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Surface changes of enamel after brushing with charcoal toothpaste

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Abstract. The aim of this study was to determine the surface roughness changes of tooth enamel after brushing with charcoal toothpaste. Thirty specimens were brushed using distilled water (the first group), Strong® Formula toothpaste (the second group), and Charcoal® Formula toothpaste for four minutes and 40 seconds (equivalent to one month) and for 14 minutes (equivalent to three months) using a soft fleece toothbrush with a mass of 150 gr. The roughness was measured using a surface roughness tester, and the results were tested with repeated ANOVA test and one-way ANOVA. The value of the surface roughness of tooth enamel was significantly different ($p<0.05$) after brushing for an equivalent of one month and an equivalent of three months. Using toothpaste containing charcoal can increase the surface roughness of tooth enamel.

1. Introduction

Dental caries is a pathologic process of tooth tissue damage caused by microorganisms. Caries is a multifactorial disease that involves four fundamental factors: the host, who consists of dental tissues and saliva; the agent, which is microflora; the environment or substrate; and time. Caries is also defined as the progressive demineralization of the hard tissue of the tooth surface by organic acids from food. Dental caries occurs in people in Indonesia with a high prevalence and severity. Based on Indonesia Basic Health Research in 2013, the index of DMF-T in Indonesia is 4.6 with D-T = 1.6, M-T = 2.9, and F-T = 0.08. This means that there are 460 damaged teeth in 100 people of the Indonesian population [1]. Most microorganisms in the oral cavity can survive and form colonies when microorganisms attach to rough surfaces, enamel, or cementum [2]. A study that entailed observing the changes in the surface roughness of tooth enamel by examining the surface topography suggested that changes in enamel surface roughness could increase the proliferation of bacteria and biofilm formation, as well as increase the resistance of bacteria [2]. Several factors influencing changes in enamel surface roughness are exposure to acid that dissolves the hydroxyapatite on the surface of the enamel, as well as exposure to the abrasive agents contained in a variety of dental materials [3-4].

Nowadays, various types of toothpaste have been created and circulated in the market, ranging from toothpastes designed to protect teeth from calculus, gum disease, bad breath, plaque, and caries, as well as whitening toothpaste [5]. The selection of the right kind of toothpaste is possible through knowing the content and the function of the content in each toothpaste. Almost all available toothpastes in the market contain more than one active ingredient, which is promoted as having some advantages for the user. Generally toothpastes in the market today are a combination of abrasive materials, detergents, and one or more therapeutic agents.

Charcoal or activated carbon is one of the materials contained in some toothpaste products. Micro charcoal in toothpaste is known to have the ability to absorb dirt and to clean the teeth as well as the gaps between the teeth, which are difficult to reach. However, charcoal's shape and composition, along with the sizes of its particles could make it abrasive, so it is suspected to increase the surface roughness of tooth enamel [6]. Toothpastes that are considered ideal are those with the ability to maximally clean teeth with a minimum degree of abrasiveness, however.

2. Materials and Methods

This study was an experimental laboratory study whose design category was a randomized pretest-post test. This study involved three groups, and each group consisted of at least nine samples. Thus, in this study, each group consisted of 10 samples, so the total number of samples used was 30. The samples were grouped into group A (brushed with distilled water), group B (brushed using Strong® Formula toothpaste), and group C (brushed using Charcoal® Formula toothpaste).

The samples for this study were the buccal surfaces of maxillary first premolar teeth that had been extracted and approved for use by the Ethics Research Committee of Faculty of Dentistry, Universitas Indonesia (Number: 96/Ethical Approval/FKGUI/XI/2016). The research was conducted at the Laboratory of the Department of Dental Materials, Faculty of Dentistry, Universitas Indonesia and at Material Physics Laboratory, Faculty of Mathematics and Natural Sciences, Universitas of Indonesia.

Specimens were made through mounting, grinding, and polishing procedures. Teeth that met the criteria for the study samples were cut at the boundary of the Cemento Enamel Junction (CEJ) in a mesiodistal direction using a low-speed micromotor and carborondum disc. Then, a paper sticker was placed on a mixing slab with the sticker surface facing upward, and the buccal enamel surfaces were affixed to the paper. A plastic ring was affixed to each paper sticker encircling the specimen. The ring was pressed to prevent the leakage of resin during filling. After that, the resin liquid and hardener liquid were mixed until homogeneous, and they were poured into the plastic ring and kept for 24 hours until the resin hardened. After the resin hardened, the specimens were removed from the plastic ring, and their thickness was measured using a digital caliper. The specimens were sanded with sandpaper numbers 800, 1500, and 2000. During sanding, the thickness of each specimen was controlled; any enamel wasted could not be greater than 100 μm (0.1 mm). After sanding, the specimens were polished with 1 micron of alumina.

