The Influence of Green Tea (*Camellia sinensis L.*) Temperature on Changes in Enamel Color

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Abstract

Extrinsic discoloration can be caused by tea. Nowadays, green tea is consumed by many people. To analyze the difference in tooth discoloration due to the various concentrations, frequency of exposure, and temperature of green tea 17 samples of tooth enamel were tested. The samples were divided into control, shame control, and groups of 1%, 2%, and 3% tea concentrate exposure with 60 and 180 times frequency. Three different levels of temperature (30°C, 37°C, and 45°C) were also used. Green tea was applied 60 and 180 times, for 60 seconds, to each group. The results were measured with a photometer and converted to cyan-magenta-yellow and black colors (CMYK). The tooth enamel become darker. Increasing the concentration, frequency, and temperature all caused the tooth enamel to become darker.

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Introduction

The color of teeth naturally darken as we age.[1,2] Factors that cause these changes are the presence of secondary dentine and extrinsic staining on the tooth.[1] There are many causative factors or etiologies which result in discoloration of the teeth, as described above, and one of these etiologies is staining. Staining, based on the etiology, can be divided into two parts; extrinsic and intrinsic.[3,4] Color changes occurs in the outer layer of the tooth surface, as seen clearly in the enamel, which is referred to as extrinsic discoloration, and can be caused by direct and indirect causes.[3] Extrinsic staining can be caused by many factors, and one of these is a person's dietary habits.[4,5] Consuming soft drinks in large amounts, tea consumption, and coffee drinking habits are factors that cause discoloration on the outer surface of the tooth.[3]

Of the various beverages consumed by people, tea is the most consumed beverage in the world besides water.[6] There are various ways and traditions of drinking tea for Indonesian people. Mostly, Indonesian people consume tea after it has been brewed in hot conditions.[7] A study showed that 22% of Indonesians brew and consume tea in hot conditions.[7] Of the various types of tea, green tea has lately received much attention because of its high level of antioxidants that are suspected to cause discoloration of the teeth, or discoloration due to its tannin compounds. Ashok et al. reported that the tea plant has a high tannin content. In addition, it has also been reported that tea leaves which are soaked at high temperature leads to a sense of "tart", which is the characteristic taste of tannin.[8] Meanwhile, another study reported that tea caused teeth discoloration because of is tannin compounds.[5] There are no recent studies of the temperature effect of tea beverages on teeth discoloration. The aim of this study was to analyze the color changes of teeth after the application of green tea at different concentration levels, frequency, and temperature.

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Materials and methods

This research, in order to determine the effect of temperature on the color change or discoloration that occurs on the enamel surface after application of green tea (*Camellia sinensis L.*) was an in vitro laboratory experiment. Color change values were observed using image processing software (Adobe Photoshop) and data processing software (SPSS). The study was conducted using 17 samples of teeth that were

divided into control, shame control, and treatment groups. Tooth samples were chosen with the criteria (by using a normal tooth as a comparison) of normal enamel, free from caries on the buccal or lingual areas as well as good microstructure free from abrasion, erosion, and tooth discoloration. One of the 17 samples was chosen randomly to be used as the enamel control, with no treatment.

Another tooth was used as a shame control that was only exposed to distilled water and not exposed to a green tea solution. The remaining 15 teeth were all smoothed with sandpaper (CC-1000 and CC-1500) on the lingual or buccal surface to eliminate any rough surfaces. Windows (5 mm x 5 mm) were then made on the enamel surface by covering the rest on the tooth with white nail polish. Pictures of the teeth before and after the application of green tea solution were taken in accordance with established standards. Green tea solution was applied in advance, in variable concentrations and frequencies, by immersing the teeth in green tea solutions with concentrations of 1%, 2%, and 3% respectively, either 60 times or 180 times. Once treated, an analysis of the teeth was done to find the optimum concentration and frequency to cause tooth discoloration. These optimum variables were then used with the further variable of temperature for three different groups; 30°C, 37°C, and 45°C.

Photos were analyzed using image processing software (Adobe Photoshop). The color values obtained from the photometer were added to a color converter and converted to cyan-magenta-yellow and black (CMYK) colors. The results of the CMYK color values were processed with data processing software. Results of the enamel color, before and after the application of the green tea solutions were compared. Color values and the data obtained can be analyzed. The color values obtained from the photometer were used as the data group and tested for normality using the Kolmogorov-Smirnov test and a homogeneity test. The results indicated that most of the data sets had a normal distribution ($p > 0.05$) and were homogeneous ($p > 0.05$). Therefore, one-way analysis of variance (ANOVA) and bivariate Pearson correlation tests could be done. The analyzed results from the first experiment were used as the reference for the selection of the concentration and frequency to be used on the three different groups for the second experiment, for temperature and CMYK color value.

