

EFFECT OF SOAKING COW'S MILK ON TOOTH ENAMEL SURFACE ROUGHNESS

Dentistry

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ABSTRACT

Tooth enamel is the outermost part of the tooth structure and is the hardest part of the body. Demineralization and remineralization are natural processes in tooth enamel. Remineralization is the first step in bringing new minerals into a demineralized tooth. Remineralization can be produced by a solution containing calcium and phosphate ions, one of which is milk. Objective: This study aims to determine the effect of soaking pasteurized cow's milk on the surface roughness of tooth enamel. 32 premolars which were divided into 2 groups consisting of a treatment group and a control group. The sample of the treatment group was soaked in pasteurized cow's milk and the control group was given 20 ml of distilled water for 5 minutes. The treatment group was repeated every 6 hours per day for a week, and the control group was repeated once a day. All samples were measured using an enamel surface roughness tester. Analysis of the data used is bivariate data to determine the differences between the two sample groups. 16 teeth soaked in milk decreased the surface roughness of tooth enamel. There was a significant decrease in tooth enamel surface roughness after soaking in pasteurized cow's milk ($p < 0.05$). There is an effect of pasteurized cow's milk drink on the surface roughness of tooth enamel.

KEYWORDS

Remineralization, Demineralization, Cow's Milk, Surface Roughness, Tooth Enamel

INTRODUCTION

Tooth enamel is the outermost part of the tooth structure and is the hardest part of the body. Roughness of the enamel surface can facilitate attachment and bacterial colonization which promotes demineralization. Enamel demineralization can occur if the enamel is in a pH environment below 5.5. The pH value plays a role in demineralization because a low pH will increase the concentration of hydrogen ions and these ions will damage the hydroxyapatite of tooth enamel, causing enamel surface hardness. (Farooq, Imran, and Amr Bugshan (2020)).

Tooth erosion is the progressive loss of tooth tissue in tissues that are not mediated by bacteria but are chemically exposed to acid on the tooth surface. The potential for erosion does not only depend on the pH value of each acid. The acid that causes erosion comes from food and drink not a product of the intraoral flora. The erosion potential in erosive drinks is reduced or even eliminated when acidic drinks are enriched with minerals, especially calcium. Research conducted by Vieira, et al. In 2018, stated that worldwide prevalence of dental erosion varies between 6% and 52% in children aged 2 years to 5 years in primary teeth and between 3% to 95% in adolescent permanent teeth. (Vieira A, Chung C, Raffensperger S, Muluk P (2018), Korner, Wiedemeier, Attin, Wegehaupt (2020))

Dental caries is a major oral health disease caused by demineralization of the hard tissue structure of teeth through organic acids produced from fermented carbohydrates by cariogenic bacteria in dental plaque. Demineralization and remineralization are natural processes in tooth enamel. Remineralization is influenced by saliva, oral hygiene, fluoride and other natural protective factors. The stability of the oral environment is affected by demineralization and remineralization. The demineralization process is greater than the remineralization process which causes tooth enamel to become caries. (Al-Ani , Al-Naimi (2020), Dzulfia, Damiyanti, Herda (2016))

One of the things that can overcome the risk of dental caries is by consuming milk. Milk can reduce erosion of tooth enamel because it contains phosphorus, calcium, lactoferrin, phosphate, fat, and natural casein which are good for health and can protect teeth from caries. Research Dzulfia, et al. In 2016, it was shown that cow's milk had an effect on increasing tooth enamel hardness.(Dzulfia, Damiyanti, Herda (2016))

Indonesia is the largest milk producer in Southeast Asia and its products are popular in Indonesia, Singapore, Malaysia, Hong Kong, Brunei and the Philippines. The need for a healthy lifestyle and materials high-quality food is increasing, but mostly met by imported products. So that Indonesia produces cow's milk to meet local and regional needs surroundings.(Dzulfia, Damiyanti, Herda (2016)).

Cow's milk is considered a complete food, providing high-quality

protein and essential micronutrients, including vitamins and minerals. Cow's milk has been shown to contain fluoride and can increase bone remineralization. Research conducted by Rahardjo, et al. in the study of Dzulfia, et al. in 2016, proved that topical application using milk had the best protective effect against demineralization compared to the combined application of milk-tea and milk with 0.2% NaF. Research conducted by Damayanti in 2011, in the research of Dzulfia, et al. in 2016 stated that in 100 grams of milk there is 125 milligrams of calcium, this high calcium content is very beneficial for bone health, dental health and children's body growth.(Dzulfia , Damiyanti , Herda (2016), Antunes, I. & Bexiga, R. & Pinto, Carlos & Roseiro, Cristina & Quaresma, Mário. (2022)).