As many as 30 tooth specimens were divided into three groups randomly. The initial surface roughness (Ra) value of each specimen was measured using a surface roughness tester with a cut-off length of a 0.25-mm and 1.25-mm evaluation length. In one specimen, three measurements were done on different surfaces. The results of the three measurements were later counted as an average.

Each specimen group was given a different brushing treatment, which were: using distilled water, using Strong® Formula toothpaste, and using Charcoal® Formula toothpaste. The toothpastes were weighed with digital scales that had each been given a base. Initial equalization was done by pressing the "tare" button until it displayed a number in the form of "0, 00 gr." Toothpaste was then applied above each specimen until the scale showed 0.10 gr, then mixed with 0.1 ml of distilled water. The brush holder was properly set and was given a load of 150 gr [7]. After that, the solution of toothpaste was applied to the surface specimen. Brushing was done for four minutes and 40 seconds, which was assumed to be the equivalent of brushing for one month, followed by brushing for nine minutes and 10 seconds, which was assumed to be the equivalent of brushing for two months, so that when totalled, the brushing time would be equivalent to brushing for three months.

A Ra measurement was taken after brushing for the equivalents of one month and three months. A contribution procedure as the initial Ra measurement before treatment was done with a surface roughness tester with a 0.25-mm cut-off length and a 1.25-mm evaluation length. In one specimen, measurements were carried out three times on different surfaces. The total measurement results were then averaged.

The results were statistically tested using parametric tests. The significance of the changes in the initial Ra value, the first-month value, and the third-month value in each group were tested by using

the ANOVA repeated test. The significance of the differences in the Ra value among the groups was tested with one-way ANOVA. In this study, particle size analysis was also conducted using a particle size analyzer *(COULTER LS 100, America)* in the Laboratory of Material Physics, Faculty of Mathematics and Natural Sciences, Universitas Indonesia.

3. Results and Discussion

3.1 Results

3.1.1 Surface Roughness Test Results

The measured roughness values were the initial value, the value after brushing for an equivalent of one month (four minutes and 40 seconds), and the value after brushing for an equivalent of three months (14 minutes) [8]. The tooth surface roughness value was measured using a surface roughness tester *(Mitutoyo SJ 301, Japan)* as seen in Table 1.

Table 1. Tooth surface roughness test results after brushing with toothpaste containing charcoal

Group	Roughness Surface Value [Ra ± SD (μm)]		
	Initial	After Brushing for Equivalent of 1 Month (4 Minutes and 40 Seconds)	After Brushing for Equivalent of 3 Months (14 Minutes)
Distilled water (A)	0.06±0.01	0.10±0.02	0.12±0.03
Strong® Formula (B)	0.06±0.01	0.10±0.01	0.12±0.02
Charcoal® Formula (C)	0.06±0.01	0.11±0.01	0.22±0.01

After the normality assumptions and homoscedasticity were tested and met, the two-way analysis of variance (ANOVA) were carried out for the study of surface roughness and hardness of the specimens. A repeated ANOVA test followed by post-hoc pairwise comparison analysis was used to analyze changes in the value of roughness in the brushing group using distilled water (group A), Formula Strong® toothpaste (group B), and Formula Charcoal® toothpaste (group C). Moreover, this analysis was used to determine the value of the significance of the changes in the value of enamel surface roughness. Observations of the roughness changes in each group of specimens were performed prior to brushing; after brushing for four minutes and 40 seconds, which was assumed to be equivalent to brushing for one month; and after brushing for 14 minutes, which was assumed to be equivalent to brushing for three months [8].