Results

Criteria	K value
Concentration	
Before Application	27.46±1.57
After 1% Application	25.50±2.63
After 2% Application	49.80±2.52
After 3% Application	46.40±2.21
Frequency	
Before Application	27.46±1.54
After 60 Applications	40.4±3.32
After 180 Applications	40.7±3.64
Temperature	
Before Application	28.1±1.18
After 30^0C Application	47.4±2.15
After 37^0C Application	42.4±2.51
After 45^0C Application	41.4±1.79

Table 1. Average K value of green tea application at different concentrations, frequencies, and temperatures.

Table 1 shows the color changes of the enamel after application of green tea with various concentrations. The change in the average K value is demonstrated. Before the application, the average K value was 27.46. After green tea was applied, at a concentration of 1%, the average K value changed to 25.50. When the green tea was applied at a concentration of 2%, the average K value changed to 49.80, which means the tooth enamel color became darker. After the application of green tea with a concentration of 3%, enamel color changed to 46.40. This means the discoloration of the tooth enamel became darker, even though the average K value was not greater than that for the 2% concentration. Enamel color changes can be seen from the comparison of average K value before and after application of the green tea. When this data group was by the ANOVA test, it showed that the average K value before the application of green tea and the average K value after application of a 1% concentration of green tea had no significant color change. This means there were no significant changes in the color of the enamel. However, the average K value before the application and the average K value after application of a 2% concentration of green tea were significantly different, and the color become darker. The average K value at a concentration of 3% as well as a concentration of 2% are also significantly different to the average K value before the application of green tea. The

bivariate Pearson correlation test of the average K value at the concentrations of 1%, 2%, and 3% showed a positive correlation of 0.681. This may imply that for the higher the concentration of green tea applied to the enamel; the average K value will also increase, causing the color to become darker.

Table 1 also shows the change in enamel color, which becomes darker, after the application of green tea with different frequencies. Before the application, the average K value was equal to 27.46. After the application of green tea 60 times, the average K value changed to 40.4. This means the color of the enamel turned darker. After application of green tea 180 times, the average K value also increased to 40.7 which means that the enamel color becomes darker compared to the prior application of green tea and a frequency of 60 times. The enamel color changes after the application of green tea displayed a wide range of frequencies compared to the average K value before application of the green tea. The ANOVA test result showed, that the difference in average K value before green tea application and average K value after green tea application, with a frequency of 60 times, was significant. This means, the enamel color change after an application of 60 times is significant. The same result is also shown for the average K value before application of green tea, and average K value after application of green tea, with a frequency of 180 times. The color becomes darker and the changes are significantly different. The bivariate Pearson correlation test result of the average K value, after green tea application with a frequency of 60 times and 180 times, showed a correlation of 0.46 and was positive. This correlation test result means the higher the frequency, or the more green tea applied to enamel, the more the average K value increased and the tooth discoloration become darker.

Further, Table 1 show the changes to enamel color, that is it turned darker, after application of the green tea at different temperatures. Before the application of green tea, the average K value was 28.1. After the application of green tea, with a temperature of 30°C, the average value K changed to 47.4, which means the color of the enamel turned darker. After the green tea was applied with an average temperature of 37°C, the average K value changed to 42.4. The average value K after the application of green tea with a temperature of 45 °C also turned out to be darker compared with the average K value before application, which was 41.4. The color changes of the tooth enamel can be observed from the average K value after the application of the green tea for the different groups of temperature compared to the average value K before the green tea application. The bivariate Pearson correlation test result showed a correlation of 0.52 and was positive. This correlation of the test results can be interpreted as, the higher the temperature of the green tea solution applied, the greater the average K value will be, and the discoloration of the enamel turns darker.

