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Effect of soaking strawberry fruit extract (*Fragaria x ananassa*) on the surface hardness of tooth enamel

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Abstract

Background The use of chemical-based tooth whitening agents such as hydrogen peroxide and carbamide peroxide has been reported to decrease enamel hardness. Therefore, natural-based alternatives that are safer and more biocompatible are needed. One such alternative is strawberry (*Fragaria x ananassa*), which contains ellagic acid and malic acid that are believed to have natural whitening effects. However, the effect of strawberry extract on enamel surface hardness remains underexplored.

Objective: This study aims to examine the effect of soaking bovine incisors in strawberry extract at concentrations of 50%, 75%, and 100% on enamel surface hardness.

Research Method: This study employed a post-test only control group design using a laboratory experimental approach. Twenty-four bovine mandibular incisors were randomly divided into four groups: 50%, 75%, and 100% strawberry extract, and a negative control group (aquadest). The specimens were soaked for 14 days, 8 hours per day. Enamel surface hardness was measured using the Micro Vickers Hardness Tester (MVHT) and expressed in Vickers Hardness Number (VHN).

Results: The LSD test indicated that the 100% extract group had a significant difference compared to all other groups ($p<0.05$), while the 50% and 75% groups did not significantly differ from the control.

Conclusion: Soaking in Strawberry extract has an effect on enamel surface hardness. Higher concentrations of Strawberry extract are associated with a greater potential for enamel demineralization.

Keywords: Bovine incisors; Enamel hardness; *Fragaria x ananassa*; Micro Vickers Hardness Tester; Strawberry extract

1. Introduction

Teeth are one of the important aesthetic factors, which must be considered especially in tooth color. Teeth that experience discoloration can reduce a person's self-confidence (Ulliana et.al., 2020). The popularity of using natural ingredients today has many advantages over chemicals, including being safer, more comfortable, cheaper, and easier to obtain. Several researchers have stated that surrounding plants can be used as natural ingredients for teeth whitening.

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One of the fruits that can play a role in whitening teeth is Strawberry (*Fragaria x ananassa*) (Yunita et.al., 2016; Hamrun et al., 2023).

This study will use ethanol extract of Strawberry fruit obtained by maceration with 96% ethanol solvent. The resulting thick Strawberry fruit extract will be dissolved with distilled water, into several concentrations, namely 50%, 75%, and 100%. The selection of the dilution concentration is based on previous studies using variations in these concentrations, so researchers want to continue previous research with different tests (Simamora, 2022).

Based on the background above, Strawberry fruit is widely used in dentistry, especially in the field of conservation to whiten teeth. However, the acid content in Strawberry fruit can also have an impact on the protection of tooth enamel, in maintaining its hardness. So researchers are interested in further researching the effect of Strawberry fruit extract on tooth enamel hardness.

The formulation of the problem in this study is How is the effect of soaking Strawberry fruit extract (*Fragaria x ananassa*) at concentrations of 50%, 75%, and 100% on reducing the hardness of tooth enamel surfaces. The purpose of this study was to determine the effect of soaking with distilled water and Strawberry fruit extract (*Fragaria x ananassa*) at concentrations of 50%, 75%, and 100% on reducing the hardness of tooth enamel surfaces. The benefits of the results of this study are expected to be a scientific contribution in Dentistry education, especially in the field of conservation science in terms of providing information on the effective time to use Strawberry fruit extract (*Fragaria x ananassa*), with concentrations of 50%, 75%, and 100% on reducing the hardness of tooth enamel surfaces.

2. Material and methods

This type of research uses true experimental laboratories designed with the posttest with control group method, to determine the effect of the experimental group after being given treatment. The location of this research was carried out in several places, namely the manufacture of extracts was carried out at the UPT Herbal Materia Medica Batu Laboratory. Sample application and sample storage were carried out in an incubator, at the Sterile Laboratory of the Faculty of Pharmacy, Bhakti Wiyata Kediri Health Sciences Institute. The hardness test of the samples used the Micro Vicker Hardness Taster tool, which was carried out at the Materials Laboratory of the Mechanical Engineering Department, Malang State Polytechnic. The time of this research was carried out in February - May 2025. The population in this study were bovine incisors (bovine incisive teeth) obtained from the UPTD Kediri City Slaughterhouse. Based on the calculation of the Fedeerer formula, the minimum number of samples was 6, for each treatment group. The total number of samples needed is 6 bovine incisors per group x 4 treatment groups = 24 bovine incisor samples, for the treatment group. Sample Criteria include Sample Inclusion Criteria, namely samples using extracted bovine incisors, caries-free bovine incisors, intact bovine incisor crowns, extracted bovine incisors without any damage, bovine incisors have never been bleached Sample Exclusion Criteria include: bovine incisors have caries, bovine incisor crowns are not intact, bovine incisors have been bleached, there are anomalies. Strawberry Inclusion Criteria include: Strawberries of the same ripeness level, California variety Strawberries, bright red Strawberries, have a sour taste, the color of the leaves is still green, Strawberries are not wilted. Strawberry Exclusion Criteria include: Strawberries are not ripe the same, Strawberries are small in size, Strawberries have wilted, Strawberries leaves have changed color and are no longer green. The sampling technique in this study used the Simple Random Sampling method.