The effect of cow's milk on the surface roughness of tooth enamel has been significantly known to cause remineralization of the tooth enamel surface. Therefore researchers were interested in conducting research on the effect of soaking cow's milk on the surface roughness of tooth enamel and by comparison with the control group.(Dzulfia , Damiyanti , Herda (2016))

METHODS

The type of research used is laboratory experimental research (in vitro) with a pretest-posttest research design with control group design. The research was conducted at the Technical Laboratory of Tarumanagara University. This laboratory was chosen because it has a tool to measure tooth surface roughness, namely a surface roughness tester. The time of the research was carried out in March 2022 – May 2022. The sample in this study were 32 premolars. Crowns were still intact, No caries, No fillings, No white spots. Sampling was done by systematic random sampling technique. Samples were taken at random in a systematic way.

The samples used were premolars that matched the inclusion and exclusion criteria. All tooth samples were cleaned with running water. Samples were washed with running water and stored in saline solution at room temperature. Every two days the saline solution was changed until the time of the study in order to maintain the humidity of the teeth so that no changes occurred. If the sample wants to be used, then the sample is washed first with running water and dried with a tissue or chip blower. The sample was divided into two groups, namely the treatment group and the control group. The treatment group and the pre-test group will be planted on wax and the initial enamel surface roughness will be measured using a surface roughness tester.

Milk drinks (packaged pasteurized cow's milk) and aquadest are first measured in the pH of the container and measured with a pH meter. The sample is immersed in 20 ml of packaged cow's milk drink which is pasteurized in a container for 5 minutes then rinsed with running water and dried with a tissue or chip blower. The sample is immersed again in saline solution and the treatment is carried out in the same way every 6

hours for 24 hours because the sample is analogous to humans who consume cow's milk in the morning, afternoon, evening and night. Samples of the control group were immersed in 20 ml of distilled water for 7 days and replaced every 24 hours at room temperature.

On day 8, the entire treatment group and post-test control groups were cleaned with running water and dried with a tissue or chip blower before being planted in wax. All samples to be measured by roughness surface tester will be planted with wax first. All samples of post-test treatment and control groups that have been waxed will be measured using a roughness surface tester to measure the final surface roughness. The specimen is placed in the roughness tester unit. The tool starts working at the time specified by the tool. The results of the roughness of the specimen will appear through the results on the screen contained in the test equipment in units of Ra (Roughness Average).

RESULTS

The data obtained in this study resulted that cow's milk had an influence on the surface roughness of tooth enamel. Soaking the teeth in milk results in a decrease in the roughness of the surface of the tooth enamel. the results of the Independent T-Test showed that soaking in cow's milk and soaking in distilled water there were significantly different results.

Table 1 - Values of Surface Roughness Treatment Group

No. Sample	Before (µm)	After (µm)	difference (µm)
1	0,025	0,022	-0,003
2	0,109	0,064	-0,045
3	0,198	0,186	-0,012
4	0,251	0,220	-0,031
5	0,174	0,128	-0,046
6	0,111	0,110	-0,001
7	0,137	0,126	-0,011
8	0,173	0,102	-0,071
9	0,240	0,143	-0,097
10	0,281	0,223	-0,058
11	0,141	0,134	-0,007
12	0,332	0,124	-0,208
13	0,121	0,115	-0,006
14	0,109	0,103	-0,006
15	0,143	0,084	-0,059
16	0,223	0,179	-0,044
Average	- 0,04343		

Group 1 was the group that had 16 teeth soaked in pasteurized cow's milk. The enamel surface roughness value obtained by calculating the buccal surface roughness before and after treatment (pasteurized greenfields milk immersion) was measured using a surface roughness tester mutitoyo SJ-210. The result data on the roughness value can be seen from table 1.

Table - Surface Roughness Values for Control Group

No. Sample	Before (µm)	After (µm)	Difference (µm)
1	0,129	0,127	-0,002
2	0,169	0,147	-0,022
3	0,211	0,156	-0,055
4	0,382	0,300	-0,082
5	0,297	0,203	-0,094
6	0,391	0,317	-0,074
7	0,299	0,277	-0,022
8	0,059	0,049	-0,01
9	0,323	0,264	-0,059
10	0,615	0,285	-0,33
11	0,300	0,316	-0,016
12	0,451	0,110	-0,341
13	0,137	0,110	-0,026
14	0,599	0,308	-0,291
15	0,121	0,113	-0,008
16	0,593	0,389	-0,204
Average			-0,1022

The control group was the group that was immersed in 20/ml/sample of distilled water and soaked for 24 hours and then replaced every day for 7 days. The surface roughness value of the enamel was obtained using the surface roughness tester mutitoyo SJ-210. The result data on the roughness value can be seen from table 2.

