Effects of Weakly Electrolyzed Water on Properties of Green Tea Infusion

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Abstract

The effect of weakly electrolyzed water (anode water and cathode water) produced by weak electrolysis of tap water on the properties of green tea infusion was examined. Instrumental measurements and sensory tests of green tea infusion revealed that the use of cathode water gave a delicious green tea infusion with a bright green color, and well-balanced astringency and bitterness. In addition, green tea beverages with high functionality could be prepared using cathode water as compared with non-electrolyzed tap water, since cathode water promoted the dissolution of tea catechins (ECG, EGCg and EC). On the other hand, the use of anode water to infuse green tea destroyed the balance of tastes, and was not considered appropriate for preparing green tea beverages.

1. Introduction

Green tea has grown to be one of the most popular beverages now even widely available bottled. The quality of green tea beverage is greatly influenced by the characteristics of water such as hardness (Horie *et al,* 1998). Therefore, soft water is recommended for preparing green tea (Torii, 1966).

In recent years, many techniques to improve the functionality of water such as tap water have been developed (Isobe, 1999). Previously, we reported that the use of weakly electrolyzed tap water improved the quality of cooked rice (Kobayashi *et al.,* 1996; Onishi *et al.,* 2000), bread (Onishi *et al.,* 1999), Japanese wheat noodles (Hara *et al.,* 2003a) and tofu (Hara *et al.,* 2003b).

In the present study, we compared the characteristics of green tea infusion prepared with weakly electrolyzed water and tap water, and discussed the possibility of the use of weakly electrolyzed water for preparing green tea beverages.

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Abbreviations: EGC, (-)-epigallocatechin; EGCg, (-)-epigallocatechin gallate; EC, (-)-epicatechin; ECg, (-)-epicatechin gallate; Asp, Aspartic acid; Glu, Glutamic acid; Ser, Serine; Arg, Arginine; The, Theanine

2. Materials and methods

2.1 Materials

Green tea leaves (cv. *Yabukita,* middle grade) grown in Shizuoka Prefecture were purchased from a local tea shop in Shizuoka City. Before infusion, green tea leaves were passed through 8.6-mesh screen to remove the broken leaves of 2 mm or less.

2.2 Water

Using an electrolyzing device, two kinds of weakly electrolyzed water were prepared from tap water (Shizuoka City, pH 7.25±0.63), weakly electrolyzed anode water (referred to as "anode water" hereafter) and weakly electrolyzed cathode water (referred to as "cathode water" hereafter).

The conditions for the electrolysis were: device, model HOX-40A (Hoshizaki Electric Co., Nagoya); electrolytic voltage, 50 V; electrolytic current, 2 A; temperature of tap water, approximately 20℃. Anode water (pH 3.50±0.25) was obtained from a positive electrolytic cell and cathode water (pH 9.25±0.11) was obtained from a negative electrolytic cell. The methods of measurement for pH, oxidation-reductive potential (ORP), electric conductivity, and concentrations of dissolved oxygen (DO), residual chlorine, cation (chloride and nitrate) and anion (potassium, calcium, magnesium and sodium) were similar to those in our previous reports (Hara *et al.*, 2003a; 2003b). Three independent measurements were made for each sample and the results were expressed as a mean (SD).

2.3 Infusion of green tea

Anode water, cathode water and tap water (180 ml) were heated to 70℃ and green tea leaves (3 g) were put into the water. After infusing for 5 min at 70℃, water with green tea leaves was filtered through a paper filter (No. 2, Toyo Roshi Kaisha, Ltd., Tokyo) under reduced pressure for 30 sec to remove the leaves. Then the filtrate was used for instrumental measurements and sensory test.

2.4 Measurement of color index

The color index (L^*, a^* and b^* values) of green tea infusion was measured with a transmutation color meter (SZ Optical Series, Nippon Denshoku Industries, Co. Ltd., Tokyo). The color difference (ΔE^*ab) between samples was calculated using the following formula. Five independent measurements were made for each sample and the results were expressed as a mean (SD).