2.1. Materials and Tools

The tools used in this study include: Measuring cup, Closed media, Dental tweezers, Incubator, Micromotor, Straight hand piece, Diamond disc, Micro Vickers Hardness taster, Digital scales, Knife, Blender, Autoclave, Petri dish, Rotary evaporator, Stirring rod, Maceration vessel, Spirit lamp, Scalpel, Test tube, Enlenmeyer, Blender, Sieve, Toothbrush, Stationery. The materials used include: Strawberry fruit (*Fragaria x ananassa*), Bovine incisors (cow incisors) as many as 24, Saline solution, 96% Ethanol, Dental Stone (dental blue plaster), Aquadest, Water, Tissue, Mask, Handscoon, Nail polish.

2.2. Working Procedures

In this work procedure, there are several stages. First of all, the preparation stage for making media includes: researchers take care of a permit to conduct research to the laboratory, introduce themselves, convey the intent and purpose of the research to be conducted, sterilize the tools to be used, by washing the tools using clean water. flowing and then dried, after that sterilized with an autoclave at a temperature of 121o C pressure of 2 atm for 20 minutes, preparing closed media that will be used for the container of cattle incisors that will be given different treatments by labeling each media, preparing samples to be used, namely cattle incisors that have been cut and in accordance with the inclusion and exclusion criteria obtained from the UPTD Kediri City Slaughterhouse. The next step is the procedure for

extracting cattle teeth, including : Selecting cattle aged 2-3 years, the head of the cattle that has been slaughtered, the jaw is separated first, then finger fixation is carried out for the lower jaw teeth of the cattle with the position of the index finger on the labial part, the middle finger on the lingual, the thumb fixes the mandible, after that the lower jaw incisor teeth of the cattle can be removed using cutting pliers, with the position of the crown of the cattle incisor teeth coated using a tampon first to prevent scratches or damage to the crown of the cattle incisor teeth, then positioning the beak axis in line with the tooth axis, the beak of the cutting pliers grips the root area of the tooth (do not grip the crown of the tooth), the beak is pressed as much as possible, and when moving the cattle teeth do it with controlled strength and direction, after the cattle incisor teeth are removed, the roots of the cattle incisors are cut to the cervical limit of the teeth using a diamond disc, then the teeth are cleaned with a toothbrush and the sharp parts are smoothed using a scalpel under running water, the cleaned cattle incisors can be stored in a closed container containing saline solution. The second stage is the Strawberry Fruit Extract Making Procedure using the maceration technique in the following manner: the extraction process begins with sorting and washing fresh California Strawberries that have been harvested using clean running water, the California Strawberries needed in this study are 1 kg, after that the cutting process, the Strawberries are cut thinly using a knife into several parts, with a thickness of approximately 0.5 mm, then dried in the sun until it feels completely dry, the dried Strawberries are then put into a blender, and ground into a powder simplicia from Strawberry fruit, after that the powder simplicia is sieved with a 100 mesh sieve, until it reaches a fine degree of powder simplicia, then prepare 1000 grams of Strawberry fruit simplicia powder, put into a 1000 ml Erlenmeyer flask. After that, 96% ethanol liquid was added and then stirred using a magnetic stirrer, approximately 30 minutes evenly, then left for 3 times 24 hours and stirred every day, after that it was filtered using filter paper until the residue and filtrate were obtained, the filtrate was evaporated using a rotary evaporator at a speed of 45 rpm at a temperature of 50 ℃, so that a thick Strawberry fruit extract was obtained and had a reddish brown color, then to obtain Strawberry fruit extract with a concentration of 50%, 75%, and 100%, a dilution process using distilled water can be carried out. The last is the Research Stage, including: after the extraction process is complete, the researcher prepares aquadest as a negative control group (concentration 0%) and Strawberry fruit extract with concentrations of 50%, 75%, and 100%, then the cow's incisors are put into a closed container that has been given a sequential number based on the treatment group, as follows: Containers with serial numbers 1-6, the entire surface of the cow's incisor crown is soaked using Strawberry fruit extract with a concentration of 100%, Containers with serial numbers 7-12 the entire surface of the cow's incisor crown is soaked using strawberry fruit extract Strawberry with a concentration of 75%, Container number 13-18 the entire surface of the crown of the cow's incisor teeth is soaked using Strawberry fruit extract with a concentration of 50%, Container number 19-24 the entire surface of the crown of the cow's incisor teeth is soaked with distilled water. Then the samples that have been given Strawberry fruit extract treatment will be rinsed using clean running water and dried using tissue, then stored in an incubator with a temperature of 37 ℃ until the next treatment time, the procedure for giving treatment to these teeth can be done every 8 hours per day for 14 days based on research by basting, et.al, 2003. After that, each treatment of each group can be measured using the Micro Vickers Hardness tester. Then the results of these measurements can be compared between the samples of the aquadest immersion treatment group and the samples of the Strawberry fruit extract immersion treatment group with concentrations of 100%, 75%, and 50%.

