

Techniques for Universal Evaluation of Astringency of Green Tea Infusion by the Use of a Taste Sensor System

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A practical method for universal evaluation of the astringency of green tea infusion by a taste sensor system was established. The use of EGCg aqueous solution as a standard enabled analysis with high accuracy and reproducibility. The sensor output was converted into taste-intensity on the basis of Weber's and Weber-Fechner laws, which was named the "EIT_{ast}" value ("EIT" and "ast" are abbreviations for "Estimated Intensity of Taste" and "astringency" respectively). It was clarified that green tea infusion is to be classified into eight grades on the EIT_{ast} scale. Furthermore, the high correlation of the EIT_{ast} value with the human gustatory sense and the high stability of the taste sensor were proved.

Key words: evaluation of astringency; taste sensor; green tea; EGCg

The taste of green tea infusion is an important factor for evaluating its quality. An organoleptic test has been considered the only means of the evaluation, because the human tongue is able to recognize the subtle difference in the taste controlled by various factors such as synergistic or inhibitory action among taste substances. However, it is not easy to obtain objectivity and reproducibility in the data by this classical method because of the effects of the test conditions and personal preferences of the panelists.

Recently, a taste sensor system which mimics the human response to food taste has been developed as an objective sensing technology by the Toko group in cooperation with Insent (Intelligent Sensor Technology, Inc., Kanagawa, Japan).^{1–3} This novel system is equipped with several sensor probes corresponding to saltiness, bitterness, sourness, and astringency, and detects the intensity of each taste as an electrical potential difference. It has been used in attempts to evaluate the taste of medicines^{4–6} and such foodstuffs as beer,⁷ mineral water,⁸ coffee,⁹ and milk.^{10,11} However,

although the results of these analyses are undoubtedly objective, it is difficult to regard them as universal data, because commercial foods have been used as a standard solution for the taste sensor analysis. Commercial products always include the possibility that the quality varies among the lots, deliberately or accidentally. In addition, there was room for improvement in the method for converting the sensor output into the corresponding intensity of the taste in order to gain more precise data.

In this study, a practical method for universal evaluation of the astringency of green tea infusion by a taste sensor system was established. We report here the technique for the precise measurement and the grading method of the astringency.

Materials and Methods

Materials. All chemicals were obtained from commercial suppliers and used without further purification. (–)-Epigallocatechin-3-*O*-gallate (EGCg) for the standard solution in a taste sensor analysis was purchased as TEAVIGO™ from DSM Nutritional Products (Heerlen, Netherlands). EGCg, (–)-epicatechin-3-*O*-gallate (ECg), (–)-epigallocatechin (EGC), and (–)-epicatechin (EC) for the samples in the taste sensor analysis and the standard solution in HPLC analysis were purchased from Nakahara Kagaku (Gifu, Japan). Crude tea leaves were manufactured at the National Institute of Vegetable and Tea Science (Shizuoka, Japan). Refined tea leaves were obtained from commercial suppliers.*

Preparation of green tea infusions for taste sensor analysis. Green tea leaves (2 g) were added to a nylon filter cup in a glass pot (type GAV-2, Selec, Gifu, Japan). Boiling water (200 ml) was poured into the pot. The mixture was allowed to stand for 5 min at ambient

* Crude tea is tea manufactured from fresh leaves by steaming, rolling, and drying. Refined tea is commercially available tea produced from crude tea by firing, sorting, blending, etc.

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temperature. The nylon filter cup was removed and the residual infusion among the tea leaves was strained from the filter cup into the glass pot without swinging. The infusion was cooled to 0°C in an ice-water bath (no precipitate apparently caused by cooling was observed in the infusion), and then the temperature was raised to ambient temperature. The infusion was passed through a paper filter (Advantec, no. 2, Toyo Roshi, Tokyo, Japan). Thirty-five ml of the filtrate was used for the taste sensor analysis.

