

Effect of Application over Time for Each Type of Blending Tea on Bovine Tooth Coloration

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Background: This study aimed to investigate the effect of selecting commercially available blending teas and applying them to bovine teeth on color change over time.

Methods: After selecting healthy bovine teeth, using a cutting-disc, 105 specimens with a dimension of 5×5×3 mm were prepared, and 15 specimens were distributed to each group. Black tea was used as a positive control, water was used as a negative control, and blended tea of five types was used as an experimental group. First, pH and buffering capacity were measured with a pH meter, and tooth color was determined using a spectrophotometer before immersion in the blending tea solution and 1, 5, 7, 14, and 21 days after immersion. Thereafter, the shape change of the enamel surface was observed using a scanning electron microscope, and SPSS ver. 26 was used to analyze the color change.

Results: The average pH of the five blending teas in the experimental group was 3.78, and the pH of group 3 (strawberry rhubarb) was the lowest at 3.22. The pH levels of black tea and water were 5.19 and 7.30, respectively. The buffering capacity was the highest in group 3 at both pH levels of 5.5 and 7.0. The L*a*b* color change according to immersion time was the largest in group 4 (rooibos yellow flower), and the amount of color change was large in black tea and group 4. As a result of observing the enamel surface of bovine teeth, changes in the surface shape were noted in all groups immersed in the experimental solution for 21 days, except for water.

Conclusion: There was a significant difference between the experimental groups in terms of color change according to the immersion time, and color and enamel surface changes were observed in black tea and all experimental groups, except for water.

Key Words: Blending tea, Bovine teeth, Color change, Titratable acidity

Introduction

As people's level of awareness increases and quality of life emerges as the most important issue owing to an increase in income, interest in health is increasing, and the demand for tea is increasing as a well-being trend. To practice health and well-being, a culture of purchasing and consuming related products and services is being established, which has resulted in changes in the lives of consumers, including living culture, lifestyle, purchasing behavior, and consumption activities¹⁾.

Quality of life can be evaluated by various factors, such as socioeconomic factors, health status, residential environment, leisure, and cultural life. In particular, health is directly related to life, so interest in and efforts to maintain health are increasing²⁾. Oral health-related factors such as dental caries, oral disease caused by periodontal disease, and the shape, arrangement, and color of the teeth can cause esthetic and psychological problems and reduce the quality of life^{3,4)}. However, bright and clean teeth without damage can not only improve social skills and self-confidence but also have a positive effect on

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appearance and impression, improving the quality of life⁵).

Oral health is closely related to factors such as the face, lips, gingiva, and bone and is related to tooth discoloration, tooth shape, tooth surface structure, surface glossiness, and tooth function⁶. In other words, despite having healthy teeth, white and beautiful teeth without discoloration, and a well-aligned oral cavity are used as symbols of beauty, which are considered a healthy oral cavity.

On the other hand, the causes of tooth discoloration are classified into extrinsic staining and intrinsic staining. Extrinsic causes of discoloration include plaque discoloration; prolonged use of mouthwashes, such as chlorhexidine; smoking (e.g., cigars and tobacco); and drinking coffee, tea, and acidic beverages⁷. Tea, one of the causes of exogenous pigmentation, is currently the second most consumed beverage in the world, after coffee. Tea is being treated as a healthy drink by people, and it has been found that drinking tea is beneficial to health. It was reported that subjects who drank more than one cup of black tea daily had a lower risk of heart attack than those who did not⁸. In addition, tea improves cardiovascular diseases or has a cancer-preventing effect in Asians who mainly drink green tea, which is easily accessible⁷. Due to the effects of tea, it has been reported that the higher the consumption of tea, the higher the interest in health⁹.

As such, there are reports that tea is effective for health, but coffee is a representative color-inducing substance that ranks first in Korea's general preference and is consumed by 70% of the world. According to previous studies, it was reported that green tea and black tea showed more coloration than coffee in the results of re-staining experiments of coffee, green tea, and black tea for 7 days after tooth whitening. It was found that the tannin component of black tea reduces plaque by attacking bacteria in the oral cavity, but the black pigment of tannin affects the tooth color¹⁰.