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

2.5 Contents of tea catechins and caffeine

Tea catechins and caffeine in the green tea infusion were measured by the method of Kinugasa *et al.* (1997). Green tea infusion diluted with distilled water was filtrated with a 0.20 μm membrane filter (DISMIC-13$_{HP}$, Toyo Roshi Kaisha, Ltd., Tokyo) and then tested by high performance liquid chromatography (HPLC). HPLC conditions were as follows: apparatus, PU-980 (JASCO Co., Tokyo); column, Wakopak 5C$_{18}$HG (4.6 × 30 mm, Wako Pure Chemical Industries Ltd., Osaka); solvent, methanol/water/phosphoric acid (22/78/0.1); flow rate, 1 ml/min; column temperature, 30℃; detector, spectrophotometer (JASCO Co., UV-970), detection wavelength, 280 nm. EGC, EGCg, EC, ECg and caffeine (Wako Pure Chemical Industries Ltd.) were used as standards. Three independent measurements were made for each sample and the results were expressed as a mean (SD).

2.6 Content of free amino acid

Polyvinylpolypyrrolidone (PVPP, 300 mg, Sigma-Aldrich Co., St. Louis) was mixed with green tea infusion diluted with distilled water (100 ml) and left standing for 30 min, it was filtrated with a paper filter (No.2, Toyo Roshi Kaisha, Ltd) and a 0.20 μm membrane filter (DISMIC-13_{HP}), and then analyzed by an automatic amino acid analyzer (L-8500, Hitachi, Ltd., Tokyo). Three independent measurements were made for each sample and the results were expressed as a mean (SD).

2.7 Content of free sugar

The green tea infusion was treated with PVPP by the method mentioned above and then tested by HPLC. HPLC conditions were as follows: apparatus, PU-980 (JASCO Co.); column, Wakosil $5NH_2$ (Wako Pure Chemical Industries Ltd.); solvent, acetonitrile/water (7/3); flow rate, 1 ml/min; column temperature, 40℃; detector, reflection indicator, 830-RI (JASCO Co.). Fructose, glucose and sucrose (Wako Pure Chemical Industries Ltd.) were used as standards. Three independent measurements were made for each sample and the results were expressed as a mean (SD).

2.8 Content of reduced *L*-ascorbic acid

The green tea infusion was treated with PVPP by the method mentioned above and then tested with an F-kit for *L*-ascorbic acid (J.K. International Inc., Tokyo). Five independent measurements were made for each sample and the results were expressed as a mean (SD).

2.9 Sensory test

The green tea infusion was tasted by a panel of 15 female students (20-22 years old). The green tea infusion prepared with tap water was used as a control (zero value). Seven factors (color, flavor, deliciousness, sweetness, astringency, bitterness and overall judgment) of green tea infusion prepared with anode and cathode waters were evaluated using a seven-grade rating scale from +3 (increased or good) to -3 (decreased or bad). The sample of green tea infusion was kept warm at 70℃ in an incubator immediately before serving to the panel members. The results were expressed as a mean (SD).

2.10 Statistical analysis

Comparisons of two means were made by Student's *t*-test (Fisher, 1958).

3. Results and discussion

3.1 Characteristics of weakly electrolyzed water

First, we examined the characteristics of the weakly electrolyzed water. (Table 1) Compared with tap water, the anode water had a low pH, high ORP, high DO and a large amount of anions such as chlorine and nitrate. On the other hand, the cathode water had a

Table 1. Characteristics of water.

Characteristic	Weakly electrolyzed[a)]		Non-electrolyzed
	Anode water	Cathode water	Tap water[b)]
	mean (SD)	mean (SD)	mean (SD)
Value of pH	3.50 (0.43)	9.25 (0.19)	7.25 (1.1)
Oxidation-reduction potential (mV)	498 (31)	-355 (48)	304 (35)
Electric conductivity (ms/m)	21.8 (2.3)	11.3 (1.0)	9.2 (2.4)
Dissolved oxygen (mg/l)	9.6 (0.9)	5.9 (0.2)	6.8 (0.3)
Residual chlorine (mg/l)	0.2 (0)	0.1 (0)	0.4 (0)
Ion concentration (mg/l)			
Cl^-	13.0 (1.0)	6.1 (0.2)	6.5 (0.5)
NO_3^-	3.1 (0.7)	1.4 (0.5)	1.5 (0.7)
Na^+	5.4 (1.0)	10.0 (0.2)	9.7 (0.2)
K^+	0.4 (0.2)	1.1 (0.2)	1.1 (0.5)
Mg^{2+}	3.5 (0.5)	5.3 (0.2)	5.3 (0.2)
Ca^{2+}	4.5 (0.7)	8.8 (0.5)	8.5 (0.5)

[a)] Using an electrolyzing device, two kinds of weakly electrolyzed water (anode water and cathode water) was obtained from the electrolysis of tap water.