Table 3 - Results of Paired T-Test Between Before Immersion in Cow's Milk and After Immersing in Cow's Milk

Paired Sample Correlation

	N	Correlation	Sig.
Before Soaking in Cow's Milk- After Soaking in Cow's Milk	16	0,742	0,004

* Sig. <0,05= There is a statistically significant difference

Based on the results of the Paired T-Test between before soaking cow's milk and after soaking cow's milk in table 3, there were significant results, namely $P = 0,004 < 0,05$. In these results, there is a significant difference between before soaking cow's milk and after soaking cow's milk. This explains that soaking teeth with cow's milk can reduce the surface roughness of tooth enamel.

Table 4 - Paired T-test Results Between Before Immersion In Aquatic And After Immersion In Aquatic

Paired Samples Test

	N	Correlation	Sig
Before Soaking in Aquades - After Soaking in Aquades	16	0,762	0,005

*Sig. <0,05= There is a statistically significant difference

Based on the results of the Paired T-Test between before immersion in distilled water and after immersion in distilled water, table 3 shows significant results, namely $P = 0,005 < 0,05$. In these results, there is a significant difference between before and after aquades immersion. This explains that soaking teeth with cow's milk can reduce the surface roughness of tooth enamel.

Table 5 - Results of Independent T-Test Analysis Between After Immersion in Cow's Milk and After Immersion in Aquades Independent Samples Test

	Sig.(2 tailed)
Independent T-Test	0,005

*Sig. <0,05= There is a statistically significant difference

Table 3 shows the results of the Independent T-Test statistical test to test whether there is a difference in the mean of the two sample groups that are not related or not paired. In the table above, the sig value is obtained. = 0,005 < 0,05, which means that the average tooth surface roughness after soaking cow's milk and after soaking in distilled water there is a statistically significant difference. Based on the hypothesis that has been applied, it can be concluded that the research hypothesis is accepted.

DISCUSSION

This study was conducted to explain the effect of soaking pasteurized cow's milk on the surface roughness of tooth enamel. Tooth enamel is the outermost covering of the anatomical crown of the tooth. Tooth enamel is the hardest part of the human body which consists of more than 96% organic matter, the main component of tooth enamel is hydroxyapatite.

Milk is one type of drink that is often and widely consumed by the community, because milk is a product that contains many benefits. The content of phosphate and calcium ions in milk can affect the remineralization process in tooth enamel. Based on a survey from AC Nielsen Indonesia, Greenfields fresh dairy products controlled 54% of the product market share in the first quarter of 2017. The data is taken from sales records in super-super markets which show that Greenfields fresh milk sales reached 900,000 liters. (Yudhit, Astrid & Imanida, Kholidina, Dewi, Yuli (2019))

The treatment group used was 16 premolars which would be immersed in a container with 20 ml of pasteurized cow's milk (greenfields) for 5 minutes per 6 hours a day. Based on the enamel surface roughness values obtained before and after being treated using a surface roughness tester, it showed a decrease. Furthermore, the results of the statistical test carried out with the paired t-test, there was a value of $p = 0,004$ ($p < 0,05$) which showed a significant difference between before and after soaking cow's milk.

The effect of decreasing the surface roughness of tooth enamel is caused by various factors, namely extrinsic and intrinsic factors.

Extrinsic factors that cause a decrease in roughness or smoother occur when consuming drinks that have a low pH. This decrease in roughness occurs when the tooth is in a remineralized state. The lower the pH in saliva, the faster the hydroxyapatite bonds to tooth enamel or the faster the remineralization process.

Saliva had a buffering ability, which allowed it to maintain the pH balance of the oral cavity and prevent tooth demineralization. The demineralization process begins when tooth degradation occurs, when the tooth is exposed to a drink or food with a pH below 5.5 it will dissolve calcium in the tooth so that the hydroxyapatite crystals dissolve or are eroded. Milk can prevent the demineralization process and result in the remineralization process. The protein in milk sticks to the tooth surface, at that time calcium and phosphate ions diffuse below the enamel surface to fill the empty hydroxyapatite crystal space as a result of the demineralization process, when calcium and phosphate ions fill the empty hydroxyapatite space, a remineralization process occurs. (Sukanto, Ibmurrafi, Sulistiyani, Lestari, Pujiastuti, Setyorini, Probosari, Budirahardjo, Prihatiningrum (2023))

Solutions containing phosphate and calcium ions can cause remineralization. According to the results of research from Wisyaningtyas, et al. in 2014, stated that the process of tooth enamel remineralization is due to the presence of calcium and phosphate ions that diffuse from pure soy milk into the microporosity of the enamel. Research conducted by Wisyaningtyas, et al. in 2014, using cow's milk immersion using premolars and the milk will be changed every 8 hours. Remineralization can occur if a neutral pH contains sufficient Ca^{2+} and PO_4^{3-} ions. Research results from Vieira, et al. in 2017, stated that milk produces enamel remineralization. (Vieira, Chung, Raffensperger, Muluk (2018), Obeid, Khalaf (2022))