In a domestic study on tooth discoloration, the application of various types of coffee to human premolars during self-whitening treatment affected tooth discoloration¹¹. In addition, the application of traditional fermented foods such as red pepper paste, soy sauce, and soybean paste to denture base resins resulted in a color change in all fermented foods¹². Cho et al.¹³ reported that fermented

food had an effect on the color change of the soft denture base material, and Lee et al.¹⁰ also evaluated re-pigmentation by applying various beverages after self-whitening. All beverages used in the experiment showed coloration. In addition, various studies have been reported, such as results on changes in the tooth color according to the application of drugs or whitening methods¹⁴⁻¹⁶.

As described earlier, several studies have reported the effects of foods containing a large number of pigments, such as coffee, cola, and red wine, on the change in the tooth color and the degree of tooth surface discoloration according to the change of the tooth surface after whitening. However, studies on the effect of blending tea with various ingredients on the tooth surface and whether tea with low acidity affects the change in the tooth color are insufficient.

Therefore, in this study, blending tea, which is the number 1 product based on online sales volume, was selected among the blending teas sold in the market, and the effect on the tooth surface color change was investigated. This study was performed to provide basic data on consumers' rational purchase and intake of blended tea and find a way to minimize tooth discoloration.

Materials and Methods

1. Materials

For the teeth subject to the experiment, healthy bovine teeth without caries or morphological damage were selected. For the experimental drink, black tea (yellow label tea; Unilever Korea, Seoul, Korea) was selected as a positive control, and water (Jeju Samdasoo, JPDC, Jeju, Korea) was selected as a negative control. The experimental group consisted of five types of blending teas with high preference among domestically marketed blending teas (group 1: pinacolada, Doori Int, Anseong, Korea; group 2: grape elderberry, Doori Int; group 3: strawberry rhubarb, Doori Int; group 4: rooibos yellow flower, Doori Int; and group 5: nable orange, Doori Int) (Table 1).

Table 1. Test Groups Used in the Experiment

Classification	Commercial name	Ingredients	Manufacturer
Black tea (positive control)	Yellow label tea	Black tea 100%	Unilever korea, Seoul, Korea
Water (negative control)	Jeju Samdasoo	Natural mineral water	JPDC, Jeju, Korea
Group 1	Pinacolada	Hibiscus flower, dried pineapple slices, dried coconut slices	Doori Int, Anseong, Korea
Group 2	Grape elderberry	Grape, hibiscus flower, black elderberry	Doori Int, Anseong, Korea
Group 3	Strawberry rhubars	Strawberry, rhubarb leaves	Doori Int, Anseong, Korea
Group 4	Rooibos yellow flower	Rooibos leaves, cornflower, sunflower, synthetic perfume (plum)	Doori Int, Anseong, Korea
Group 5	Nable orange	Dried orange peel, rose hip, orange	Doori Int, Anseong, Korea

2. Methods

1) Specimen preparation

After selecting healthy bovine teeth without caries or morphological damage, calculus removal, and surface washing were performed using an ultrasonic scaler and pumice, respectively. After cleaning, using a cutting disc (Ruby cutting discs No. 1; Ruby Dental Products, Osaka, Japan), 105 specimens with a dimension of 5×5×3 mm were prepared, and 15 specimens were distributed to each group. Prepared specimens were stored in saline until the start of the experiment.

2) Preparation of the blending tea solution

Five types of blending tea solutions were used in the experiment (pinacolada, grape elderberry, strawberry rhubarb, rooibos yellow flower, and nable orange). Each blending tea bag was brewed in 230 ml of water at 85 to 90°C for 3 minutes, according to the manufacturer's instructions. After cooling the brewed solution to the same temperature, 30 ml of each sample was dispensed into a beaker, and the solution was replaced every immersion.

