



Color Changes Of Discolored Human Teeth After Immersion In Spinach Extract In Different Durations

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Abstract

Introduction: Recently, natural dental bleaching agents are being developed to replace chemical dental bleaching agents. Chemical dental bleaching agents containing hydrogen peroxide have the potential to cause toxicity as a side effect. Spinach leaves are natural ingredients that contain oxalic acid which can whiten teeth. **Purpose:** To investigate the color changes in discolored human teeth after immersion in spinach extract in different durations. **Methods:** 30 human premolar samples were divided into 3 groups, group A (immersion in 28 hours), group B (immersion in 42 hours), and group C (immersion in 56 hours). All 30 samples were discolored with coffee solution for 12 days and color status (L_1, a_1, b_1) were measured using the chromameter. Then, each 10 samples were immersed in spinach extract according to their treatment group, and the color status (L_2, a_2, b_2) were measured again. Color changes (ΔE) were calculated with a formula concerning L, a, b data measured. **Results:** A significant color change is observed between group A, group B, and group C (p-value= 0.002), and further comparison between three groups showed that group A differed significantly with group C (p-value=0.000), group B also showed significant difference with group C (p-value=0.038). However, there was no significant difference between group A and group B (p-value=0.121). **Conclusion:** The significant color changes can only be observed after 56 hours of immersion. The spinach leaf extract has the potential to be used as a natural dental bleaching agent.

Keywords: Color change; spinach extract; discolored human teeth

Introduction

Changes in teeth color could cause lower self-esteem and could have a psychological impact on patients.¹ Alterations in teeth color have two main factors, extrinsic factor such as food and beverage stains, and intrinsic factor such as dental caries.^{2,3} During the last two decades, dental bleaching has been one of the most popular dental esthetic improvement. There are several dental bleaching methods practiced, one of which is home bleaching.^{4,5} It is more widely preferred because patients find it more convenient and it doesn't require an expensive cost.⁶

Chemical dental bleaching agents generally contain hydrogen peroxide which can whiten teeth effectively so they have been widely used in recent times. However, it turns out that the hydrogen peroxide used and produced from these chemical teeth whitening agents can cause



adverse effects such as tooth sensitivity and gingival irritation during and after use. Even high levels of hydrogen peroxide can produce local oral toxicity after prolonged exposure if mishandled.⁷ This is the reason that many studies have been developed to create dental bleaching products made from natural ingredients so that they can be used as an alternative to chemical dental bleaching agents.

One of the alternatives considered for use as a natural bleaching agent is spinach leaves. Spinach (*Amaranthus hybridus L*) is vegetable most widely consumed.⁸ Spinach is known to possess the highest level of oxalic acid compared to other plants. High levels of oxalic acid could be located at the leaves of the spinach plant (around 39%).^{9,10} The whitening reaction conducted by oxalic acid is called oxidation. In this reaction, electrons are released against the organic molecules called chromophores. These electrons would bind with three C-tertiary molecules in the chromophores. These new bonds would cause an electron disturbance in the chromophores and produce a brighter structure.^{11,12}

Based on the universal home bleaching durations of 2-3 hours per day for 2-4 weeks¹³, we chose 28 hours (2 hours per day for 2 weeks), 42 hours (2 hours per day for 3 weeks), and 56 hours (2 hours per day for 4 weeks) as the immersion durations. The aim of this study was to investigate the color changes of discolored human teeth after immersion in spinach extract in different durations.

Methods

Preparation Sample

The study was an experimental laboratory research with a pretest-posttest group design. The population of the study was human premolar teeth (first and second premolars, maxillary and mandibular premolars) extracted for orthodontic needs in Medan, Indonesia. Sampling criteria were: intact crown and root; free of caries and restoration; and less than 3 months post-extraction. The final samples consisted of 30 premolars, randomly chosen for each treatment group. All participants who agreed to give away their extracted premolars have been informed about the study and have signed informed consent. The study has been approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Sumatera Utara, Medan-Indonesia.



First, the samples were prepared by cleaning the crowns using a brush bur and abrasive paste. The roots of the premolars were covered with clear nail polish to prevent pigments from diffusing through the dentin tubules and apical foramen. Then, all samples were labeled according to their treatment groups. There were three treatment groups: group A (28 hours immersion), group B (42 hours immersion), and group C (56 hours immersion).