2.3. Ethical Clearance

Ethical Clearance has been obtained from the research ethics commission of the Bhakti Wiyata Kediri Health Sciences Institute with the number: 404/FKG/EP/IV/2025.

2.4. Data Analysis

Data analysis is presented in the form of tables, diagrams, and images to show the amount of data in each category. This data processing is done using the Statistical Package for the Social Science (SPSS). The data obtained is tested for normality first, namely using the Shapiro Wilk test, if the data is normally distributed, it can be continued by conducting a homogeneity test with the Levene's test. If the data is not normally distributed, the Chi-square test can be used. Then, if normal and homogeneous data are obtained, it can be continued with the One-Way ANOVA test, which is used to compare the averages of three or more groups in a study. Then the next data analysis can be tested with the Post-Hoc test in the form of Least Significance Difference (LSD) which aims to determine a significant difference in each group. If the data is not normally distributed, then a non-parametric test can be used in the form of Mann-Whiteney for independent data and the Wilcoxon test for dependent data.

2.4.1. Normality Test

The normality test aims to determine whether the data is normally distributed or not. In this study, the normality test used Shapiro-Wilk, because the number of samples in this study was less than 50, with the criteria that if the significance value or p-value> 0.05 then the data is declared normal.

2.4.2. Homogeneity Test

The homogeneity test aims to determine whether the existing data has a homogeneous variety or not. In this study, the homogeneity test uses Levene's Test.

2.4.3. One-Way ANOVA Test

The One-Way ANOVA test is used to compare the means of three or more different groups of data based on one independent variable, which aims to determine whether there are significant differences between the groups.

2.4.4. Post-Hoc Test in the form of Least Significance Difference (LSD)

Post-Hoc Test in the form of Least Significance Difference (LSD) which aims to determine a significant difference in each group.

3. Results and discussion

This study was conducted to determine the effect of soaking Strawberry fruit extract on tooth enamel hardness. The manufacture of Strawberry fruit extract was carried out at the UPT Herbal Medica Batu Laboratory using the Maceration method, then for tooth soaking it was carried out in the Sterile Laboratory Incubator of the Faculty of Pharmacy, Bhakti Wiyata Kediri Health Sciences Institute, and tooth enamel hardness testing using the Micro Vicker Hardness Taster tool was carried out at the Materials Laboratory of the Department of Mechanical Engineering, Malang State Polytechnic. Based on the results of the study, the data obtained from the hardness test of cow incisor tooth enamel are presented in table V.1 below

Tabel 1 Enamel Hardness Test Result Data

Tooth Enamel Hardness (VHN) Test Results				
Replication	*Aquadest*	50%	75%	100%
1	347.07	334	330.93	247.77
2	336.1	314.5	294.1	249.1
3	305.2	325.63	307.8	244.57
4	344.23	357.13	316.93	242.87
5	355.27	338.17	336.73	238.87
6	362.3	293.6	346.17	243.53
Rerata	341.70	327.17	322.10	244.05
Standard Deviation	20.04	21.69	19.42	3.65

Based on the table above, it can be seen that group 4 as the treatment group of 100% concentration Strawberry fruit extract has the lowest average value of tooth enamel hardness, which is 244.05 VHN. While in group 1 as the negative control group (aquadest) has a hardness value of 341.70 VHN. This shows that Strawberry fruit extract with a concentration of 100% has a significant decrease in tooth enamel hardness.