Measurement of astringency of green tea infusion by the taste sensor system. The electrical potential difference corresponding to the astringency of a sample solution was measured by the SA402B taste sensor system (Insent, Kanagawa, Japan), fitted with a sensor probe for astringency (SB2AE1) and a reference probe.¹²⁾

The electrical potential difference (δE_{ast}) of a solution, which is the information on astringency, was recorded as the difference between the potential detected by the sensor electrode and the potential detected by the reference electrode. Figure 1 shows the measuring procedure for a sample. After the sensor electrode and the reference electrode were dipped into the sample solution (or the standard solution) for 30 s and briefly washed in 30 mM KCl aqueous solution including 0.30 mM tartaric acid, $\delta E_{ast}^{sam(CPA)}$ (or $\delta E_{ast}^{std(CPA)}$) was

acquired in 30 mM KCl aqueous solution including 0.30 mM tartaric acid. This is the potential difference generated by the chemical substance adsorbed on the lipid membrane of the sensor in the sample solution (or the standard solution). Here, “CPA” is an abbreviation for Change of membrane Potential caused by Adsorption. The astringency of the sample solution was defined as the difference ($\Delta E_{ast}^{sam(CPA)}$) between $\delta E_{ast}^{sam(CPA)}$ and $\delta E_{ast}^{std(CPA)}$, that is, $\Delta E_{ast}^{sam(CPA)} = \delta E_{ast}^{sam(CPA)} - \delta E_{ast}^{std(CPA)}$, because the CPA method is excellent in that it is able to detect astringency selectively.¹³⁾

The SA402B taste sensor system is able to analyze eight samples or fewer of the tea infusion at a time. The one-measurement cycle was performed in the following order: the standard solution (0.65 mM EGCg aqueous solution including 5 mM KCl), 0.26 mM EGCg aqueous solution including 5 mM KCl (for calculating the EIT_{ast} values), the first sample solution, the second sample solution, . . . , and the last sample solution. The measurement was automatically carried out for five cycles at 298 K. The average of three data excluding those in the first and last measuring cycles was adopted as the $\Delta E_{ast}^{sam(CPA)}$ value of each sample solution.^{**}

Measurement of the $\Delta E_{ast}^{sam(CPA)}$ values for the various concentrations of EGCg. EGCg aqueous solutions of the following concentrations including 5 mM KCl were prepared: (i) 2.2 μ M, (ii) 6.5 μ M, (iii) 22 μ M, (iv) 65 μ M, (v) 0.22 mM, (vi) 0.65 mM, (vii) 2.2 mM, and (viii) 6.5 mM. The $\Delta E_{ast}^{sam(CPA)}$ values for each solution were measured under the foregoing conditions for the green tea infusions on the basis of 5 mM KCl aqueous solution.

Measurement of astringency of the catechins (EGCg, ECg, EGC, and EC) by the taste sensor system. Four 10 mM KCl aqueous solutions (10 ml) including the following compounds were prepared: (i) EGCg (1.00 mg, 2.18 μ mol), (ii) ECg (0.964 mg, 2.18 μ mol), (iii) EGC (0.668 mg, 2.18 μ mol), and (iv) EC (0.633 mg, 2.18 μ mol). The $\Delta E_{ast}^{sam(CPA)}$ values for each solution were measured under the foregoing conditions for the green tea infusions on the basis of 10 mM KCl aqueous solution.

Organoleptic test. The 15 green tea samples for the organoleptic test were prepared by the following procedure: Green tea leaves (5 g) were added to a nylon filter cup in a glass pot (type GV-3, Selec, Gifu, Japan). Boiling water (500 ml) was poured into the pot. The mixture was allowed to stand for 5 min at ambient temperature. The nylon filter cup was removed and the residual infusion among the tea leaves was strained from the filter cup into the glass pot without swinging. The infusion was cooled to 0°C in an ice-water bath (no