Then, all samples were discolored by immersion in a coffee solution by dissolving 300 grams of coffee powder (Kapal Api©, Indonesia) in 300 mL of boiling water. Each sample was immersed in a 10 mL coffee solution for 24 hours and repeated for 12 days (Figure 1). After 12 days, the samples were rinsed under running water and dried with tissue paper. Color status (L1, a1, b1) was measured for all samples using the chromameter. (Figure 2) The L value is the brightness level, ranging from 0 (darkest) to 100 (brightest). The a value is the red-green quality of the sample, ranging from 0-100 (red) and 0- -80 (green). The b value is the yellow-blue quality of the sample, ranging from 0-70 (yellow) and 0- -80 (blue).

Extraction Procedure

The spinach extract was made through a maceration process. Approximately 7.5 kg of spinach leaves were washed and separated from debris, then were put inside a drying cabinet with a temperature of 40-50°C for 1 week (Figure 3a). The dried leaves were crushed into powder with an electric blender (Figure 3b). The maceration process was started by dissolving 1.5 kg of the powder and 15 L of ethanol 70% inside a closed container, and the mixture was left overnight.

The mixture was transferred to a percolator bottle, and at the bottom of the bottle, an IV-drip was set up to allow the liquid to filter through (Figure 3c). This process was repeated by adding 7.5 L of 70% ethanol into the mixture. Then a water-bath system was installed to evaporate the rest of the solvent to produce a concentrated spinach extract (Figure 3d), and then the pH was measured.

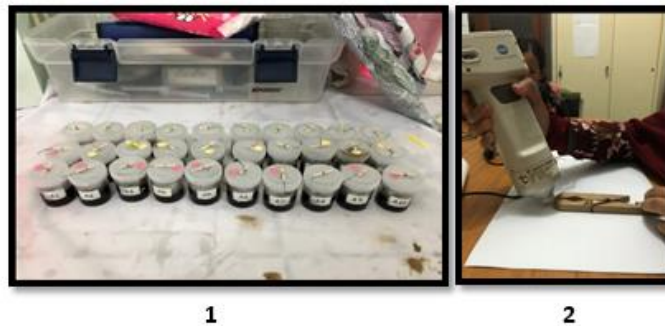


Figure 1. The samples were immersed in coffee solution for 12 days; 2. The color status for each sample was measured using the chromameter.

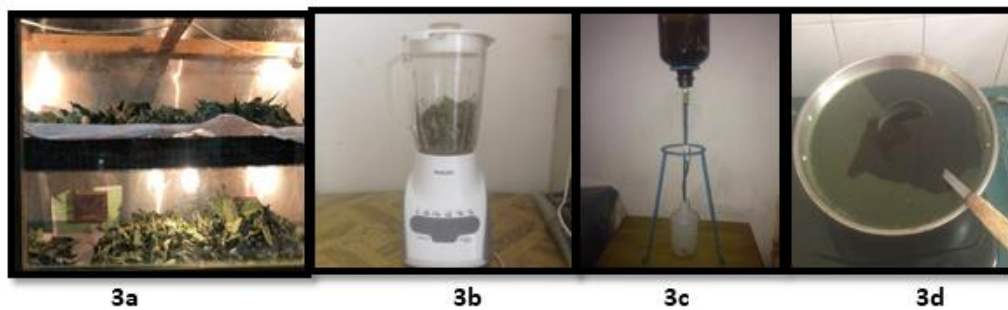


Figure 3a. The spinach leaves were put inside a drying cabinet of temperature 40-50°C for 1 week; **3b.** The spinach leaves were made into powder with an electric blender; **3c.** The mixture in a percolator bottle, and at the bottom of the bottle, an IV-drip was set up to allow the liquid to filter through; **3d.** A water-bath system was installed to evaporate the rest of the 70% ethanol solvent, and a thick spinach extract was produced.

Immersion Procedure

The samples were immersed in the thick spinach extract according to their treatment groups, group A (28 hours), group B (42 hours) and group C (56 hours). The samples were immersed for two hours per day for 2, 3, and 4 weeks for group A, group B, and group C, respectively. After every immersion, the samples were rinsed under running water and dried with a tissue paper.