This study used premolars including posterior teeth and used pasteurized milk. The difference lies in the research subject and the type of milk conducted by Fadil, et al. in 2021, using resin-modified glass ionomer cement using UHT milk and the comparison is fresh cow's milk. The results were not significant in both milks, but similar to this study, there were significant results of remineralization between the fresh cow's milk group and the control group and UHT milk and the control group. (Yudhit, Astrid & Imanda, Kholidina, Dewi, Yuli (2019))

In group 2, which is the control group, treated with distilled water resulted in a decrease in roughness or a smoother surface than the results of the paired t-test (table 4) of $p = 0.005$ ($p < 0.05$). The decrease in surface roughness of tooth enamel is caused by the pH factor in the solution that plays a role. The type of solution used for the control group was distilled water, where the composition of the distilled water resembled pure water. Aquades has a pH that is close to normal (pH 7). Research according to Hughes cited by S Kazmi in 2016 explains that pH has an important role in increasing roughness. The smaller the pH in the feeding solution, the higher the roughness level, the higher the pH in the solution, the lower the roughness that occurs.

The results of this study indicate that there are significant differences between the treatment group and the control group due to the phosphate, protein, and calcium content in pasteurized cow's milk. This research is strengthened by research from Amaliyah, et al. in 2021, which showed a decrease in tooth enamel surface roughness due to the protein content in cow's milk. (Farooq, Imran, Bugshan (2020))

CONCLUSION

Based on the results of research that has been carried out, there is a change in the form of a decrease in the surface roughness of tooth enamel after soaking in pasteurized cow's milk drink (greenfields), because the pH of the drink contained in this drink is 6.5. The content in milk is one of the substances that are good for bone remineralization, so it can cause a decrease in tooth roughness caused by:

The immersion time used in this study was short so that there was no change in pH which would cause the pH to become acidic and cause demineralization of the teeth.

Proteins attached to teeth cause calcium and phosphorus ions in cow's milk to diffuse below the tooth enamel surface so that phosphorus and calcium ions can occupy empty or eroded hydroxyapatite crystal spaces, resulting in a decrease in tooth enamel surface and a remineralization process.

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Potency of Acid Content in Local and Imported Strawberry Fruit Extract (*Fragaria X Ananassa*) as Natural Ingredients for Teeth Whitening

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Research Article

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Abstract

Background: Bleaching treatment is a whitening treatment for discolored teeth to restore their aesthetic function. The bleaching agent that is commonly used is 35% carbamide peroxide. Strawberries contain malic acid which can whiten teeth.

Purpose: This study aims to find out acid potential on strawberries as a natural ingredient for teeth whitening.

Methods: 48 post-extraction permanent single-rooted teeth that had been coated with clear nail polish were soaked in black coffee for 2 days, then 16 teeth were immersed in a local strawberry extract solution, 16 teeth were immersed in an imported strawberry extract solution and the other 16 teeth were immersed in a 35% carbamide peroxide gel solution and then observed and measured the color of the teeth after immersion at 24 hours and 48 hours.

Results: There is potential for local and imported strawberry fruit extract in whitening teeth but the whitening potential when compared to carbamide peroxide is not significant ($p>0.05$). The whitening potential of imported strawberry juice is higher than that of local strawberry juice based on the average value.

Conclusion: Based on research results, local and imported strawberry juice extracts have the potential to whiten discolored teeth.

Keywords: Whitening Treatment; Strawberries

Introduction

Tooth bleaching has become one of the most successful and well-accepted aesthetic dental treatments over the past decades and has become an important part of aesthetic dentistry, representing the most conservative method of treatment of dental discoloration. Special interest enjoyed this type of dental treatment, especially among patients and physicians, has led to numerous market profiles for bleaching

products and application techniques [1,2].

The 2015 survey data states that more than 1 million Americans are interested in carrying out teeth whitening treatment at a cost of up to 600 million dollars per year [3].

Tooth discoloration is caused by stains which may be intrinsic, extrinsic or a combination of both in terms of source [4]. Intrinsic factors such as congenital, systemic,

hematologic factors, disorders and exposure to phenolic or iodoformic drugs and conditions caused by extrinsic factors, namely pigments in food, beverages such as tea, coffee, tobacco, red wine, and other substances that often come into contact with the hard tissues of the teeth in the oral cavity. The use of tobacco products is also a risk factor for tooth discoloration [5-7].

The color of the tooth structure results from the interaction of different light phenomena through hard tissues including dispersion, refraction, transmission and absorption. The final observed color is determined by a combination of the optical properties of the enamel and dentine tissue including translucency and chromaticity [8]. Bleaching involves an oxidation process in which the molecules causing the discoloration are chemically modified [9].