Table 2. Statistical test result for repeated ANOVA with value of $p<0.05$ in three experiment groups

Variable	p-value	Test Group	Mean Difference	Mean Difference (%)	p-value among Groups
Distilled Water Group (A)	0.001*	A1 with A2	-0.031	51.6	0.016*
		A1 with A3	-0.057	95	0.010*
		A2 with A3	-0.026	26	0.187
Strong® Formula Group (B)	0.000*	B1 with B2	-0.038	63.3	0.000*
		B1 with B3	-0.060	60	0.000*
		B2 with B3	-0.022	22	0.006*
Charcoal® Formula Group (C)	0.000*	C1 with C2	-0.053	88.3	0.000*
		C1 with C3	-0.158	143.6	0.000*
		C2 with C3	-0.105	95.5	0.000*

Note: A,B,C=brushing group with distilled water (A), Strong® Formula toothpaste (B), Charcoal® Formula toothpaste (C); **1,2,3=**initial (1), brushing time equivalent to 1 month (2), brushing time equivalent to 3 months (3); ***p*<0.05→*=**statistically significantly different

The repeated ANOVA test results for the negative control group with distilled water (A) in Table 2 show a change in the value of surface roughness (Ra), which is statistically significantly different overall ($p<0.05$). These test results indicate that at least two different measurements were significantly different. Therefore, the post-hoc pairwise comparison test was conducted to determine the significance among the groups. The results of this analysis show that there was a statistically significant difference ($p<0.05$) between the initial Ra (A1) and the Ra after brushing for the equivalent of one month (A2). Significant differences were also found between the initial Ra (A1) and the Ra after brushing for three months (A3). Meanwhile, the values of Ra between brushing for the equivalent of one month (A2) and brushing for the equivalent of three months (A3) were not significantly different ($p>0.05$).

The repeated ANOVA test results for the positive control group with Strong® Formula toothpaste (B) in Table 2 show a change in the value of surface roughness (Ra), which is statistically significantly different overall ($p<0.05$). The results of the post-hoc pairwise comparison test show statistically significant differences ($p<0.05$) in the surface roughness values for all surfaces, including for the initial group (B1) and the group after brushing for the equivalent of one month (B2), for the initial group (B1) and the group after brushing for the equivalent of three months (B3), and the group after brushing for the equivalent of one month (B2) and the group after brushing for the equivalent of three months (B3).

The repeated ANOVA test results for the positive control group with Charcoal® Formula toothpaste (C) in Table 2 show a change in the value of surface roughness (Ra), which is statistically significantly different overall ($p<0.05$). The results of the post-hoc pairwise comparison test show statistically significant differences ($p<0.05$) in the surface roughness values for all surfaces, including for the initial group (C1) and the group after brushing for the equivalent of one month (C2), for the initial group (C1) and the group after brushing for the equivalent of three months (C3), and for the group after brushing for the equivalent of one month (C2) and the group after brushing for the equivalent of three months (C3).

The one-way ANOVA hypothesis test was also used to analyze the results of this study. Specifically, it was used to determine the significance of the differences in the average values of enamel surface roughness among the three groups of A, B, and C before treatment, after brushing for the equivalent of one month, and after brushing for the equivalent of three months. This was followed by Tukey post-hoc analysis to see which treatment groups had significant differences.

Table 3. Statistical test result for one-way ANOVA with value of $p<0.05$ in three experiment groups

Treatment	p-value	Test Group	Mean Difference	Mean Difference (%)	p-value among groups
Initial (1)	0.501	A1 and B1	-0.00500	8	0.473
		A1 and C1	-0.00300	5	0.760
		B1 and C1	-0.00200	3	0.884
Brushing Equivalent to 1 Month (2)	0.020*	A2 and B2	-0.00200	2	0.955
		A2 and C2	-0.01900	19	0.028*
		B2 and C2	-0.01700	17	0.052
Brushing Equivalent to 3 Months (3)	0.006*	A3 and B3	-0.00900	7.5	0.597
		A3 and C3	-0.08700	72.5	0.000*
		B3 and C3	-0.09600	80	0.000*

Note: A,B,C= Brushing group with distilled water (A), Strong® Formula toothpaste (B), Charcoal® Formula toothpaste (C); **1,2,3=** initial (1), brushing time equivalent to 1 month (2), brushing time equivalent to 3 months (3); ***p*<0.05→*** statistically significantly different

Table 4. Differences in the average tooth surface roughness values among groups before treatment (A1, B1, C1)

	ΔRa Distilled Water Group	ΔRa Strong® Formula Group	ΔRa Charcoal® Formula Group
ΔRa Distilled Water Group (µm)	-	0.00500	0.00300
ΔRa Strong® Formula Group (µm)	-0.00500	-	-0.00200
ΔRa Charcoal® Formula Group (µm)	-0.00300	0.00200	-

Initial roughness before brushing in the three groups did not differ significantly

In Table 4, we can see the differences in the mean values of enamel surface roughness among the groups before treatment (A1, B1, and C1). Based on the results of the post-hoc Tukey HSD in Table 3, the roughness values of the three groups were not statistically significant at 0.501 ($p>0.05$). This was consistent with the previous assumption that every sample was in the same state before the study.