Discussion

The 17 tooth samples were prepared by smoothing the surface of the lingual or buccal with sandpaper (CC-1000 and CC-1500) to eliminate the rough surface. The purpose of this was to cut the curved enamel rods, on the outer surface, so that the color molecules could easily infiltrate into the micro-pore structures of the enamel crystals. In addition, a window was created on the enamel to identify the area for before and after application of green tea image shooting. The type of green tea used for this experiment was brewed green tea (*teh hijau tubruk*) that comprises pure green tea leaves. Brewed loose leaf green tea was chosen over tea-bag green tea because the brewed loose leaf green tea maintains its purity during tea manufacture, while green tea-bags are manufactured from tea processing left-overs. The difficulties encountered in this research were maintaining the camera settings, the lighting, and the positioning of the tool while taking the pictures to obtain the color values, to be analyzed with Adobe Photoshop. Besides this, the method used to apply the green tea 180 times to the samples required very high precision and concentration.

The experimental treatment was soaking tooth enamel in green tea solutions with concentrations of 1%, 2%, and 3% in repetitions of 60 and 180 times. Concentrations of 1%, 2%, and 3% were obtained by dissolving 0.5, 1, and 1.5 g of green tea, each, with 50 ml of water. The amount of green tea (in grams) was determined by the consumption of tea per capita per year in Indonesian society, as published by the

Secretariat General of the Ministry of Agriculture of the Republic of Indonesia (2015). In 2014, the tea consumption in Indonesia increased by 0.61 kg per capita per year.[9] If 0.61 kg is converted into grams, it means that the consumption of tea in Indonesia is 610 mg/year. From these results, it can be concluded that the average tea consumption by Indonesian society is about 1.671 g/day. Therefore, this experiment divided the three concentrations of green tea by 1.671 g. To compare the effect of the enamel color changes, the samples were soaked, in two groups, 60 and 180 times. The frequency of soaking for 60 times was to replicate a two month consumption of green tea assumed as a person's daily intake. This also applied to 180 times soaking, in assumption of how green tea a person consumes, daily, over six months. This experiment showed which frequency of exposure will cause color changes on tooth enamel.

The duration of one immersion was 60 seconds. It was assumed that 60 minutes was the average time each person spends drinking one cup of green tea. Exposure to the green tea, in the experiment, was followed by immersion in distilled water for 10 seconds, assuming that after every gulp, the enamel will be back-washed by saliva. The color changes were assessed using a CMYK color space model. Theoretically, cyan, magenta, and yellow can be combined to produce the color black.[10] Magenta and yellow will produce red; cyan and yellow will produce green; cyan and magenta will produce a blue color; and the combination of cyan, magenta, and yellow produces black.[10] This experiment demonstrated that the application of green tea solutions, at concentrations of 1%, 2%, and 3%, changed the color of tooth enamel. The average K value before the application was 27.46. The average K value after application of green tea solution, at a concentration of 1%, was equal to 25.50. In this experiment, the average K value decreased after application of green tea solution at a concentration of 1%. This can be explained by the theory that at a concentration of 1% the tannin contained in the green tea, and deposited onto the enamel surface, was too small to cause significant color change. The ANOVA test result showed that there were no significant changes between the different colors of enamel before the application of green tea and the color of enamel after the application of green tea with a concentration of 1%.

The average K value, before and after application of green tea with a 2% concentration, changed from 27.46 to 49.80. The ANOVA test result showed that there was a significant color change between the average K value, before application of green tea, and the average K value, after application of green tea with a 2% concentration. This means, that tannin successfully infiltrated the micro-structure tubule of the tooth enamel and caused a significant color change on the enamel when a green tea solution with a concentration of 2% applied. The average K value before and after application of green tea with a concentration of 3% changed from 27.46 to 46.40. The ANOVA test result showed there was a significant change in color between the average K value before and after the application of a green tea solution with a concentration of 3%. These results showed that tannin had successfully entered the micro-structure tubule of the tooth enamel. However, the change in the average value from the previous condition did not increase beyond a concentration of 2%. This can be explained by the theory of saturation of an object. The tannin in the green tea solution that successfully infiltrated at a concentration of 3% is not as great as in the 2% concentration because the micro-structure tubule of the tooth enamel had already been filled with tannin from the previous concentration. Therefore, the tannin cannot further infiltrate into the micro-structure of the tubule, or cannot infiltrate as much as before.