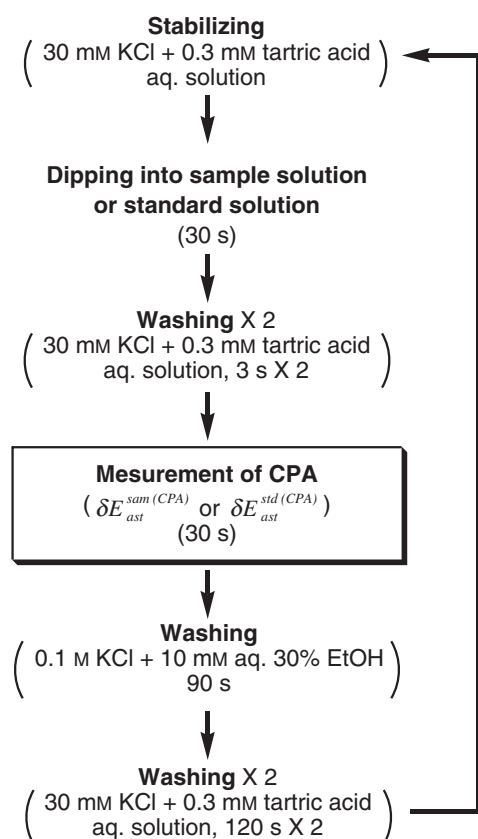


Fig. 1. Measurement Procedure by the Taste Sensor System.

** The first measurement cycle was carried out to stabilize the sensor electrode and the reference electrode. The last measurement cycle was performed to obtain spare data in case of trouble.

precipitate apparently caused by cooling was observed in the infusion), and then the temperature was raised to ambient temperature.

Organoleptic tests were performed with six healthy (trained and expert) panelists. Each sample was kept in the panelist's mouth for 5 s, and then was graded according to relative astringency.

Preparation of sample solutions for HPLC analysis.

The sample solution prepared for the taste sensor analysis was diluted 10 times. The solution was passed through a syringe filter (DISMIC-13HP, pore size: 0.45 μm , Advantec, Toyo Roshi, Tokyo, Japan), and then used for HPLC analysis.

HPLC conditions.¹⁴⁾ HPLC was performed on a system composed of a pump (PU-986, Jasco, Tokyo, Japan), an ultraviolet-visible detector (SPD-10Avvp, Shimadzu, Kyoto, Japan), a low-pressure gradientor (LPG-1000, Eyela, Tokyo Rikakikai, Tokyo, Japan), a column oven (CTO-10Asvp, Shimadzu, Kyoto, Japan), and a degasser (GASTORR 102, Flom, Tokyo, Japan). A column (Wakopak Navi C18-5, ϕ 4.6 mm \times 150 mm, Wako, Osaka, Japan) and pre-column (Wakopak Navi C18-5, ϕ 4.6 mm \times 10 mm, Wako, Osaka, Japan) were installed in a column oven and then maintained at 40 $^{\circ}\text{C}$. The samples (20 μl) were injected and eluted with a gradient solvent system. Solvents A and B consisted of acetonitrile/water/phosphorous acid = 10/400/1 (v/v/v) and solvent A/methanol = 2/1 (v/v) respectively. The gradient program was the following: 0–2 min, solvent A/solvent B = 100/0; 2–40 min, solvent A/solvent B = 100/0 to 20/80 at linear rate; 40–50 min, solvent A/solvent B = 20/80; 50–60 min, solvent A/solvent B = 100/0. The flow rate was 1.0 ml/min. Detection was carried out at 272 nm.

Results and Discussion

Determination of the standard solution for the taste sensor analysis

Fixing a standard point for a measurement is one of the most important processes in a quantitative analysis. In taste sensor analysis, the standard solution is required to possess the property that the $\delta E_{ast}^{std(CPA)}$ value and the chemical behavior are close to the object of the analysis in order to obtain more accurate data. However, in the case of green tea infusion, the standard solution such as a commercially available tea drink is not adequate for the reason described in the introduction. Accordingly, an EGCg aqueous solution was taken to be the most proper candidate, because EGCg is regarded as one of the main constituents which contribute to the astringency of green tea infusion. The diagram in Fig. 2 shows the relation between the concentration of EGCg and the corresponding $\Delta E_{ast}^{sam(CPA)}$ value on the basis of 5 mM KCl aqueous solution. On the other hand, the taste sensor analysis of the fifty sample solutions prepared from the crude tea

