three continuous tooth brushing cycles as shown in Table 2 and Figure 2. This was in accordance with Sharif et al.<sup>21</sup> who concluded that only a small number of whitening toothpastes had the potentiality of chemical stain removal. Moreover, Nam et al.<sup>22</sup> stated that whitening toothpastes could produce a significant whitening effect after 6 weeks of tooth brushing of the unstained specimens.

For charcoal-based toothpaste, the color change was attributed to the ability of charcoal powder to absorb stains and pigments from the enamel surface. In addition, the presence of small amount of hydrated silica share in stain removal by a mechanical way. This color change was noticed only after the third cycle of tooth brushing with a significant tooth whitening effect although this effect could not reach the baseline values. This proved that charcoal-based toothpaste had a minimal tooth whitening effect and it needed almost 12 weeks to entail such a result. This was in accordance with Vaz et al.<sup>7</sup> and Dionysopoulos et al.,<sup>23</sup> who proved that charcoal-based toothpaste could induce minimal tooth whitening effect after tooth brushing for 2 and 3 months, respectively. On the other hand, Palandi et al.<sup>24</sup> concluded that charcoal-based toothpaste did not enhance any color change either alone or in combination with other toothpastes. Moreover, an integrative review<sup>25</sup> stated that the effectiveness of charcoal-based whitening toothpaste is still questionable and controversial besides its possible side effect.

For the sea salt-lemon based toothpaste, a significant tooth whitening effect was due to the ability of marine salt to remove the extrinsic stains in a mechanical abrasion way. In addition, the presence of hydrated silica in their formulation shares in stain removal in a similar abrasive action. Where obvious color change was recorded after the second and third tooth brushing cycles (equivalent to 4 and 8 weeks, respectively), and the color change after the third cycle was close to the baseline color. Little evidence was available about such a new product; however, Ramadan et al.<sup>26</sup> proved that brushing for 8 weeks using sea salt-lemon-based toothpaste was able to change the color significantly.

In this study, Signal Complete 8 was used as a control toothpaste as it does not contain any active whitening ingredient in its formulation. However, previous studies found that silica and hydrated silica particles present in the regular toothpaste were capable of removal of the extrinsic stains by abrasive action.<sup>4,21</sup> Our findings revealed that no whitening effect could be achieved even after continuous tooth brushing cycles (12 weeks). This was in accordance with Vaz et al.<sup>7</sup> who proved that regular toothpaste was not capable of stain removal even after continuous tooth brushing.

Perceptibility and acceptability thresholds determine if a color variation is perceptible and whether it is acceptable or not. The perceptibility threshold ( $\Delta E_{00}$ ) in the current study was set at 0.8 whereas the 50:50% acceptability threshold ( $\Delta E_{00}$ ) was 1.8.<sup>14</sup>

When the ( $\Delta E_{00}$ ) values were assessed in our study, it was obvious that the perceptibility threshold values of the dental enamel were higher than 0.8 for all kinds of toothpastes, regardless of brushing time as shown in Table 2. Furthermore, the acceptability threshold values were higher than 1.8 after the third tooth brushing cycle with all toothpastes.

Regarding the surface roughness results of our study, as shown in Table 3 and Figure 4, they revealed that all of the tested toothpastes increase the degree of surface roughness by continuous tooth brushing cycles. However, sea salt-lemon-based toothpaste showed the highest degree of surface roughness after the third tooth brushing cycle.

For charcoal-based toothpaste, there was a gradual increase in surface roughness after each tooth brushing cycle and this increase was obviously marked after the third tooth brushing cycle (12 weeks equivalent). However, this increase in surface roughness was not statistically significant from the control group. This was in accordance with Franco et al.<sup>27</sup> study showed that there was no obvious difference in terms of surface roughness between charcoal-based toothpaste and regular toothpaste after only 2 weeks of tooth brushing. While Ghajari et al.<sup>6</sup> and Vural et al.<sup>8</sup> who tested different types of charcoal-based toothpastes proved that charcoal-based toothpastes could significantly increase the degree of surface roughness even more than the control group. Mehrgan et al.<sup>3</sup> attributed the conflicting results concerning charcoal-based toothpaste to different factors including size, shape, and the degree of abrasiveness of charcoal particles.

For sea salt-lemon-based toothpaste, surface roughness increased after each cycle of tooth brushing and this increase was markedly noticed not only after the third tooth brushing cycle (12 weeks) but also after the second tooth brushing cycle (8 weeks). This marked increase in surface roughness might be due to the presence of both citrus lemon extract and marine salts in the ingredients of this toothpaste which cause more tooth abrasion during continuous tooth brushing cycles. This was in accordance with Yilmaz et al.<sup>28</sup> who proved that toothpaste containing marine salts was capable of increasing the degree of surface roughness after continuous tooth brushing cycles due to the presence of salt components in its formulation. On contrary Nanian et al.<sup>9</sup> stated that salt and lemon toothpaste had shown less reduction microhardness than other whitening toothpastes.

Concerning Signal Complete 8 Original conventional toothpaste, it showed a marked increase in surface roughness by increasing the number of tooth brushing cycles. This was in accordance with Rahardjo et al.<sup>29</sup> who proved that nonwhitening toothpaste could increase the degree of surface roughness after continuous tooth brushing cycles for 3 months due to the presence of abrasive particles, such as hydrated silica with mean size 10  $\mu\text{m}$ , in their formulation. In addition, Roopa et al.<sup>30</sup> stated that the increase of the surface roughness could be either due to the brushing action itself or due to the abrasive particles present in the formulation of different toothpaste. The same postulation was introduced by Bolay et al.<sup>31</sup> who found that tooth brushing procedures enhanced the enamel surface roughness. Thus, the null hypothesis was accepted for the whitening effect and rejected regarding the enamel surface roughness.

There were some limitations in this *in vitro* study; the inherent differences found between the strict laboratory conditions vs the natural dynamic conditions of the oral cavity and patient oral hygiene habits, also the deep staining protocol used which created a marked darkening of the tooth color. Thus, in order to confirm our results, clinical studies are highly advocated to correlate these findings with the real clinical condition where the presence of dynamic saliva may alter the degree of tooth stain and the whitening efficacy of different toothpastes. Moreover, further investigations are needed to assess the stability of whitening results of these products.

## CONCLUSION

With the limitation of this *in vitro* study, none of the tested whitening toothpastes can produce complete removal of the tea stains and return the tooth color back to its initial state. However, sea salt-lemon-based whitening toothpaste had a better whitening effect than charcoal-based whitening toothpaste after three cycles of tooth brushing. However, both charcoal- and sea salt-lemon-based whitening toothpastes as well as the conventional toothpaste increased the degree of surface roughness by increasing the number of tooth brushing cycles.

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