Table 5. Differences in the average tooth surface roughness values among groups after brushing (A2, B2, C2)

	ΔRa Distilled Water Group	ΔRa Formula Strong® Group	ΔRa Formula Charcoal® Group
ΔRa Distilled Water Group (µm)	-	-0.00200	-0.01900*
ΔRa Strong® Formula Group (µm)	0.00200	-	-0.01700
ΔRa Charcoal® Formula Group (µm)	0.01900*	0.01700	-

(*)Value statistically differs significantly. Result from one-way ANOVA test ($p<0.05$)

We can see the mean value of surface roughness among the groups that brushed for the equivalent of one month (A2, B2 and C2). Based on the ANOVA test, the significance value was 0.020 ($p<0.05$), which means that there were significant differences in the data group. Based on the post-hoc Tukey HSD test's significance value in Table 3, there were statistically significantly different average roughness values—between the distilled water group (A2) and Charcoal® Formula toothpaste group (C2)—with a significance value of 0.028 ($p<0.05$). Meanwhile, the average value of the distilled water (A2) with Strong® Formula toothpaste group (B2) and the average values of roughness between the Strong® Formula toothpaste group (B2) and the Charcoal® Formula toothpaste group (C2) were not significantly different. This is seen from the significant values between the respective groups, which were 0.955 and 0.052 ($p>0.05$).

Table 6. Differences in the Average Tooth Surface Roughness Value among Groups after Brushing (A3, B3, C3)

	ΔRa Distilled Water Group (µm)	ΔRa Strong® Formula Group (µm)	ΔRa Charcoal® Formula Group (µm)
ΔRa Distilled Water Group (µm)	-	0.00900	-0.08700*
ΔRa Strong® Formula Group (µm)	-0.00900	-	-0.09600*
ΔRa Charcoal® Formula Group (µm)	0.08700*	0.09600*	-

(*)Value statistically differs significantly. Result from one-way ANOVA test ($p<0.05$)

In Table 6, there are differences in the mean surface roughness values among the groups after brushing for the equivalent of three months (A3, B3 and C3). Based on the ANOVA test, the significance value was 0.000 ($p<0.05$), which means that there were significant differences in the data group. Based on the post-hoc Tukey HSD test's significance value in Table 3, there were statistically significantly different average roughness values between the distilled water group (A3) and the Charcoal® Formula toothpaste group (C3), with the significance value being 0.000 ($p<0.05$). The average surface roughness value of the Strong® Formula toothpaste group (B3) and the Charcoal® Formula toothpaste group (C3) was also significantly different. Mean-while, the average surface roughness value of the distilled water group (A3) and the Strong® Formula (B3) was not statistically significantly different with a p value of 0.597 ($p>0.05$).

3.1.2 Particle Size Analyzer Analysis Results

The result of the particle size test using the particle size analyzer showed that the particle size of the Charcoal® Formula toothpaste was greater than that of the Strong® Formula toothpaste. The Charcoal® Formula toothpaste had an average particle size of 7.853 µm, while the Strong® Formula toothpaste had an average size of 4.625 µm. This was presumably because there were charcoal particles in large quantities in the toothpaste. Several previous studies mentioned that large particle size in toothpaste can affect the value of the tooth surface roughness (Ra).