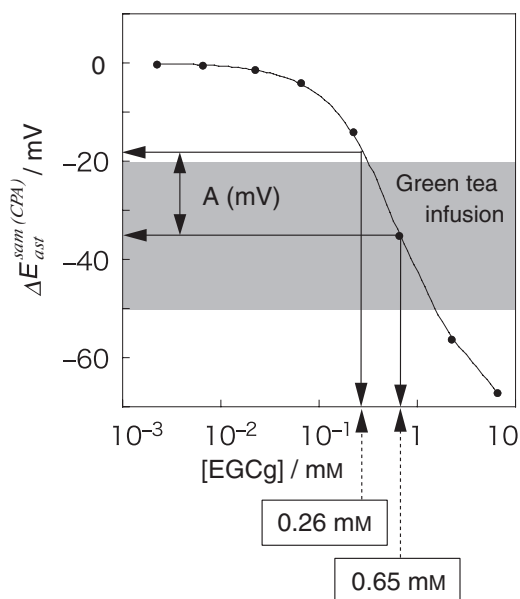


Fig. 2. The Relation between the $\Delta E_{ast}^{sam(CPA)}$ Value and the Concentration of EGCg.

leaves revealed that the $\Delta E_{ast}^{sam(CPA)}$ values of the green tea infusion are approximately within the range of -50 to -20 mV under the same conditions (the shaded zone in Fig. 2). If the EGCg solution whose $\Delta E_{ast}^{sam(CPA)}$ on the basis of 5 mM KCl aqueous solution is the midpoint between -50 mV and -20 mV is used as a standard solution, it is assumed that the measurement error is minimal, because only this can most effectively shorten the distance between every $\delta E_{ast}^{sam(CPA)}$ and $\delta E_{ast}^{std(CPA)}$. Therefore, 0.65 mM EGCg aqueous solution was adopted as the standard (the concentration of the EGCg solution whose $\Delta E_{ast}^{sam(CPA)}$ value on the basis of 5 mM KCl aqueous solution is the midpoint between -50 mV and -20 mV is about 0.65 mM), and the astringency of the green tea infusion was defined as the difference ($\Delta E_{ast}^{sam(CPA)}$) between the $\delta E_{ast}^{sam(CPA)}$ of the infusion and the $\delta E_{ast}^{std(CPA)}$ of 0.65 mM EGCg aqueous solution.

Astringent intensity notation

Although the taste sensor system records taste information as the electrical potential difference as described above, the relation between the difference in the $\Delta E_{ast}^{sam(CPA)}$ values and the discrimination threshold of astringency is unclear. It has been reported that the intensity of basic taste feeling is proportional to the logarithm of concentration of taste substances,¹⁵⁾ and human beings can generally distinguish a difference of 1.2-times concentration of taste substances¹⁶⁾ (these follow Weber-Fechner and Weber's laws). Therefore, in this study, it was hypothesized that intensity of astringency also is proportional to the logarithm of concentration of the astringent substances, and one unit of astringent intensity was defined as the amount of $\Delta E_{ast}^{sam(CPA)}$ corresponding to a difference of 1.2-times concentration of astringent substances. The values on

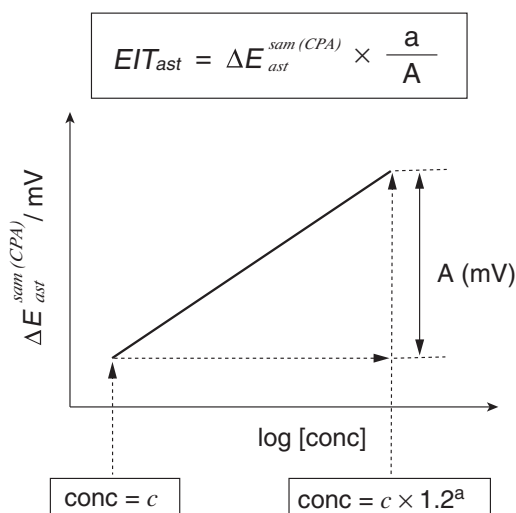


Fig. 3. The Concept of EIT (Estimated Intensity of Taste).