3) pH and buffer capacity

To measure the pH of the experimental solution, it was left at room temperature of 25°C for 6 hours, and then 10 ml was dispensed into a beaker. The pH of each solution was measured using a pH meter (S20K pH meter; Mettler-Toledo, Greifensee, Switzerland) calibrated with a pH standard buffer solution. The buffering ability was determined using a micropipette (PIPETMAN G, Wilson, France) in which 0.05 ml of 1-M NaOH was added to the

experimental solution to measure the amount of 1-M NaOH added until the pH levels reached 5.5 and 7.0. All measurements were performed three times in the same manner to calculate the average values.

4) Application of blending tea solution

To determine the degree of coloring of the surface of bovine teeth over time, the intake time of 1 day (three times) was calculated as an average of 10 minutes, and the intake time of 1 day was defined as 30 minutes. In other words, it was divided into immersion before and after 1 day (30 min), 5 days (150 min), 7 days (210 min), 14 days (420 min), and 21 days (630 min). The immersion time was set based on a previous study that calculated the average food intake time as 10 minutes¹⁷⁾. After immersing the specimen in a beaker containing 30 ml of precipitation solution, the separated specimen was washed in running water for 1 minute each time, moisture was removed with a paper towel, and the specimen was dried to measure the color tone.

5) Color measurement

A spectrophotometer (CM-700d; Konica Minolta Inc., Tokyo, Japan) was used to measure tooth color before and after immersion in the blending tea solution on days 1, 5, 7, 14, and 21. Standard adjustment was performed on the L*, a*, and b* values with standard white and black plates before color tone measurement. The center of the teeth was measured by dividing the teeth into three parts in the vertical and horizontal directions, and the measurement tip was perpendicular to the teeth. To minimize error, the average value was calculated after repeated measurements

were performed three times by the same person, and the International Commission on Illumination (CIE) L*a*b* system prescribed by the International Lighting Commission was used for color tone measurement. L* represents the brightness of the tooth color by brightness and has a range of 0 to 100, with a high value indicating light and a low value indicating dark. a* indicates red saturation; (+), red; (-), green; b*, yellow saturation; (+), yellow; and (-), blue. The amount of color change (ΔE^*) was calculated using the following formula:

$$\Delta E^* = \{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\}^{1/2}$$

6) Observation with scanning electron microscopy

To examine the morphological changes in the enamel surface, the treated specimens were dehydrated in the order of increasing alcohol concentrations and then dried using a critical point dryer (HCP-2; Hitachi, Tokyo, Japan). Next, the specimen was fixed on an aluminum stub and coated with gold-palladium to 200-nm thickness using an ion sputter (E-1030; Hitachi) for observation under a scanning electron microscope (SEM) (S-4700; Hitachi) at 10-kV voltage and 1,000× magnification.

7) Data analysis

For effective verification of the change in color tone measurement values, SPSS ver.26 (IBM Corp., Armonk, NY, USA) was used for analysis. Repeated measures analysis of variance (ANOVA) was used to determine the difference in the amount of change in brightness and

saturation over time within the same treatment group, and one-way ANOVA was used to determine the amount of the final change in color tone. For the post hoc test, Tukey's multiple comparisons test was performed and verified at a 95% significance level.

Results

1. pH and buffer capacity

The average pH of the five blending teas in the experimental group was measured to be 3.78. The pH of group 3 was the lowest at 3.22 (group 1: 3.23, group 2: 3.41, group 5: 4.19, and group 4: 4.87). The pH of the positive control, black tea, was measured to be 5.19, and that of the negative control, water, was measured to be 7.30 (Table 2).