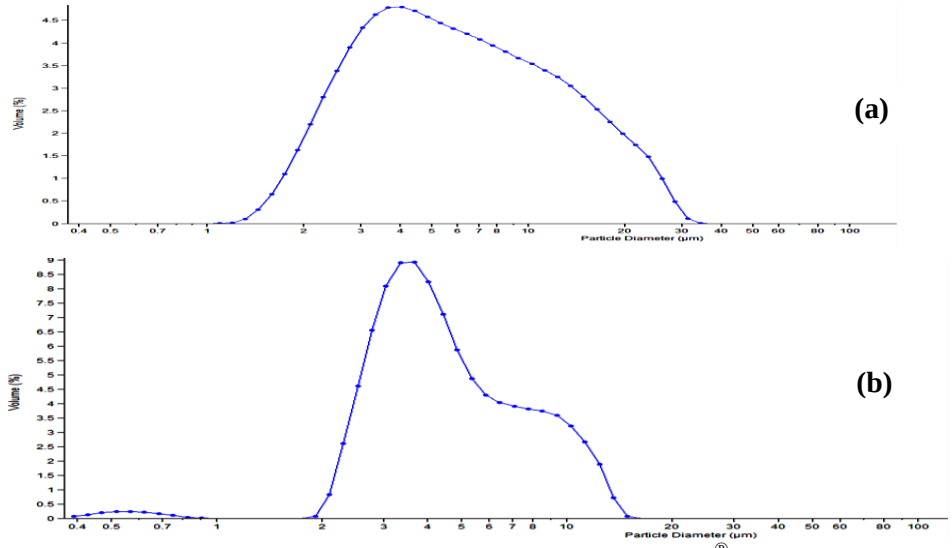

Figure 1. (a) Particle Size Analysis Result for Charcoal® Formula, (b) Particle Size Analysis Result for Strong® Formula

3.2 Discussion

The goal of this study was to observe changes in enamel surface roughness after brushing with toothpaste containing charcoal, then evaluated after brushing for the equivalent of one month and three months. This study used parameters adapted to the clinical recommendation, such as the volume of toothpaste, given load, and brushing time for each specimen. In this study, the toothpaste was diluted using distilled water at a ratio of 1:1. Loading was at 150 gr [7]. Also, in this study, brushing teeth simulation was done according to tooth brushing recommendations for 120 seconds (two minutes) for the entire surface of the tooth, with each quadrant brushed for 30 seconds [9]. A study involving the use of an electric toothbrush on a subject who had an average of six teeth in each quadrant explained that the size of the head of the electric toothbrush was capable of reaching at least more than one tooth surface, buccal/labial or lingual/palatal, so as to brush two tooth surfaces simultaneously. This took an estimated five seconds, the same amount of time estimated for brushing one tooth surface [7]. Some studies also used five seconds on each tooth surface in their research methods [9]. Thus, if brushing one tooth surface is assumed to take five seconds, with the recommendations stating that the teeth should be brushed twice a day, brushing a tooth in one day was assumed to take 10 seconds, brushing for one week was assumed to take 70 seconds, brushing for one month was assumed to take 280 seconds (four minutes and 40 seconds), and brushing for three months was assumed to take 840 seconds (14 minutes). In this study, brushing was conducted for 280 seconds (equivalent to one month), followed by brushing for 560 seconds (equivalent to two months) for each treatment, so the total time would become 840 seconds (equivalent to three months).

The results showed that the values of surface roughness of all three groups after brushing for the equivalent of one and three months were significantly different. The increased Ra value was caused by the action mechanism of the toothbrush bristles, the applied load while brushing, and the content of abrasive material contained in the toothpaste. In this study, the type of toothbrush bristle and brushing load were controlled accordingly and were the same for the three groups—a soft bristle brush with a load of 150 gr.

The smallest increase in Ra was found in the brushing group using distilled water (group A), which was a negative control. Based on the results of the study, an increase in Ra was significantly different between before brushing and brushing time equivalent to one month, but it was not significantly different between brushing time equivalent to one month and brushing time equivalent to three months. This showed that the brushing load and soft bristle brush affected the release of minerals and the increase of the roughness of tooth enamel. A toothbrush with a soft bristle brush has a greater contact surface area with the surface of the tooth, thus increasing surface roughness [8]. In addition, the level of abrasiveness of the tooth surface is also influenced by the length of the brushing time [8].

Increased Ra also occurred in the group involving brushing using Strong® Formula toothpaste (group B), which was a positive control, with there being similarities in the types of abrasive material content found in Strong® Formula and Charcoal® Formula toothpaste, which are silica and hydrated silica. Strong® Formula toothpaste did not claim to contain charcoal to clean the teeth and to remove stains found on the surface of a tooth; thus, it was assumed that the increasing Ra value in this group was due to the content of the abrasive material contained in toothpaste. When the process of abrasion occurred, abrasive materials contained in toothpaste would cause scratches in the enamel surface and cause the loss of some minerals in the enamel surface. The loss of tooth minerals would lead to an increase in the value of Ra. Some of the factors influencing the improvement of the abrasion of the material include abrasive material hardness. On the Mohs hardness scale, silica and hydrated silica have a value of 5-7, whereas the tooth enamel has a value of 5. We should beware if tooth enamel contacts materials with greater Mohs hardness values, as this could create scratches on the surface of the tooth enamel and increase the Ra value. In addition, the shape, size, and number of particles also determine the abrasive properties of a material. Based on the results of the particle size analyzer test, the average size of the particles in Strong® Formula toothpaste is 4.625 μm.