3.1. Normality Test

Tabel 2 Shapiro-Wilk Normality Test Results

Group	***Shapiro-Wilk Test***
	p-value
Negative control (Aquadest)	0.337
Treatment of strawberry fruit extract with 50% concentration	0.983
Treatment of strawberry fruit extract with 75% concentration	0.912
Treatment of 100% concentration strawberry fruit extract	0.842

Based on the table above, it can be seen that the results of the normality test using Shapiro-Wilk show a significant value from each group, namely p-value> 0.05. It can be concluded that the data from all groups are normally distributed, so it can be continued with the next test, namely the homogeneity test using Levene's Test.

3.2. Homogeneity Test

Tabel 3 Levene's Test Homogeneity Test Results

Levene's Statistic	df1	df2	Sig.
2.340	3	20	0.104

Based on the table above, it can be seen that the homogeneity test uses Levene's Tset which shows a significance value or p-value of 0.104. This shows that the data has a homogeneous distribution, because the p-value is > 0.05.

Statistical tests can be carried out using the One-Way ANOVA parametric statistical test, because the requirements for normal data and homogeneous data have been met.

3.3. One-Way ANOVA Test

Tabel 4 One-Way ANOVA Test Results

	Sum of Square	Df	Mean Square	F	Sig.
Between Groups	34412.392	3	11470.797	36.321	0.000
Within Groups	6316.307	20	315.815		
Total	40728.698	23			

Based on the table above, it can be seen that the results of the One-Way ANOVA statistical test show a p-value = 0.000, where the p-value is <0.05 so it can be concluded that there is a significant difference between each group. Furthermore, a follow-up test in the form of Post-Hoc LSD can be carried out, which aims to find out more specifically which groups show significant differences.

3.4. Post-Hoc Test is Least Significance Difference (LSD)

Tabel 5 Least Significance Difference (LSD) Post-Hoc Test Results

Group	K (-)	50%	75%	100%
K (-)	-	0.172	0.071	0.000*
50%	0.172	-	0.627	0.000*
75%	0.071	0.627	-	0.000*
100%	0.000*	0.000*	0000*	-

Note: An asterisk (*) indicates groups that have significant differences.

Based on the table above, it can be seen that the p-value that has been marked with an asterisk in the Post-Hoc LSD test is a p-value <0.05, so that in the compared groups there is a significant difference. While the p-value that is not marked has a p-value> 0.05, so that in the compared groups there is no significant difference or the same.

In the negative control group (aquadest) there was a significant difference in hardness value compared to the 100% concentration Strawberry fruit extract treatment group. However, it was not significantly different when compared to the 50% concentration Strawberry fruit extract group or the 75% concentration Strawberry fruit extract group. These results indicate that 100% concentration Strawberry fruit extract in its use as a tooth whitener provides the most significant side effect of reducing hardness. This is indicated by a smaller hardness value compared to the negative control which was only given aquadest.

There was no significant difference between the negative control group and the treatment group of Strawberry fruit extract with a concentration of 50% or Strawberry fruit extract with a concentration of 75%. This shows that in the treatment group of Strawberry fruit extract with both concentrations, the side effect of decreasing hardness was not too great, which was caused by the low concentration used so that the hardness decrease value was still the same as the control group which was only given distilled water. The significance value of the Strawberry fruit extract group with a concentration of 50% when compared to the distilled water group was 0.172, where this value was greater when compared to the significance of the 75% extract when compared to distilled water, which was 0.071. These results indicate that Strawberry fruit extract with a concentration of 75% gave a greater side effect of decreasing hardness compared to the 50% extract. Where the higher the concentration of extract used, the greater the side effect of decreasing hardness caused.

This study aims to determine the effect of soaking Strawberry fruit extract (*Fragaria x ananassa*) on the enamel hardness of lower jaw cattle incisors. This study used four treatment groups, namely Strawberry fruit extract with concentrations of 100%, 75%, and 50%, negative control (aquadest). Measurement of tooth enamel hardness was carried out using the Micro Vickers Hardness Tester (MVHT) and the results were expressed in Vickers Hardness Number (VHN) units. The results of the Shapiro-Wilk test obtained a p value> 0.05 in all treatment groups, which means that the data was normally distributed. The results of the homogeneity test using the Levene Test showed a p value = 0.104 (> 0.05), which means that the data has a homogeneous variance. The results of the ANOVA test showed a significance value of 0.000 (p <0.05), which means that there was a significant difference between groups. The analysis was continued with the post hoc Least Significant Difference (LSD) test to determine which group had a significant difference.