Shade guides is the one commonly used and commercially available is Vitapan Classical with 16 shade guides (Vita Zahnfabrik) arranged from lightest to darkest, namely B1, A1, B2, D2, A2, C1, C2, D4, A3, D3, B3, A3 .5, B4, C3, A4 and C4. Vitapan 3D master with 29 colors including three colors of bleach (Vita Zahnfabrik) which is used as a benchmark for measuring tooth color. Each tab is placed in the inspection box and measured using a spectrophotometer [10,11].

Color measurement is based on the use of 3 or 4 color discs, each of which has been calibrated using 3 methods, namely chromatic, value and chrome. Chromatic is a color intensity that distinguishes strong colors from weak colors which is described as the distance of the circle from the center (Munsell color ball). Value is a color quality associated with lighting and the system's color measurement for visually matching product colors to Munsell colors using the sense of sight [12].

Indications for bleaching teeth that have undergone discoloration are tooth discoloration caused by pulp chambers, discolored dentin, discolored due to calcification metamorphosis, and discolored due to hypoplasia/hypomineralization. Hypoplasia and hypomineralization may require additional treatment to completely cover the discoloration [13].

The side effect of using hydrogen peroxide and carbamide peroxide as teeth whitening agents are that they can cause penetration of the pulp chamber, increase sensitivity to temperature making it easier to ache, decrease enamel hardness and changes in enamel layer roughness [14].

The side effects resulting from the use of chemicals as tooth whiteners have made many researchers look for alternative materials to replace chemicals, namely by using

natural ingredients that are safer and at affordable prices [14].

Indonesia imports around 150 tons of premium strawberries per year from South Korea, the United States and Australia. Strawberry fruit is a fruit that is widely consumed as fresh or processed food with a distinctive aroma, red color and sweet taste. In 2019 world strawberry production reached 8.9 million tonnes, of which China contributed 35.9%, the United States 11.2% and Mexico 10.11%. Strawberry is a fruit that is valued because of the nutritional content contained in the fruit [15,16].

Indonesia is one of the fruit-producing countries with diversity and rich in taste when compared to fruit from other countries. Based on data from the Food and Agriculture Organization (FAO). In 2014 Indonesia was ranked in the top 20 as a world-level fruit producing country. Tomatoes and strawberries are types of fruit that are quite popular and are known to have a fairly high water content. Strawberries and tomatoes are considered fruits that are widely consumed in Indonesia, but both have a short shelf life due to their high water content [17].

There are natural ingredients from strawberries as a natural teeth whitener because strawberries (*Fragaria x ananassa*) contain pectin (natural fiber) which helps clean teeth themselves and strawberries are fruits that are rich in phytochemicals such as ellagic acid (ellagic acid), malic acid (malic acid), anthocyanins, quercetin, catechins and vitamins such as ascorbic acid and folic acid. Ellagic acid has a potential OH group which acts as a strong oxidizer. Stephanie et al noticed that the more ellagic acid, the more OH groups produced and the more effective the bleaching process. OH and H radicals produced from ellagic acid react with organic enamel molecules, disrupting electron conjugation and changing the absorption of energy in enamel by forming smaller organic molecules with a lighter color [18,19] (Figure 1).

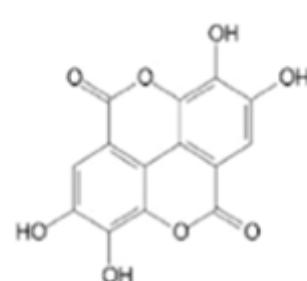


Figure 1: Ellagic Acid Structure [20].

Materials and Methods

In this study, 48 single-rooted teeth were used which had been coated with clear nail polish on the roots of the teeth Figure 2.



Figure 2: Single-rooted teeth.

Inclusion Criteria

Caries free teeth, extracted single-rooted teeth, teeth free from calculus. Exclusion criteria: fracture teeth, stained crown.

Carbamide peroxide 35% is the bleaching agent used in this study. A digital spectrophotometer is used to measure the color of the sample, which will take the initial and final colors as a comparison of changes.

The samples used local strawberry juice extract (holibert strawberry, bandung, Indonesia) and imported strawberry fruit juice extract (evergood brand, South Korea). The bleaching potential was compared with 35% carbamide peroxide.

Conducted an analysis test for malic acid content in the strawberries used in this study at the Saraswanti Indo Genetech Bogor laboratory with the result that there was a malic acid content of 0.15% for local strawberries and 0.0885% for imported strawberries.

Making a strawberry fruit extract paste is by preparing 200 grams of strawberries which have been cleaned, diced and mixed with 15 ml of distilled water and blended to get about 100 ml of strawberry concentrate. This concentrate was filtered then transferred into a cooling centrifuge to be processed at 2000 rpm for about 20 minutes at room temperature.4oC. Liquid is collected in a container and stored at 4oC.