The buffering capacity of blending tea was highest at pH 5.5 in group 3 at 3.5 ml, followed by groups 1, 2, 4, and 5. At pH 7.0, group 3 was the highest at 5.0 ml, followed

Table 2. The pH and Buffer Capacity of Tea Used in the Experiment

Classification	Commercial name	pH	Buffer capacity (ml)	
			pH 5.5	pH 7.0
Black tea	Yellow label tea	5.19	0.5	1.0
Water	Jeju Samdasoo	7.30	-	-
Group 1	Pinacolada	3.23	3.13	3.43
Group 2	Grape elderberry	3.41	1.9	2.2
Group 3	Strawberry rhubars	3.22	3.5	5.0
Group 4	Rooibos yellow flower	4.87	0.65	1.31
Group 5	Nable orange	4.19	0.3	0.6

Table 3. Chromaticity L* of Bovine Teeth after Immersion in Blending Tea

Group	Time					
	Before	1 day	5 day	7 day	14 day	21 day
Black tea	80.53±0.62	74.05±1.26 ^a	72.03±2.30 ^a	70.82±2.90 ^a	69.77±4.08 ^a	68.75±4.31 ^a
Water	80.48±0.56	80.25±0.81 ^d	80.18±0.92 ^b	80.11±0.68 ^b	80.06±0.49 ^b	80.01±0.87 ^b
Group 1	82.89±1.34	75.58±1.85 ^b	75.45±2.32 ^c	74.48±1.99 ^c	73.83±2.73 ^c	71.10±2.79 ^{ac}
Group 2	83.22±1.01	80.55±0.76 ^{de}	76.92±1.55 ^c	75.34±1.73 ^c	75.30±1.93 ^{cd}	73.58±2.32 ^c
Group 3	82.71±1.19	79.99±0.65 ^{de}	76.30±2.37 ^c	74.60±2.54 ^c	74.18±3.32 ^{cd}	72.25±3.44 ^c
Group 4	82.68±0.73	80.47±0.58 ^{de}	77.43±1.38 ^{cd}	76.40±1.66 ^c	76.88±1.69 ^{bd}	70.32±2.09 ^{ac}
Group 5	80.34±0.78	78.69±0.88 ^c	79.25±1.16 ^{bd}	79.34±1.17 ^b	79.06±1.35 ^b	78.12±1.45 ^b

Values are presented as mean±standard deviation.

^{a~c}Significant differences (p < 0.05) found by Tukey's multiple comparisons are indicated by values which have the same letter. p-values are statistically significance according to stain time by repeated measures ANOVA procedure (p < 0.05).

by groups 1, 2, 4, and 5, and the buffering capacity of black tea, a positive control, was measured at pH 5.5 at 0.5 ml and pH 7.0 at to 1.0 ml (Table 2).

2. Color measurement

1) Change in brightness (L*) over time

There was a significant difference over time in the change in group brightness according to the type of blending tea for 21 days ($p < 0.05$). A clear change in brightness was observed in the positive control black tea and the five experimental groups, and the overall brightness tended to decrease. However, no change in brightness was observed in the negative control group. Among the experimental groups, group 4 showed the largest change in brightness, indicating the darkest, followed by groups 1, 3, 2, and 5 (Table 3).

2) Change in saturation over time

Repeated measures ANOVA was performed to measure the change in saturation over time. The a^* value indicating red saturation showed a statistically significant change over time in groups 2, 3, 4, and 5 ($p < 0.05$), and all four groups showed a tendency to increase overall red saturation. Among the experimental groups, group 4 showed the largest change, and group 5 showed the smallest change (Table 4).

The b^* value, indicating yellow saturation, showed a statistically significant change over time in the positive control black tea and groups 1, 3, 4, and 5 ($p < 0.05$), and all five groups showed a tendency to increase overall yellow saturation. However, there was no significant change in group 2. Among the experimental groups, group 4 showed the largest change, and group 2 showed the smallest change (Table 5).