The decrease in enamel hardness value along with the increase in the concentration of Strawberry fruit extract. The group soaked in 100% Strawberry extract showed the lowest enamel hardness value, which was 244.46 VHN. The results of the LSD test showed that there was a significant difference between the 100% group and all other groups (p <0.05). Nuerhaeni (2017) stated that the decrease in enamel hardness was caused by the organic acid content in Strawberry fruit such as malic acid and ellagic acid. According to Fredericks J. (2015) in Karmawati et al. (2015), malic acid has the ability to erode and remove some stains on the surface of tooth enamel. According to Marcella et al. (2014) stated that malic acid can reduce the tooth enamel layer, causing irreversible tooth decomposition or commonly called Enamel Erosion.

Ellic acid has the ability to break down chromogens, namely dyes that adhere to the surface of tooth enamel through an oxidation process, resulting in changes in enamel color. This acid also contributes to tooth erosion (Juanita, 2017). This study is in line with Hamrun's (2023) research which states that malic acid is involved in the process of tooth erosion. This acid can bind calcium to tooth enamel which causes the release of the main components of hydroxyapatite crystals (demineralization). Inorganic minerals in the form of calcium and phosphate can cause porosity crystals that affect tooth erosion and reduce the hardness of the tooth enamel surface. Demineralization that occurs continuously can cause the formation of small pores or porosity on the surface of the tooth enamel, so that there can be a decrease in the hardness and integrity of the tooth enamel (Pambudi, 2009; Wahyuni et al., 2022). This is supported by Hartanto et al. (2012), which states that the application of Strawberry paste for more than two weeks causes a significant decrease in enamel hardness.

In the 75% strawberry extract group, the average enamel hardness was 297.52 VHN. The results of the LSD test showed that the difference with the control group was not statistically significant (p = 0.071), although there was a tendency for a decrease in the hardness value. Firdausi (2020) explained that strawberry extract at a concentration of 75% still has an effect that can reduce hardness, but its impact on enamel hardness is not as high as a concentration of 100%. Simamora et al. (2022) stated that 75% Strawberry extract was able to fade denture discoloration due to tea, although not as strong as concentrations of 85% and 95%. The 75% concentration still has a mild abrasive effect, but does not cause significant damage to the microscopic structure of the enamel.

The 50% strawberry extract group had an average enamel hardness of 320.34 VHN and showed no significant difference with the negative control group (p = 0.172). This indicates that the 50% concentration is not strong enough to cause enamel demineralization. This finding is supported by the study of Firdausi (2020) which stated that low concentration strawberry extract has a minimal abrasive effect on enamel. In addition, Karmawati et al. (2024) stated that the use of strawberry gel with a concentration of 50% is more brightening than damaging, and does not cause a significant difference in enamel hardness when compared to the control material. Similar research by Amelia (2022) also showed that exposure of enamel to natural ingredients with a relatively higher pH and low acid concentration did not affect the morphological structure or microcomposition of the enamel. It can be concluded that a 50% concentration of strawberry

extract is still within safe limits for tooth enamel, and can be considered as an alternative natural tooth whitening ingredient with minimal risk to the hardness of the tooth surface structure.

The negative control group (aquadest) had the highest enamel hardness value, which was 341.70 VHN. Aquadest is pure water obtained from sterile distillation by distillation. Aquadest consists of one group, namely water molecules (H2O) which have a neutral compound content, so it is often used as a solvent or other mixed ingredients (Khotimah et al. 2018). Aquadest does not contain active substances or organic acids, so it does not affect the integrity of the enamel structure. This shows that soaking without acid or other active ingredients does not affect the enamel mineral structure. Amelia (2022) stated that aquadest can be used as a negative control because it does not cause changes in the enamel microstructure in in vitro conditions.

Overall, the results of the study showed that there was an inverse relationship between the concentration of strawberry extract and tooth enamel hardness. The higher the concentration of the extract, the lower the enamel hardness. These results are consistent with Mahardini (2016), Hartanto et al. (2012), and Firdausi (2020), who concluded that exposure to organic acids from strawberries can significantly reduce enamel hardness, especially at high concentrations and long exposure durations.

4. Conclusion

Based on the results of the research that has been conducted, it can be concluded that soaking in 100% concentration of Strawberry fruit extract has a significant effect on reducing the hardness of the tooth enamel surface, while soaking in 50% and 75% concentration of Strawberry fruit extract does not show much difference with the aquadest control.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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