Study Design

This type of research is a laboratory experiment with a pre-test-post research design with a control group design, namely in a simple experimental design that is chosen randomly. One group acts as the control group and the other group acts as the experimental group. Sampling was carried out using systematic random sampling technique. Samples taken randomly but systematically.

In this study, immersion was carried out 16 tooth samples in coffee solution (pre-test) and then soaked with local strawberry juice (post-test). The second sample group consisted of 16 other tooth samples which were soaked in coffee solution (pre-test) and then soaked with carbamide peroxide. Then there is a third sample group of 16 samples soaked in coffee solution (pre-test) and then soaked with imported strawberry juice (post-test).

After soaking, the light color (L) was measured after being soaked with local strawberry juice which was done 2 times, namely at 24 hours and 48 hours of immersion. The analysis was carried out by carrying out univariate analysis, namely descriptive statistical analysis by calculating the mean of the research data and also carrying out a normality test. Then do the data comparison between the pretest and posttest by using paired t-test and comparison test between treatment groups using the One Way ANOVA test.

Results

Descriptive Statistics

The following is the average value of the research data, namely the average color (L) of the teeth by soaking local strawberry juice, imported strawberry juice and carbamide peroxide gel (Figures 3-5).

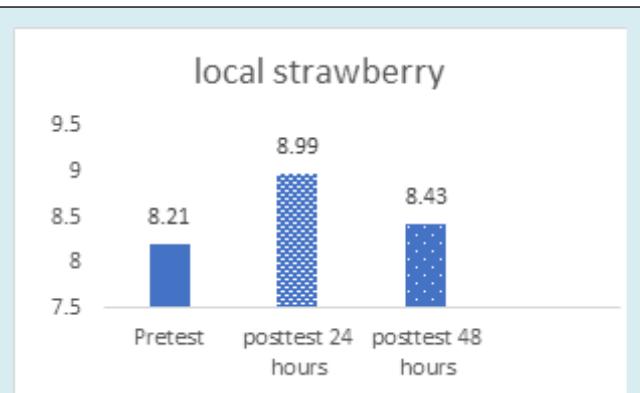


Figure 3: Bar chart of average tooth color values with local strawberry juice immersion treatment.

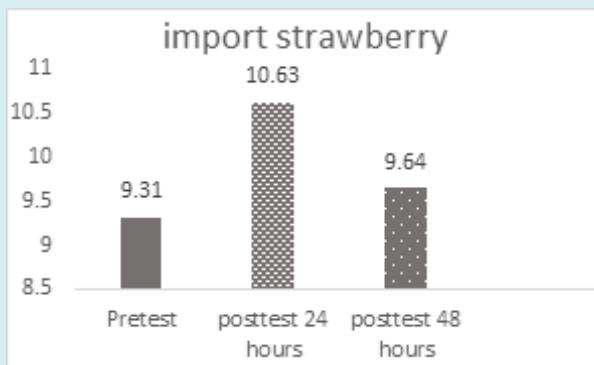


Figure 4: Bar chart of the average value of tooth color by immersion treatment of imported strawberry juice.

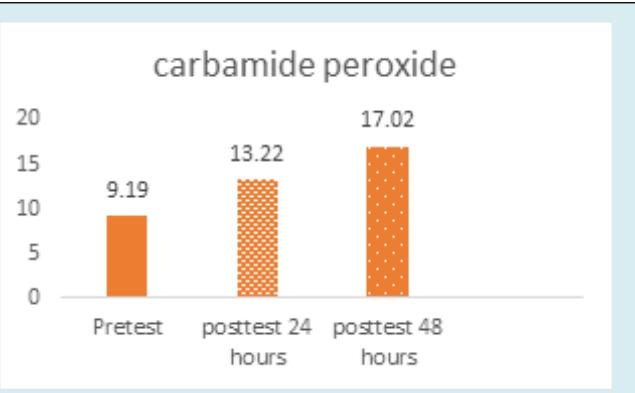


Figure 5: Bar chart of the mean values of teeth with carbamide peroxide immersion treatment.

It can be seen from Figures 3-5 that the color (L) of the teeth tends to increase at 24 hours and decrease at 48 hours if soaked with local strawberry juice and imported strawberry juice. On the other hand, the color (L) of the teeth tends to increase if soaked in carbamide peroxide gel solution.

Normality Test

The data normality test is a hypothesis test to find out whether the data obtained is normally distributed or not. This test is carried out to determine whether parametric statistical methods or non-parametric statistics are more suitable to be used in conducting the analysis. The normality test was carried out using the Shapiro-Wilk test method because the data samples totaled less than 50 data. The results of data normality test can be seen in Tables as follows.