this scale were named EIT_{ast} . The $\Delta E_{ast}^{sam(CPA)}$ value is converted easily into the corresponding EIT_{ast} value if the difference in $\Delta E_{ast}^{sam(CPA)}$ between the two concentrations is available. This concept is illustrated in Fig. 3. If the difference in the $\Delta E_{ast}^{sam(CPA)}$ value corresponding to the difference in 1.2^a -times concentration is A mV, the EIT_{ast} value is calculated by multiplying a/A by the $\Delta E_{ast}^{sam(CPA)}$ value (in the present study $-a/A$ is multiplied in practice, because the $\Delta E_{ast}^{sam(CPA)}$ value decreases with increasing astringency). However, there have been two problems in the previous method for calculating a/A . One is that one of the two points for the calculation has been set to 0 mM ($= 0$ mV). By this method the precise a/A value is not always obtained, because the relationship between the electrical potential difference and the concentration is not necessarily linear, as shown in Fig. 2. The other is that the measurements to gain a/A have not been executed in the same cycle with the sample solutions, but have been carried out separately. In this study, in order to realize a more accurate conversion, a/A was calculated from two points which show a linear relationship on the graph in Fig. 2 and

were almost covered by the range of the $\Delta E_{ast}^{sam(CPA)}$ of the green tea infusion (-50 to -20 mV): the 0.65 mM (the standard solution) and 0.26 mM EGCg aqueous solution including 5 mM KCl were selected as the two points. Furthermore, the $\delta E_{ast}^{std(CPA)}$ values of these solutions were always measured in one cycle with the sample solutions (see “Materials and Methods”). In the present case, $-5.03/A$ was multiplied by the $\Delta E_{ast}^{sam(CPA)}$ value to obtain the corresponding EIT_{ast} value, because 0.65 mM is 2.5 ($= 1.2^{5.03}$) times as much as 0.26 mM (the value of A was obtained in the each measurement).

Grading of green tea astringency

According to the foregoing concept, the EIT_{ast} values of the 80 green tea samples were calculated. The EIT_{ast} values ranged from about -3 to about $+3$, as shown in Fig. 4. It was thought that the EIT_{ast} value of every green tea infusion was not far from the range (-3 to $+3$), because these samples were composed of infusions prepared from the tea leaves (crude tea leaves and refined tea leaves) with as many properties as possible, which were attributed to the cultivar, the cultivation, manufacturing, etc. The samples whose EIT_{ast} values are more than $+3$ or less than -3 should be classified into the extra astringent or the extra non-astringent level respectively, because further grading in these excessive areas appears to be meaningless in practice. Hence, it was proposed that the astringency of the green tea infusion be classified into eight grades by the taste sensor system: in non-astringent order, level 1 ($EIT_{ast} < -3$), level 2 ($-3 \leq EIT_{ast} < -2$), level 3 ($-2 \leq EIT_{ast} < -1$), level 4 ($-1 \leq EIT_{ast} < 0$), level 5 ($0 \leq EIT_{ast} < +1$), level 6 ($+1 \leq EIT_{ast} < +2$), level 7 ($+2 \leq EIT_{ast} < +3$), and level 8 ($+3 \leq EIT_{ast}$).

The relation between the EIT_{ast} values and the human gustatory sense

Fifteen of the samples used in Fig. 4 were graded organoleptically according to relative astringency. Figure 5 shows a plot of EIT_{ast} values against scores on the organoleptic test, where a higher score represents greater astringency. These results show the high corre-

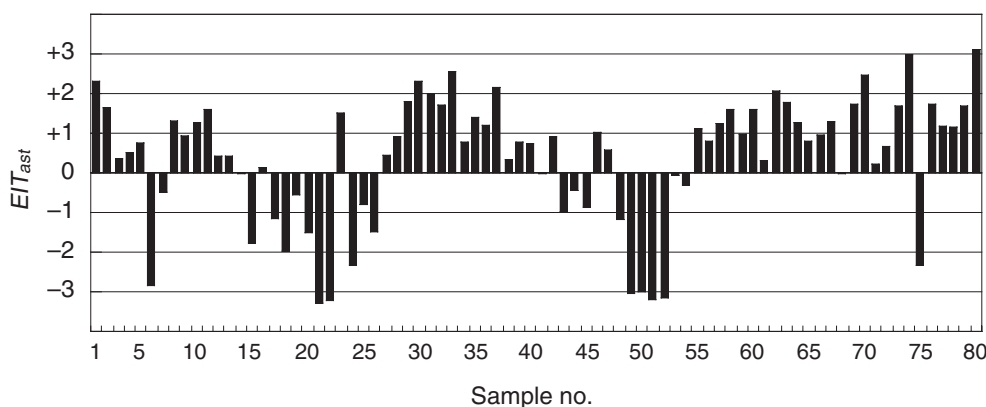


Fig. 4. The EIT_{ast} Values of the 80 Green Tea Infusions.