Color Measurement

The color status (L_2 , a_2 , b_2) of the samples were measured again. Color changes (ΔE) were calculated with the formula $\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$, whereas ΔL is $L_2 - L_1$, Δa is $a_2 - a_1$, and Δb is $b_2 - b_1$. L_1 , a_1 and b_1 values were obtained at the first measurement after immersion in coffee



solution, and L_2 , a_2 and b_2 values were obtained at the second measurement after immersion in spinach extract.

Statistical Analysis

The statistical tests used in this study were: Kruskal Wallis test to determine the significance of color changes between group A, group B and group C; Mann-Whitney test to determine further comparison between group A, group B and group C; Wilcoxon test to determine the significance between the brightness value before (L_1) and after (L_2) immersion in spinach extract in each group. All statistical analyses were done using SPSS 25.0 software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.).

Results

There were a total of 30 samples observed in the study, and color changes (ΔE) data for 30 samples were obtained. The comparison between three treatment groups (group A, group B and group C) were tested using the Kruskal Wallis test. Further comparisons of color changes within the three treatment groups were done by the Mann-Whitney test. The comparison of brightness value before (L_1) and after (L_2) immersion in spinach extract (pH=6) in each treatment group was done using the Wilcoxon test.

As shown in Table 1, the mean and SD value of color changes (ΔE) of group A, group B and group C respectively were $3,38 \pm 1,57$; $5,44 \pm 2,53$; and $8,63 \pm 2,25$. The Kruskal Wallis test revealed that there were significant differences in color changes between group A, group B, and group C, with a p-value 0.002 ($p < 0.05$). Results are shown in Table 2.

Table 1. Mean and SD values of L, a, b before and after immersion in spinach extract and color changes (ΔE) of group A, group B and group C

Treatment Groups	Mean Value of L, a, b						ΔE
	L_1	L_2	a_1	a_2	b_1	b_2	
Group A	49.38 ± 3.89	50.01 ± 4.08	2.25 ± 0.63	2.60 ± 0.37	5.21 ± 2.31	4.13 ± 1.69	3.38 ± 1.57
Group B	50.30 ± 3.89	52.49 ± 4.79	2.45 ± 0.41	2.08 ± 0.38	2.54 ± 2.15	6.24 ± 2.45	5.44 ± 2.53
Group C	49.60 ± 5.30	56.23 ± 4.31	2.47 ± 0.41	2.01 ± 0.30	2.29 ± 2.18	5.23 ± 3.73	8.63 ± 2.25



Table 2. Analysis using the Kruskal Wallis test, between the color changes (ΔE) of group A, group B and group C

Treatment Groups	n	Mean	SD	p-value
Group A	10	3,387	1,575	0.002*
Group B	10	5,441	2,537	
Group C	10	8,639	2,256	

*significant ($p < 0.05$), n: number of samples

As shown in Table 3, The Mann-Whitney test were used to further compare the differences of color changes between two specific groups. The results showed that there were significant differences when group A and group B were compared with group C ($p < 0.05$). However, there was no significant difference in the color changes between group A and group B ($p > 0.05$). Results are shown in Table 3.

Table 3. Further comparisons of the color changes (ΔE) between all groups

Treatment Groups	p-value
Group A vs Group B	0,121
Group A vs Group C	0,000*
Group B vs Group C	0,038*

*significant ($p < 0.05$)

Table 4. Analysis using the Wilcoxon test, between the brightness value before (L_1) and after (L_2) immersion in spinach extract in all groups

Treatment Groups	n	Before (L_1)		After (L_2)		p-value
		Mean	SD	Mean	SD	
Group A	10	49,38	3,89	50,01	4,08	0,285
Group B	10	50,30	3,89	52,49	4,79	0,074
Group C	10	49,60	5,30	56,23	4,31	0,007*

*significant ($p < 0.05$), n: number of samples

The Wilcoxon test was done to compare the brightness value before (L_1) and after (L_2) immersion in spinach extract in all groups. As shown in Table 1, the mean and SD value for the brightness value before and after for group A, group B and group C respectively were: $49,38 \pm 3,89$ and $50,01 \pm 4,08$; $50,30 \pm 3,89$ and $52,49 \pm 4,79$; $49,60 \pm 5,30$ and $56,23 \pm 4,31$. The Wilcoxon test showed that there was a significant difference of brightness value in group



C, with p-value 0.007 ($p < 0.05$). However, there were no significant differences in brightness value of group A and group B, with respective p-value 0.285 ($p > 0.05$) and p-value 0.074 ($p > 0.05$). Results are shown in Table 4.