It can be seen from the results of the normality test in Table 1 that all data groups produce a p-value for the Shapiro-Wilk test greater than 0,05 which indicates that the data is normally distributed. Because the pair of pre-test and post-test data on immersion with strawberry juice

is normally distributed, the Light (L) color comparison test between the pre-test and post-test of local strawberry juice immersion is carried out using the parametric method, namely the dependent t-test or Paired T-test. Likewise with the pre-test and post-test of carbamide peroxide immersion and also imported strawberry juice which can also be carried out using the parametric method namely the dependent t test or Paired T-test.

Perlakuan	Data Group	P-values
Local Strawberry Juice	Pre-Test	0,144
	Post-Test 24 Hour	0,325
	Post-Test 48 Hour	0,352
Carbamide Peroxide	Pre-Test	0,062
	Post-Test 24 Hour	0,702
	Post-Test 48 Hour	0,646
Imported Strawberry Juice	Pre-Test	0,993
	Post-Test 24 Hour	0,166
	Post-Test 48 Hour	0,459

Table 1: Normal Distribution Test Results.

Meanwhile, it can be seen that in the post-test pair of local strawberry juice immersion, post-test data of carbamide peroxide immersion and also post-test data of imported strawberry juice immersion were also normally distributed, so a comparison test between the Light color (L) between the three groups was carried out by parametric method namely One Way ANOVA.

Paired T-test

Paired T-test comparison was conducted to determine whether or not there was an effect of soaking with local strawberry juice, then whether or not there was an effect of soaking with carbamide peroxide and whether or not there was an effect of soaking with imported strawberry juice on the Light (L) color on the teeth. The results of the Paired T-test can be seen in Tables 2-4 as follows.

	P-values	Information
24 Hour Post-Test	0.408	Not Significantly Different
48 Hour Post-Test	0.837	Not Significantly Different

Table 2: Results of paired t-test immersion with local strawberry juice.

Based on Table 2, it can be seen that on soaking with local strawberry juice, both after soaking for 24 hours and 48

hours, the resulting p-value is greater than 0.05, this means that there is no significant difference between the Light (L) before and after soaking with local strawberry juice either at 24 hours, 48 hours or 72 hours. So it can be concluded that “local strawberry juice has no significant effect on the color of Light (L) on the teeth either after 24 hours or 48 hours of soaking”.

	P-values	Information
24 Hour Post-Test	0.196	Not Significantly Different
48 Hour Post-Test	0.743	Not Significantly Different

Table 3: Test results of paired t-test immersion with imported strawberry juice.

Meanwhile in Table 3 it can be seen that in soaking with local strawberry juice, after soaking for 24 hours and 48 hours, the resulting p-value is greater than 0.05, this means that there is no significant difference between Light (L) before and after soaking with imported strawberry juice at 24 hours and 48 hours. This shows that “imported strawberry juice did not have a significant effect on the color of Light (L) at 24 hours and 48 hours of immersion”.

	P-values	Information
24 Hour Post-Test	0.014	Significantly Different
48 Hour Post-Test	0	Significantly Different

Table 4: Test results of paired t-test immersion with carbamide peroxide gel.

		Sum of Squares	df	Mean Square	F	P Value
Posttest 24jam	Between Groups	145,666	2	72,833	1,783	0,180
	Within Groups	1,837,992	45	40,844		
	Total	1,983,658	47			
Posttest 24jam	Between Groups	692	2	345.943	15.402	0,000
	Within Groups	1,011	45	22.460		
	Total	1,703	47			

Table 5: ANOVA test results.

Based on Table 5 it can be seen that the P-value generated for the 24-hour immersion posttest data is greater than 0.05. This means that “the type of treatment given is immersion in juice. Local strawberries, carbamide peroxide and imported strawberry juice did not give a significant difference to the

Whereas in Table 4 it can be seen that in immersion with carbamide peroxide, both after immersion for 24 hours, 48 hours and 72 hours, the resulting p-value is less than 0.05, this means that there is a significant difference between Light (L) before and after immersion with carbamide peroxide both at 24 hours, 48 hours and 72 hours. So it can be concluded that “carbamide peroxide has a significant effect on the color of Light (L) on the teeth both after soaking for 24 hours, 48 hours and 72 hours”.

ANOVA Test

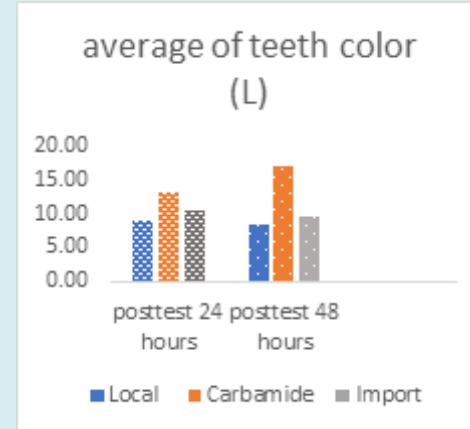


Figure 6: The average value of tooth color after soaking with local strawberry juice, imported strawberries and carbamide peroxide.