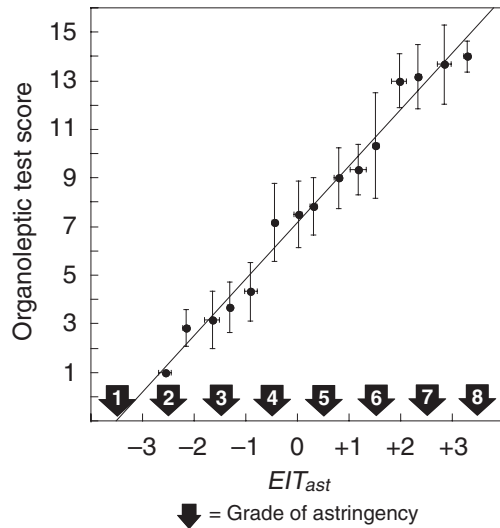


Fig. 5. The Correlation between the EIT_{ast} Values and the Human Gustatory Sense.
The error bars indicate standard deviations.

lation of the EIT_{ast} value with the human gustatory sense (linear correlation coefficient = 0.99), and show that the EIT_{ast} value correctly evaluated the astringency of the green tea infusion. Hence, it was concluded that the foregoing eight-step grading of the astringency reflected the human gustatory sense and was an appropriate method.

Stability of the taste sensor

The stability of the taste sensor under the foregoing conditions was inspected. Sixteen sensor probes were used in 15 successive measurements for three tea samples (one measuring experiment consisted of 5 cycles; see *Measurement of astringency by the taste sensor system* in “Materials and Methods”). Figure 6

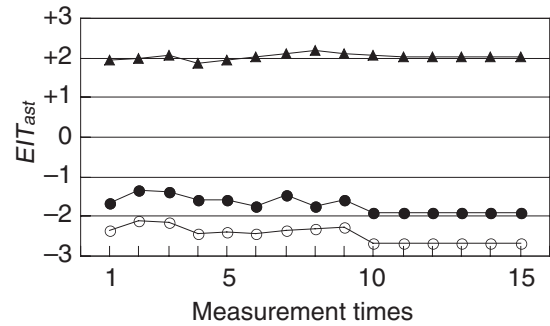


Fig. 6. The Stability of the Taste Sensor.
Changes in the Averages of the EIT_{ast} Values from 16 Sensors.
Sample A (○), Sample B (●), and Sample C (▲).

shows the changes in the averages of the EIT_{ast} values from 16 sensors. The amount of the change for each sample was within one unit on the EIT_{ast} scale. Both the measurement errors and the sensor errors were also within one unit (Table 1), where the measurement errors and the sensor errors are the averages of the 16 standard deviations of the EIT_{ast} values from each sensor and the standard deviations of the 16 EIT_{ast} values from each sensor, respectively. These results show that the present analytical method is accurate and reproducible.

The relation between EIT_{ast} values and amount of the catechins

The relation between the EIT_{ast} values and the amount of the four major catechins (EGCg, ECg, EGC, and EC), which are regarded as the main astringent substances in green tea infusion, was examined for the samples shown in Fig. 4. The amount of the catechins was determined by HPLC. In Fig. 7, the EIT_{ast} values are plotted against the concentration of the catechins. Here, the concentration was not calculated by the simple