DISCUSSION

This study was conducted with 30 premolars extracted for orthodontic reasons, and the samples were first discolored using coffee solution. Coffee solution was used as discoloring agent because it contains tannin (tannic acid) that could deposit brown pigments onto teeth enamel.¹⁴ The samples were then immersed in spinach extract in different durations: 28 hours, 42 hours and 56 hours. The color status were measured using the chromameter and the color changes (ΔE) were calculated from the color status (L, a, b). As shown in Table 1, the mean and SD values of color changes (ΔE) increased with the increase of the immersion duration.

As shown in Table 2, there were significant differences between group A, group B and group C. The longer the duration of bleaching, the better results it will produce. The longer the bleaching ingredient comes in contact with the tooth surface, the better the ability of the bleaching ingredient to generate more free radicals and to form more bonds with the chromophore on the tooth surface.¹³

Spinach is known as a plant containing the highest oxalic acid compared to other plants. The highest content of oxalic acid can be found in the leaves, approximately 39%. Oxalic acid is a type of organic acid classified as an organic compound and does not have acidic oxides. Oxalic acid with the formula $H_2C_2O_4$ decomposes to $2H^+$ and $2CO$. It contains anions and tends to release an electron. This process of releasing electrons is called an oxidation reaction, where oxalic acid is referred to as a reducing agent. In the oxidation reaction, there is a process of releasing electrons to the chromophore organic molecules. The electron will then bind to the three molecules of tertiary C contained in the chromophore on the enamel surface. This bond will cause disruption of electron conjugation in organic molecules to produce a new brighter structure.⁹

Lumuhu et al. investigated the effects of tomato juice and apple juice on dental bleaching. They discovered that along with the increase in the duration of application of tomato and apple



juice, teeth color improved into a brighter color.¹⁵ Prastiwi et al. also investigated the effectivity of starfruit extract in teeth brightening, varying in several time durations. They used different duration in the study: 56 hours, 88 hours, and 124 hours. They concluded that the longer the bleaching agent came in contact with the teeth surface, the better effect it had on improving teeth color.¹⁶

As shown in Table 4, there were significant differences in brightness value before (L1) and after (L2) immersion in spinach extract in group C. This meant that color change in group C led to a brighter color change, with a significant increase of brightness value before and after. The increase in brightness was due to the presence of oxalic acid.^{11,12}

In this study, the pH measurement of spinach leaf extract was 6. A dental bleaching agent with a relatively neutral pH or close to pH 7 is a better bleaching agent to avoid damage caused by ingredients that are too acidic or too alkaline. Several factors, such as pH, concentration, exposure time, and frequency can contribute to the erosion of tooth enamel. This current study showed that the pH of spinach leaf extract did not cause teeth erosion.

Other natural bleaching agents that have been studied previously were strawberries and pineapples. The pH of strawberries ranges from 3.6-3.7, while the critical pH of enamel was 5.5 which may be causing enamel solubility resulting in erosion. The acidic nature of the fruit, especially those with a low pH, can easily erode the enamel surface. Spinach leaves are a natural ingredient relatively safe and effective for changing the color of teeth to become brighter.¹⁷

CONCLUSION

There is a significant difference of color changes in discolored human teeth after immersion in spinach extract in different durations (p-value=0.000), and there is a significant difference of brightness value before and after immersion in spinach extract for 56 hours (p-value=0.007). The spinach leaf extract has the potential to be used as a natural dental bleaching agents. The results of this study can be used to develop a new study to test further physical properties of spinach extract and to consider the clinical usage of spinach extract as a bleaching agent.



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Conflict of interest

All authors have none to declare.

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