Table 4. Chromaticity a^* of Bovine Teeth after Immersion in Blending Tea

Group	Time					
	Before	1 day	5 day	7 day	14 day	21 day
Black tea	-0.76±0.41	1.04±0.81 ^a	2.11±0.99 ^a	2.13±1.13 ^a	2.84±1.61 ^a	2.69±1.40 ^a
Water	-0.80±0.37	-0.75±0.34 ^b	-0.76±0.32 ^b	-0.68±0.32 ^c	-0.71±0.28 ^c	-0.70±0.27 ^b
Group 1	-0.82±0.33	-0.73±0.27 ^b	-0.91±0.29 ^b	-0.86±0.24 ^c	-0.85±0.47 ^c	-0.61±0.49 ^b
Group 2	-0.93±0.37	-0.73±0.38 ^b	-0.65±0.36 ^b	-0.45±0.41 ^c	-0.54±0.42 ^c	-0.35±0.49 ^b
Group 3	-0.65±0.28	-0.31±0.40 ^b	-0.07±0.43 ^c	0.17±0.46 ^b	0.31±0.62 ^b	0.53±0.70 ^c
Group 4	-0.71±0.24	-0.62±0.30 ^b	-0.65±0.39 ^b	-0.45±0.40 ^c	-0.51±0.42 ^c	0.52±0.61 ^c
Group 5	-0.74±0.32	-0.60±0.33 ^b	-0.61±0.29 ^{bc}	-0.68±0.29 ^c	-0.57±0.34 ^c	-0.55±0.38 ^b

Values are presented as mean±standard deviation.

^{a~c}Significant differences ($p < 0.05$) found by Tukey's multiple comparisons are indicated by values which have the same letter. p-values are statistically significance according to re-stain time by repeated measures ANOVA procedure ($p < 0.05$).

Table 5. Chromaticity b^* of Bovine Teeth after Immersion in Blending Tea

Group	Time					
	Before	1 day	5 day	7 day	14 day	21 day
Black tea	7.12±1.35	9.41±1.53 ^a	11.20±1.52 ^a	10.97±1.42 ^a	11.75±1.52 ^a	12.32±1.32 ^a
Water	6.59±1.18	6.92±0.84 ^b	6.94±0.87 ^c	7.00±0.79 ^b	7.10±0.72 ^b	7.10±0.68 ^d
Group 1	6.26±0.96	6.05±0.93 ^b	5.69±0.85 ^c	5.69±0.84 ^c	5.50±0.83 ^c	5.47±0.80 ^c
Group 2	5.75±1.33	5.97±1.05 ^b	5.77±0.88 ^c	5.79±0.77 ^{bcd}	5.78±0.83 ^{bc}	6.08±0.74 ^{de}
Group 3	5.73±1.13	6.57±1.00 ^b	6.88±1.00 ^c	7.12±1.08 ^{bc}	7.57±1.34 ^{bd}	8.42±1.31 ^b
Group 4	6.12±1.14	8.28±1.61 ^a	9.62±1.80 ^b	9.77±1.84 ^a	10.78±1.85 ^a	10.71±1.54 ^c
Group 5	5.66±0.91	6.92±0.98 ^b	6.73±0.95 ^c	6.86±1.10 ^{bcd}	6.99±1.12 ^{bcd}	7.00±1.21 ^d

Values are presented as mean±standard deviation.

^{a~c}Significant differences ($p < 0.05$) found by Tukey's multiple comparisons are indicated by values which have the same letter. p-values are statistically significance according to re-stain time by repeated measures ANOVA procedure ($p < 0.05$).

3) Amount of color change over time

As a result of performing one-way ANOVA to determine the amount of color change over time, the ΔE^* value, indicating the color change amount, showed a statistically significant difference between all groups ($p < 0.05$). After immersion for 21 days, the change in color tone increased the most in the positive control group, black tea, followed by groups 4, 1, 3, 2, and 5 and water, the negative control group (Table 6).