The ANOVA test result from the comparison of the color change for each concentration showed that there was a significant color change between the concentrations of 1% and 2%, and between the concentrations of 1% and 3%. However, there are no significant differences in the color changes between the concentration of 2% and 3%. The bivariate Pearson correlation test between the K values from the application of green tea solution concentrations of 1%, 2%, and 3% showed a correlation of 0.681 and was positive. From this results, it was determined that the higher the concentration of green tea solution applied to the tooth enamel, the higher the K value, which means the tooth enamel turns darker. The analysis of the experimental results from the soaking frequency of 60 and 180 times showing that there was an enamel color change after the application of green tea at both frequencies. The

average K value before the application was 27.46. The average K value after application of green tea 60 times was 40.4. This result showed that the tannin in the green tea solution when applied 60 times had successfully infiltrated into the micro-structure tubules of the enamel and had deposited there, making the enamel color turn darker. The ANOVA test result showed that there was a significant change in color between the average K value, before the application of green tea, and the average K value after the application of the green tea with a frequency of 60 times. This result also occurred after the application of the green tea with a frequency of 180 times. This means, after the samples were soaked 180 times, tannin in the green tea has infiltrates and made the enamel color darker.

The ANOVA test result for each frequency showed significant differences between the color changes from a frequency of 60 times and 180 times. The bivariate Pearson correlation test result between the K values from the frequency of 60 times and 180 times was 0.47 and was positive. These means that the more the frequent the application of green tea solution onto enamel, the higher the K value, and the color of the teeth turn darker as the application frequency increased. The results of this study showed that the discoloration of teeth tends to move towards a darker line as the green tea concentration and frequencies increased. A similar study which used different types of tea, also demonstrated color changes on the teeth after presentation to tea solution, regardless of the type of tea used.[5] Therefore, it can be shown that tea, or any kind of chemical substance that contains tannin can cause discoloration of the teeth. Another study also mentioned that among all of the tea used, red tea caused the most discoloration to the teeth, so it is advisable to reduce the frequency of drinking tea and replace the type of tea products.[5]

Some areas in Indonesia have a tradition of drinking tea in hot conditions, that is often called *Teh Nasgitel* (*Panas, Legit dan* Kental), such as in Solo, Tegal and Padang. Based on the results of research by the *Asosiasi Minuman Ringan* Indonesia, as many as 22% of Indonesians consume hot tea.[7] This proves that the tradition of drinking tea in various regions of Indonesia cannot be separated from the temperature used to brew the tea. To replicate these conditions, this research inserted variable temperatures into the application of green tea solution to determine the effect of temperature on color changes that occur on tooth enamel. Similar research suggested that teas should be made at room temperature, but people habitually drink tea under hotter conditions. Additionally, previous study showed that tea leaves of any kind brewed with hot water will increase the incidence of the "tart" taste or astringency which is a primary characteristic of tannin.[8] Therefore, this study used three distilled water temperatures to make the tea solutions, being: 30°C, 37°C, and 45°C. For this temperature experiment, the concentration of 3% was used with a frequency of 180 times in reference to the preceding result that the higher the concentration and frequency of application of green tea to enamel the darker the enamel will become. The average K value before the application was 28.1. After the application of green tea, with an average temperature of 30°C, the K value became 47.4. This may imply that the change in enamel color becomes darker when the green tea solution is applied at this temperature.

Changes also occurred in the teeth color when the green tea was applied with a temperature of 37°C, where the average K value changed to 42.4. After the application of green tea at 45°C, the average K value was 41.4. The bivariate Pearson correlation test result for the K value of the group temperature was 0.52 and was positive. This may imply that the higher the temperature used to brew the green tea, the darker the tooth color becomes. This study's results are consistent with the theory that when any kind of tea leaves are brewed in hot water, it will lead to the emergence of a sense of "tart" or sour/bitter taste that is a key characteristic of tannin.[8] This makes the color changes on tooth enamel become darker. In addition, when the temperature used to brew the green tea is hotter than room temperature, the complex molecules in tannin compounds contained in green tea can be easily broken down to the simple molecules. After decomposition of the tannin to simpler molecule, it is easier for the color to infiltrate the micro-structure tubule shaft that exists in the structure of the enamel. These two factors are the reason for the darker color changes when hot tea is consumed.

Conclusion

It can be concluded that green tea solution can change the color of tooth enamel darker with increasing concentration and frequency of exposure. The frequency and maximum concentration of green tea can cause discoloration of the enamel with different levels of temperature. It is necessary to undertake further studies with other types of tea. In addition, people need to be informed not to brew their tea under high temperatures to avoid their teeth changing color. The results of this study should be published as a source of information for further research.

Declaration of Interest

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