The highest increase in the Ra value occurred in Charcoal® Formula toothpaste. This was thought to result from the content of the abrasive material contained in toothpaste. In addition to having silica and hydrated silica as abrasive materials, Charcoal® Formula toothpaste also contains activated

carbon or charcoal. Based on visual observation, the color of Charcoal® Formula is identical to the color of charcoal, which is black. Charcoal particles are star shaped or so-called fractal shaped. A study once explained that the irregularity of particle shape could affect the value of surface roughness on a tooth [6]. In addition, based on the test results of the particle size analyzer, the average size of the particles in Charcoal® Formula toothpaste was greater than that of Strong® Formula, which was 7.853 μm. Larger particle size was expected to increase the value of Ra on the enamel surface after brushing with Charcoal® Formula toothpaste. After statistical tests, it was shown that the values of Ra after brushing for the equivalent of one month and three months among distilled water, Strong® Formula toothpaste, and Charcoal® Formula toothpaste were significantly different. This indicates that the materials contained in toothpaste affect changes in the roughness of tooth enamel. The statistical testing of Strong® Formula toothpaste (positive control) and Charcoal® Formula toothpaste, which had similar types of abrasive materials, also showed significantly different Ra value changes after brushing for the equivalent of three months. This indicates that the charcoal allegedly played a role in the change of surface roughness of tooth enamel.

The duration of toothpaste application could also affect tooth enamel, which is divided into good influences, such as the effect of the removal of food debris and stains attached to the surface of the teeth, and bad influences in the form of the side effects of the use of toothpaste. Charcoal® Formula toothpaste claims that the content of charcoal or activated carbon contained in the toothpaste was able to clean teeth and remove dirt on the surface of the tooth, so more time is needed to see the long-term effects. Therefore, in this study, the brushing times were the equivalent of one month and three months to see their effect on Ra.

The results showed increasing Ra values for all treatment groups after brushing for the equivalent of one month and three months. A tooth's rough enamel would be a strategic place for bacteria to adhere to the tooth's surface. The presence of bacteria in the oral cavity is one of the causes of high caries and periodontal disease risk [8]. The threshold value for Ra that might cause the retention of bacteria is 0.2 μm [8]. An Ra value above the threshold would be a strategic place for bacterial growth and the accumulation of plaque causing a high risk of caries and periodontitis [8].

Based on the research that has been done, after brushing for the equivalent of one month, the Ra values of the three groups were still below the threshold. However, after brushing for the equivalent of three months, the Ra value of Charcoal® Formula toothpaste crossed the threshold of bacterial retention. The increased Ra value nearly doubled from the initial roughness. Therefore, people need to reconsider the use of toothpastes containing charcoal for the long term (more than three months).

This research study had some limitations. First, the teeth were randomly selected, so researchers did not know the previous conditions of the teeth, such as dental age and interventions that had been conducted on the teeth. This caused the initial Ra values to vary. However, after being statistically tested, the initial Ra values of the three treatment groups did not differ significantly; the average values were similar. Second, there were differences between the research conditions and the natural atmosphere of the oral cavity. In the natural condition of the oral cavity, the demineralization-remineralization process occurs continuously, and there are also many other interventions. In in vitro studies, it is difficult to recreate such conditions [10].

Further research is needed to investigate each toothpaste composition and surface topography analysis can be further analysed with Scanning Electron Microscope. For the community, it is suggested to compensate for the long-term use of toothpastes containing charcoal, considering the increased risk of tooth surface roughness, through supporting enamel remineralization, such as the use of topical fluoride [10].

4. Conclusion

The research concluded that there were increasing surface roughness values of tooth surfaces after the use of toothpaste containing charcoal, and the increased surface roughness was statistically significant after brushing for four minutes and 40 seconds (equivalent to one month of brushing) and 14 minutes (equivalent to three months of brushing) using toothpaste containing charcoal.

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