3. Observation using an scanning electron microscope

As a result of observing the bovine tooth enamel surface using an SEM, changes were observed in all groups immersed in the experimental solution for 21 days, except for water, which was used as a negative control. Before immersion in the solution, the bovine tooth surface was covered with amorphous enamel, showing a smooth overall

pattern (Fig. 1A), and the negative control group also appeared similar to the specimen before immersion (Fig. 1B). In the positive control group, black tea, a honeycomb-shaped enamel rod cross section was observed in the area where the amorphous enamel was lost, but most showed a smooth pattern (Fig. 1C). In the experimental group, group 1 lost amorphous enamel, the overall surface was rough, and a honeycomb-shaped enamel rod cross section was observed (Fig. 1D). In group 2, some smooth surfaces and mostly honeycomb-shaped enamel rod cross sections were observed (Fig. 1E), and in group 3, partially smooth surfaces were observed, but in some cases, honeycomb shapes and pores were observed (Fig. 1F). Group 4 mostly showed a smooth surface, but a honeycomb-shaped enamel rod cross section and cracks were observed in the area where the amorphous enamel was lost (Fig. 1G). Group 5 showed a partially smooth surface, but most enamel rod cross sections were observed (Fig. 1H).

Table 6. Chromaticity Δ of Bovine Teeth after Immersion in Blending Tea

Amount of color change	Group							p-value ^a
	Black tea	Water	Group 1	Group 2	Group 3	Group 4	Group 5	
ΔE	13.40±4.8	1.08±0.33	11.85±2.79	9.73±2.73	10.96±3.76	13.30±2.23	2.74±1.03	0.001

Values are presented as mean±standard deviation.

^ap-values are statistically significance among the groups at each groups by one-way ANOVA procedure ($p < 0.05$).

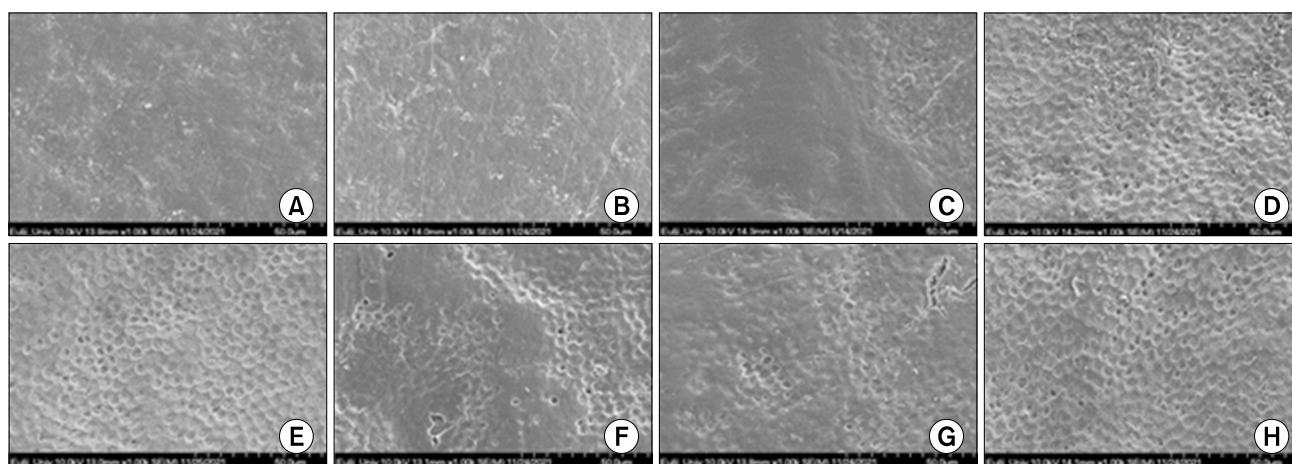


Fig. 1. Scanning electron microscope image of bovine teeth surface after immersion for 21 days. Surface changes were observed in all groups, except for water. In black tea, a honeycomb-shaped enamel rod cross section was observed in some samples but mostly smooth. There were some differences between the experimental groups, but honeycomb-shaped enamel rod cross sections were observed in all groups. (A) Before immersion. (B) Water (negative control). (C) Black tea (positive control). (D) Group 1 (pinacolada). (E) Group 2 (grape elderberry). (F) Group 3 (strawberry rhubarb). (G) Group 4 (rooibos yellow flower). (H) Group 5 (noble orange). All magnifications are $\times 1,000$.