Based on Figure 6 it can be seen that there is a gap or quite a difference in the color of the teeth between after being soaked with local strawberry juice, imported strawberries and carbamide peroxide. To see the significance of these differences, a comparison test was carried out with one way ANOVA.

color of the teeth at 24 hours immersion.

Meanwhile, the P-value generated for the 48-hour immersion posttest data is less than 0.05. This means that “the type of treatment given, i.e. immersion with local strawberry

juice, carbamide peroxide and imported strawberry juice, produced tooth color that was significantly different from 48-hour immersion".

Because the results of the ANOVA test show that the type of treatment given, namely immersion with local strawberry juice, carbamide peroxide and imported strawberry juice, produces a significantly different tooth color from the 48-hour immersion, the analysis is continued with a posthoc test to find out more precisely which type of treatment which are significantly different from each other Table 6.

		P-values
Posttest 48 hours	Local vs Carbamide	0.000*
	Local vs Import	0.736
	Carbamide vs Import	0.001*

Table 6: 48 hour immersion posthoc test results.

P-values followed by a sign (*) indicates a significant difference. Based on the posthoc test, it was found that the color of the teeth soaked in carbamide peroxide for 48 hours differed significantly from the color of the teeth soaked in local strawberry juice or imported strawberry juice. However, it was also known that there was no significant difference in the color of teeth soaked in local strawberry juice and the color of teeth soaked in imported strawberry juice for 48 hours. That is, the effect of soaking with local strawberry juice and imported strawberry juice for 48 hours on tooth color is the same, but the effect is different from soaking with carbamide peroxide.

Discussion

This study aims to determine the potential of the acid present in local strawberries and imported strawberries. The local strawberries used were Holibert strawberries originating from Garut and Ciwidey Bandung, Indonesia which had been examined for the malic acid content contained in these fruits of 0.15% and the imported strawberries used were strawberries with the Evergood brand originating from South Korea with a malic acid content of 0.0885%. This study used 35% carbamide peroxide bleach.

If you look at the average data in this study for the local strawberry juice group, if you compare the pretest and posttest at 24 hours, there is a difference of 0.78, while at 48 hours there is a difference of 0.22. The imported strawberry juice group when comparing the pretest and posttest at 24 hours there was a difference of 1.32 while at 48 hours of immersion there was difference of 0.33. The 35% carbamide peroxide group when comparing the pretest and posttest at 24 hours there was a difference of 4.03 while at 48 hours of

immersion there was a difference of 7.83. If seen from the difference in the average of the data, the immersion group with significant results occurred in the 35% carbamide peroxide group. The group of local strawberries and imported strawberries did not have a significant difference, but when compared through the difference in the average group data, imported strawberries were higher than the local strawberry group.

When compared through data from the average value of these groups, the imported strawberry juice group had a superior effect on teeth whitening results compared to the local strawberry juice group, where the malic acid content in the imported strawberry juice group was lower than the local strawberry juice group with a difference of 0.0615%. This is likely to occur due to the presence of other acids in imported strawberries, thus increasing the potential for teeth whitening, namely the ellagic acid content. Research by Selva Mutkumaran, et al. [20].

In Senthilkumar's research, et al. in 2021 stated that the average value obtained by using carbamide peroxide was more significant than strawberry extract ($p < 0.01$). In comparison, the carbamide peroxide group was statistically more significant than strawberry extract ($p < 0.01$) [21].

The results of this study are in accordance with the results of research conducted by Senthilkumar, et al. namely "the comparison in the carbamide peroxide group statistically has a significant effect compared to the strawberry fruit extract" but the difference is the soaking time of the samples. Radhakrishnan, et al. namely the use of hydrogen peroxide bleaching agent, while in this study carbamide peroxide was used. Another thing that distinguishes it is the acid content in the strawberries used as research material. It is proven that local strawberries originating from Indonesia, the holibert type, have a malic acid content of 0.15% and imported strawberries originating from South Korea, the evergood brand, have an acid content of 0.15%. 0.0885% while the strawberries used in the study by Radhakrishnan, et al. [21,22].

Conclusion and Recommendation

Strawberry fruit juice extract with malic acid content in it of 0.15% in local strawberry juice and 0.0885% in imported strawberry fruit juice has the potential to whiten discolored teeth but not significant ($p > 0.05$). Imported strawberries have a higher potential to whiten teeth compared to local strawberries.

Further research is needed to determine the ellagic acid content in local and imported strawberries at the Saraswanti Indo Genetech Laboratory, Bogor, West Java, Indonesia.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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