Table 1. Measurement Error^a and Sensor Error^b in Stability Test

Times	Sample A		Sample B		Sample C	
	Measurement error	Sensor error	Measurement error	Sensor error	Measurement error	Sensor error
1	0.0654	0.3033	0.0715	0.1715	0.0425	0.1427
2	0.0678	0.1727	0.0402	0.1913	0.0411	0.1799
3	0.0437	0.1075	0.0742	0.1139	0.0904	0.1240
4	0.0461	0.2838	0.0350	0.1248	0.0294	0.2877
5	0.0462	0.1274	0.0223	0.0410	0.0503	0.1049
6	0.0355	0.0610	0.0316	0.0733	0.0675	0.1138
7	0.0331	0.0738	0.0466	0.0792	0.0349	0.1522
8	0.0761	0.1345	0.0530	0.0868	0.0510	0.1738
9	0.0311	0.0700	0.0381	0.1478	0.0760	0.2420
10	0.0310	0.0813	0.0364	0.0773	0.0308	0.1994
11	0.0370	0.0657	0.0554	0.0438	0.0312	0.1484
12	0.0370	0.0657	0.0554	0.0438	0.0312	0.1484
13	0.0370	0.0657	0.0554	0.0438	0.0312	0.1484
14	0.0370	0.0657	0.0554	0.0438	0.0312	0.1484
15	0.0370	0.0657	0.0554	0.0438	0.0312	0.1484

^aAverages of the 16 standard deviations of the EIT_{ast} values from each sensor.

^bStandard deviations of the 16 EIT_{ast} values from each sensor.

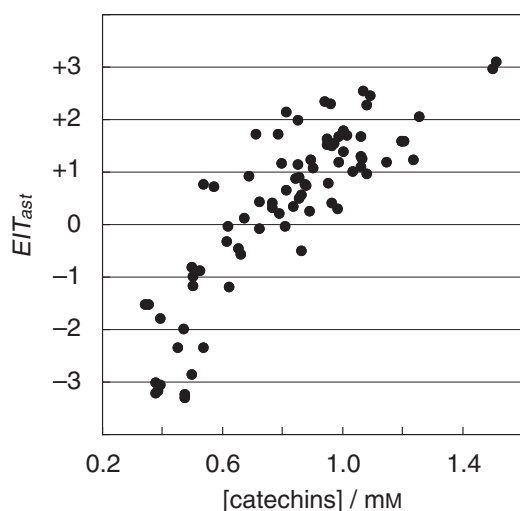


Fig. 7. The Relation between the EIT_{ast} Value and the Concentration of the Catechins ([catechins]/mM).
 $[\text{catechins}] = [\text{EGCg}] + 0.94[\text{ECg}] + 0.036[\text{EGC}] + 0.087[\text{EC}]$.

addition of the concentration of each catechin, because differences in astringency among the catechins at the same concentration must be considered. The ratio of the $\Delta E_{ast}^{sam(CPA)}$ of the catechins at the same concentration was $\text{EGCg}/\text{ECg}/\text{EGC}/\text{EC} = 1/0.94/0.036/0.087$. Consequently, the concentrations of the catechins ([catechins]) in Fig. 7 were evaluated in terms of that of EGCg by the following equation: $[\text{catechins}] = [\text{EGCg}] + 0.94[\text{ECg}] + 0.036[\text{EGC}] + 0.087[\text{EC}]$. Figure 7 showed that, although the EIT_{ast} values were macroscopically correlative to the [catechins] values, there is microscopic dispersion of more than two units on the EIT_{ast} scale in spite of almost the same concentration. These results demonstrate that, although the intensity of the astringency of green tea infusion mainly depended on the amounts of the major catechins, other factors (the other polyphenols, the unknown astringent substance, and the inhibitory or synergistic action of astringency by interaction among the taste substances) also could not be disregarded.¹²⁾ Analysis by the taste sensor system appears to be able properly to estimate such overall astringency of green tea infusion in contrast with any previous analytical method.

Conclusion

Several techniques for universal evaluation of the astringency of green tea infusion by the taste sensor system have been described. The use of the standard solution (0.65 mM EGCg aqueous solution) made it possible to obtain universal data with high accuracy and reproducibility. The astringency of the green tea infusion was classified into eight grades on the EIT_{ast} scale. Furthermore, the high correlation of the EIT_{ast} value with the human gustatory sense and the high stability of the taste sensor were proved. The methodology reported here is applicable to evaluating systems for other foods or tastes.

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