Discussion

The concept of health has changed with the trend of the times, and the meaning of oral health has expanded. The oral cavity performs the functions of mastication, pronunciation, and esthetics. In particular, as for the esthetic function, defects and deformation of teeth have a great influence on the facial appearance, which can significantly interfere with social activities as well as individual mental stress. In modern society, its importance is increasingly emphasized, and interest in esthetic treatment such as tooth whitening or orthodontic treatment is increasing^{10,18)}. Therefore, this study focused on the tooth color among the esthetic factors of oral tissue and analyzed the characteristics of “blending tea,” which has been steadily increasing in consumption due to the growing interest in health, and its effect on the color change of the tooth enamel surface.

According to a study on beverages, low-pH acidic beverages cause tooth corrosion in the mouth, and low-pH drinks such as soda, sports drinks, and energy drinks reduce the surface hardness of teeth, causing corrosion^{19,20)}. In addition, it has been reported that low-pH beverages affect durability and lifespan by chemically decomposing and lowering the physical properties of composite resin, a dental restoration material, including teeth²¹⁾. The possibility of tooth corrosion by acidic beverages is related to factors such as temperature, contact time, pH, and titratable acidity²²⁾. In this study, pH and titratable acidity were measured to observe the degree of tooth coloration according to the pH of the blending tea. The pH of the positive control group, black tea, was 5.19; the average pH of experimental group 5, blending tea, was 3.78; and the pH of group 3 (strawberry rhubarb) was the lowest at 3.22. The buffering capacity was measured to be the highest in group 3 at both pH levels of 5.5 and 7.0, and except for group 5 (noble orange), the lower the pH, the higher the amount of NaOH required to increase the titratable acidity. Because the higher the titratable acidity, the longer the maintenance time of the acidic environment in the oral cavity, we analyzed whether such an acidic environment could affect the change in tooth tone. Nam and Choi²³⁾ tested the re-staining of drinks with various pH levels after

self-whitening and found that coffee and green tea had the greatest effect on coloring, and the color of the teeth darkened. However, orange juice brightened the color of the teeth, which was interpreted as the lightening of the color due to the low pH of the orange juice corroding the teeth. In addition, the pH of orange juice used in previous studies was 3.84, which was similar to the average pH of the five blending teas used in this study, but all five blending teas showed a darker color. Therefore, it is thought that there is no correlation between the acidity and coloring degree of blending teas.

In previous studies that measured the color tone of natural teeth, dental colorimeters and spectrometers were used, and most of the results were analyzed using the CIE color system²⁴⁾. The CIE color system expresses color using the Lab* value, where L indicates brightness, a* indicates red-green saturation, and b* indicates yellow-blue saturation. The amount of color change is expressed as ΔE^* , which indicates the minimum distance between two colors and can be calculated from the Lab* colorimeter. Based on this, we measured the degree of coloration using the CIE color system.

The L* value representing brightness is brighter and whiter as the number increases within the range of 0 to 100, and the lower the value, the darker and closer it is to black. Lee et al.¹⁰⁾ compared the degree of coloration using coffee, green tea, and black tea and reported that the change in brightness (ΔL^*) for re-staining after tooth whitening showed statistically significant differences over time and that the brightness index decreased. Kim et al.¹¹⁾ also reported that the brightness change according to the type of coffee during the whitening processing period was significantly different over time and that the brightness index clearly decreased. In this study, there was a statistically significant difference in the L* value of brightness over time in all groups, except for the negative control group, water (Samdasoo). That is, the positive control group, black tea, and five types of blended tea showed a clear change in brightness and a tendency to decrease overall brightness. Among the blending teas, group 4 (rooibos yellow flower) showed the largest change in brightness and was found to be the darkest.