[b)] Tap water was supplied from Shizuoka City.

high pH, low ORP, low DO and a large amount of cations such as sodium, potassium, magnesium and calcium. Both anode and cathode waters had higher electric conductivity and lower concentrations of residual chlorine than tap water.

3.2 Characteristics of green tea infusion prepared with weakly electrolyzed water

Table 2 shows the pH value, color index and major components of green tea infusion prepared with weakly electrolyzed water and tap water. The pH value of the green tea infusion prepared with anode water was significantly lower and that prepared with cathode water was significantly higher than that prepared with tap water. Thus, the use of electrolyzed water effectively changed the pH of green tea infusion.

Electrolyzed water has no buffer capacity. It is known that when electrolyzed water comes into contact with organic substances, the H^+ and OH^- ions generated by electrolysis are gradually eliminated and eventually the pH of the electrolyzed water returns to that before electrolysis. In this study, however, we did not use a large amount of tea leaves for infusion, so that the pH value of green tea infusion prepared with weakly electrolyzed water was assumed to be affected by the initial pH of the weakly electrolyzed water. The change in pH of the green tea infusion caused by preparation with anode water was significantly larger than that caused by preparation with cathode water.

No significant difference was caused by using weakly electrolyzed water in the L^* value that indicates transparency of the green tea infusion. The $-a^*$ value that represents green and the b^* value that represents of yellow of green tea infusion were significantly decreased

Table 2. Effects of weakly electrolyzed water on pH, color index and major component of green tea infusion.

	Weakly electrolyzed		Non-electrolyzed
	Anode water	Cathode water	Tap water[a]
	mean (SD)	mean (SD)	mean (SD)
Value of pH	5.45 (0.22)**	7.17 (0.11)*	6.75 (0.02)
Color index			
*L**	94.9 (0.9)	95.4 (0.9)	94.7 (0.4)
*a**	-1.1 (0.2)***	-4.2 (0.2)*	-3.7 (0.2)
*b**	3.9 (0.9)**	10.6 (0.4)*	9.3 (0.4)
*ΔE*ab*	6.0 (0.7)	1.7 (0.4)	–
Component (mg/100 ml)			
EGC	35.9 (0.9)*	38.6 (3.1)*	32.6 (1.0)
EGCg	39.7 (0.9)	41.9 (1.0)*	38.9 (0.9)
EC	6.4 (0.2)	7.0 (0.2)*	6.4 (0.2)
ECg	4.4 (0.2)	4.7 (0.3)	4.6 (0.3)
Caffeine	22.4 (0.3)	24.5 (0.7)*	22.4 (0.5)
Free amino acid			
Asp	1.7 (0)	1.7 (0.2)	1.7 (0.2)
Glu	2.0 (0.2)	2.1 (0.2)	2.0 (0)
Ser	0.7 (0.2)	0.7 (0)	0.6 (0.2)
Arg	0.7 (0.2)***	0.3 (0)	0.3 (0.2)
The	7.1 (0.2)	7.2 (0.2)	7.1 (0.3)
Free sugar			
Fructose	1.2 (0.2)	1.3 (0.2)	1.3 (0.2)
Glucose	1.6 (0.3)	1.6 (0.3)	1.7 (0.2)
Sucrose	10.7 (0.3)	11.5 (0.2)*	10.5 (0.5)
Reduced *L*-ascorbic acid	2.3 (0.2)*	2.5 (0)	2.6 (0.2)

[a] Control.

*, ** and ***: Significantly different from the control (tap water) value. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

by using anode water and significantly increased by using cathode water. The color difference (ΔE^*ab) between green tea infusion prepared with anode water and tap water was 6.0. This difference in color of green tea infusions was easily recognized by the naked eye, because the visual color recognition limit of the difference in value of color is about 2.5 (Nayatani, 1993).

The color of green tea infusion prepared with anode water faded foster compared with that prepared with tap water, since the chlorophyll of green tea changed to pheophytin at a low pH. On the contrary, the color of green tea infusion prepared with cathode water was brighter green than that prepared with tap water, since the chlorophyll of green tea changed to chlorophyllin at a high pH.