The a* value, representing red saturation, indicates the

degree of redness as a positive value and the degree of greenness as a negative value. In this study, red saturation showed a statistically significant change over time in group 2 (grape elderberry), group 3 (strawberry rhubarb), group 4 (rooibos yellow flower), and group 5 (noble orange), except for the control group and group 1 (pinacolada). Moreover, all four groups showed a tendency to increase overall red saturation. Among the experimental groups, group 4 showed the largest change, and group 5 showed the smallest change. The b^* value, representing yellow saturation, indicates the degree of yellowness as a positive value and the degree of blueness as a negative value. In this study, yellow saturation showed a statistically significant change over time in black tea as a positive control and groups 1, 3, 4, and 5, and all five groups showed a tendency to increase overall yellow saturation. Among the experimental groups, group 4 showed the largest change, and group 2 showed the smallest change. Kim et al.¹¹⁾ reported that in a study on tooth staining with coffee applied during self-whitening treatment, there was a significant difference in the color change for each group according to the type of coffee over time. Nam and Choi²³⁾ observed the change in the tooth color for 20 days and found that there was a significant difference over time. In addition, in the study of Lee et al.¹⁰⁾, L^* , a^* , and b^* all showed statistically significant differences in the Lab^* values according to the period and group for re-staining after teeth whitening, which is similar to this study. However, in a study by Kang and Lim²⁵⁾ that tested the color changes of whitened teeth using soy sauce, red pepper paste, and soybean paste, yellow saturation tended to decrease significantly over time after precipitation, which differs from the results of this study. The cause of the increase in both red and yellow saturations in this study is thought to be the dark yellow pigment from petals and black tea leaves among the blending tea ingredients.

According to previous studies related to shade, brightness shows constant results according to shade changes, but saturation has a high rate of change due to various variables, and the change is not stable; therefore, most studies calculate and analyze saturation¹⁴⁾. As a result of examining the amount of change in color tone (Δ

E^*) of teeth over time after immersion in blending tea, statistically significant differences were found between the groups. In addition, the difference was large in the order of black tea (brown), group 4 (yellow), and group 1 (red). This is consistent with the fact that a study comparing the degree of coloring using coffee, green tea, and black tea showed statistically significant differences in the amount of color change for repainting after tooth whitening¹⁰⁾. In addition, in this study, the amount of color change after precipitation for 21 days increased the most in black tea, a positive control, and in the study by Lee et al.¹⁰⁾, not only coffee but also green and black teas contain tannins, which can cause tooth discoloration. Nam and Choi²³⁾ evaluated the effect of using whitening toothpaste and the re-staining of drinks with various pH, which can be compared with the result that green tea showed more color than coffee.

Meanwhile, as a result of observing the surface of the bovine teeth before and after immersion using an SEM, surface changes were observed in all groups immersed in the experimental solution for 21 days, except for the negative control, water. Before immersion in the solution, the surface of the bovine teeth was covered with amorphous enamel; therefore, the overall pattern was smooth, and the water, a negative control group, was also similar to that before immersion. Black tea, the positive control group, showed a smooth pattern, although some honeycomb-shaped enamel rod cross sections were observed. There were some differences in the five blending teas in the experimental group, but honeycomb-shaped enamel rod cross sections and rough surfaces were observed in all groups. In particular, severe surface changes were observed in groups 1 and 3, which had low pH. Jeong et al.²⁶⁾ studied the coloring and erosion of enamel surfaces by immersing premolars in commercially available oral brushing solutions and reported that the erosion pattern of enamel surfaces could be confirmed in all groups. In particular, erosion of the enamel surface was confirmed in the Nexcare and Listerine groups at low pH. In this study, it is thought that low pH is a factor in the change of the enamel surface.

The limitation of this study is that the surface of the sample was polished without considering the coloring

method that may occur in the actual oral cavity and then immersed in the blending tea solution for coloring to induce artificial discoloration. Because this is an in vitro experiment, it is thought that it will be somewhat difficult to apply the same results to clinical practice. Summarizing the results of this study, the coloration of the five types of blending teas increased over time, and the amount of change was particularly large in the blending tea with a deep color. Therefore, it is thought that it will help to prevent tooth discoloration if consumers refrain from consuming the dark blending tea and rinse their mouth with water after drinking the blending tea.

Notes

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

This article does not require for IRB screening because human origin is not used.

Author contributions

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