The concentrations of major tea catechins known as EGC, EGCg, EC and ECg were measured in green tea infusions prepared with anode and cathode waters or tap water. The green tea infusion prepared with cathode water had significantly higher concentrations of EGC, EGCg and EC than that prepared with tap water. Only the concentration of EGC was significantly higher in the green tea infusion prepared with anode water. These results showed that the use of electrolyzed water (especially cathode water) to infuse green tea is effective for increasing the concentration of major catechins in green tea.

By electrolysis, hydrogen particles are generated on the cathode side (Kikuchi *et al.*, 2001) and the surface tension of cathode water is decreased (Inata *et al.*, 2002). The increase of tea catechins by the use of cathode water may be attributed to a decrease in the surface tension. The reason for the increase in the concentration of EGC by the use of anode water is not yet clear.

In addition to catechins, caffeine gives bitterness to the green tea infusion. Preparation of green tea infusion with cathode water significantly increased the caffeine content compared with that with tap water, but that with anode water did not. Thus, cathode water was more effective than tap water for the extraction of caffeine. This may also be caused by an increase in surface tension.

The concentrations of five kinds of free amino acids (Asp, Glu, Ser, Arg and The) related to deliciousness and sweetness of green tea infusion (Kato and Suzuki, 1971; Ikeda *et al.*, 1972) were measured. The green tea infusion prepared with anode water contained a significantly larger amount of Arg than that prepared with tap water. No significant difference was observed between the contents of free amino acids of green tea infusion prepared with cathode water and tap water. These results indicated that the use of anode water increased the solubility of Arg compared with that of tap water.

The isoelectric point of Arg is 10.8. Since the isoelectric point of Arg was the most distant from the (low) pH of anode water, using anode water might increase the solubility of Arg. The use of weakly electrolyzed water did not significantly affect the concentration of amino acids other than Arg in the green tea infusion. This is probably due to the difference in solubility of each amino acid.

Nakagawa *et al.* (1973) reported that free amino acids are easily extracted though tannin is comparatively difficult to extract. In this study, the green tea was extracted for 5 minutes, which was considered long enough to extract most of the free amino acids irrespective of the kind of sample water. When a shorter infusion time is used, the concentration of free amino acids might also be influenced by the use of cathode water like the major catechins and caffeine.

The main component of free sugars in green tea is sucrose which occupies 60 - 80% of the total free sugars, and the remaining sugars are glucose and fructose (Nakagawa, 1997). No significant difference was observed between the concentrations of free sugars in the green tea infusion prepared with anode water and tap water, but in the infusion prepared with cathode water, the concentration of sucrose was significantly higher than that in the infusion prepared with tap water. This may be attributed to the effect of cathode water on the surface tension.

Most of the *L*-ascorbic acid contained in green tea is of the reduced type. The use of anode water significantly reduced the content of reduced *L*-ascorbic acid in the green tea infusion. No significant difference was observed between the concentration of reduced *L*-ascorbic acid in the green tea infusion prepared with cathode water and that prepared with tap water. During infusion with anode water, due to the effect of high ORP, the reduced *L*-ascorbic acid in green tea might be converted to oxidic *L*-ascorbic acid. These results indicated that the use of anode water decreases the concentration of reduced *L*-ascorbic acid in the green tea infusion.

3.3 Sensory characteristics of green tea infusion prepared with weakly electrolyzed water

The sensory test was carried out for green tea infusion prepared with anode water and cathode water serving tap water as a control (zero value). Figure 1 shows the results. The green coloring of the tea was significantly scored negative (loss of vividness) by infusion with anode water, and was significantly scored positive (became vivid) by infusion with cathode water. The flavor of the tea infused with anode water was significantly scored negative (bad) and that with cathode water scored slightly positive (good). The deliciousness of the tea infused with anode water was significantly scored negative (weak) and that infused with cathode water scored slightly positive (strong). The sweetness of the tea infused with anode water was scored slightly positive (strong) and that infused with cathode water scored slightly negative

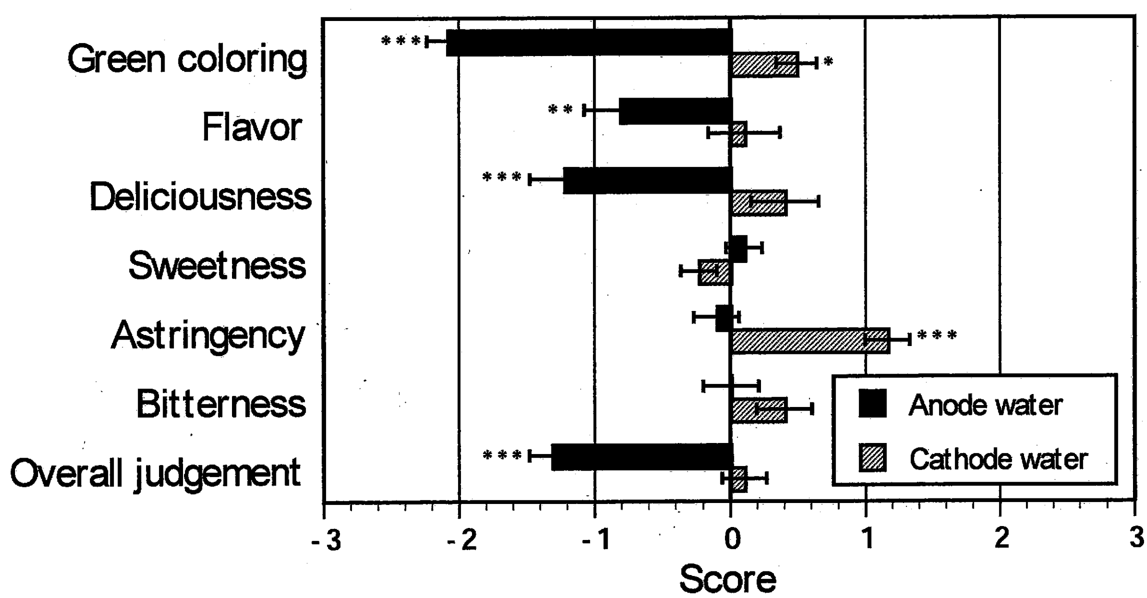

Figure 1. Sensory scores of the green tea infusion prepared with weakly electrolyzed water compared with those prepared with tap water (zero value).

*, ** and ***: Significantly different from the control (tap water) value. *$P<0.05$, **$P<0.01$, ***$P<0.001$. Positive score, increased (or good); negative score, decreased (or bad).

(weak). The astringency of the tea infused with cathode water was significantly scored positive (strong) and that infused with anode water scored slightly negative (weak). The bitterness of the tea infused with cathode water was scored slightly positive (strong) and that infused with anode water scored almost zero (equal). The overall judgment (palatability) of the tea infused with anode water was significantly scored negative (unfavorable) and that infused with cathode water scored slightly positive (favorable).

These results showed that the sensory characteristics of the green tea infusion prepared with weakly electrolyzed water were different from those prepared with tap water. The use of cathode water gave green tea infusion brighter green, astringency, deliciousness and bitterness. Astringency and bitterness might be caused by the presence of a large amount of tea catechins and caffeine. Deliciousness was increased by the use of cathode water probably because the panelists evaluated sweetness caused by increase of sucrose over the taste of amino acids, as deliciousness. No significant difference was observed between the scores for overall judgment of green tea prepared with cathode water and tap water. It was assumed that although the green tea infusion prepared with cathode water contained an increased amount of tea catechins and caffeine, it also contained an increased amount of sucrose, and the taste balance was hardly disturbed.

On the other hand, the green tea infusion prepared with anode water had slightly increased sweetness over that prepared with tap water, but was evaluated equal or negative in other characteristics. This may be attributed to the great difference in pH value and increased amount of Arg, which suppresses the bitterness and astringency that disturb the original taste of green tea. Since the taste of Arg is different from the deliciousness of green tea, the panelists might have recognized it somewhat differently.

From the results of the analytical data and sensory test, we conclude that the use of weakly electrolyzed water changes the quality of the green tea infusion. Especially, the use of cathode water for preparing the green tea infusion improved the solubility of tea catechins, which are expected to have special functions such as antioxidant (Yokozawa, *et al,* 2000; Kawase, *et al,* 2000), antitumorigenic (Agarwal, *et al.,* 1992; Wang, *et al.,* 1992), antihypercholesterolemic (Muramatsu *et al,* 1986) and antiallergic activity (Ohmori *et al.,* 1995; Matsuo, *et al.,* 1997) etc. Therefore, we suggest that green tea beverage with high functional qualities should be prepared using cathode water. On the contrary, the use of anode water for preparing green tea beverages disturbed the balance of tastes and is not recommended from the standpoint of palatability. In addition, anode water did not improve the extraction of the active substances in green tea.

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