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Evaluation of the Umami Taste Intensity of Green Tea by a Taste Sensor

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A method for evaluating the umami taste intensity of green tea by a taste sensor system was established. Interference in the measurement from catechins was solved by removing the catechins from sample solutions with poly(vinylpolypyrrolidone). A 5.00 mM aqueous solution of glutamic acid monosodium salt was used as the standard solution. Sensor outputs were converted into EIT_{uma} (estimated intensity of taste concerning umami) values. One unit on the EIT_{uma} scale was defined as the amount of the sensor output corresponding to a difference in 1.2 times the concentration of the standard substance (glutamic acid monosodium salt). The umami taste intensity of green tea was classified into six grades on the EIT_{uma} scale. Sensory tests proved that the EIT_{uma} value had a high correlation to the human gustatory sense.

KEYWORDS: Umami taste; taste sensor; poly(vinylpolypyrrolidone)

INTRODUCTION

The taste of food is one of the most important factors in its quality and generally has been evaluated by a human gustatory sense. However, the subjectivity and low reproducibility in this sensory test often have been pointed out as faults. To solve these problems, an objective evaluation method using a taste sensor has recently attracted attention. The taste sensor system developed by the Toko group in cooperation with Insent (Intelligent Sensor Technology, Inc.) is a biomimetic sensing device (*1–3*) that detects taste information as electrical potential changes with several sensor probes corresponding to human taste cells and has been applied to evaluations of various drinks, for example, mineral water (*4*), coffee (*5*), and milk (*6, 7*). Our recent studies have revealed that the taste sensor system is also extremely useful for an objective evaluation of the astringent taste intensity of tea (*8–10*). The use of a standard solution prepared from pure chemicals (in the past, commercially available tea drinks have been used as standard solutions) and introduction of an EIT (estimated intensity of taste) scale made universal evaluation of the astringent taste intensity of green tea infusions possible (*8*). One unit in the EIT scale was defined as the amount of sensor output corresponding to a difference of 1.2 times the concentration of the standard solution. As soon as the evaluation method for green tea astringency was established, we embarked upon the development of an evaluation method for the umami taste intensity of green tea. The umami taste is one of the five basic tastes and essential to an evaluation of the taste of Japanese green tea. However, obstacles were encountered at once. Curiously, the umami sensor output tends to be large when the astringency of the tea infusion is strong, even though the umami taste is in fact weak.

In this work, we revealed that the above discord between the umami taste sensor outputs and the results of human sensory tests was caused by catechins in the green tea infusion, and we succeeded in evaluating the umami taste intensity of green tea using the taste sensor system by removal of the catechins. The measurement method, the grading of the umami taste intensity of green tea, and the relationship between the sensor output and the human gustatory sense are reported here.

MATERIALS AND METHODS

Materials. All chemicals and green tea leaves were obtained from commercial suppliers. (−)-Epigallocatechin-3-*O*-gallate (EGCg) was obtained by recrystallizing TEAVIGO (DSM Nutritional Products, Heerlen, Netherlands) from hot distilled water. Tea infusions and aqueous solutions were prepared with distilled water.

Preparation of Green Tea Infusions for Taste Sensor Measurement. Green tea leaves (2.00 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, and the mixture was allowed to stand for 5 min at ambient temperature (25 °C). 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 agitation. The infusion was cooled to ambient temperature in an ice−water bath. Poly(vinylpolypyrrolidone) (PVPP) (2.00 g) was added to a 100 mL aliquot of the infusion. The resulting mixture was shaken every 10 min for 1 h and filtered through filter paper (Advantec, no. 2, Toyo Roshi, Tokyo, Japan). The filtrate was used for the taste sensor measurement.

Measurement of the Umami Taste Intensity by the Taste Sensor System. The electrical potential corresponding to the umami taste intensity

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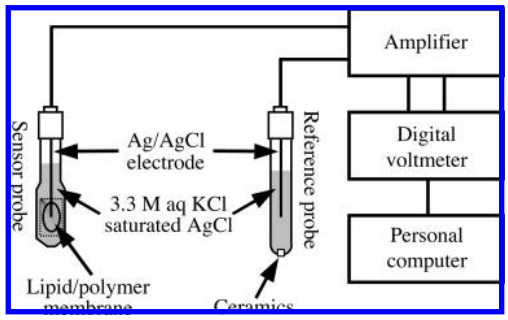

Figure 1. Schematic diagram of the taste sensor system.

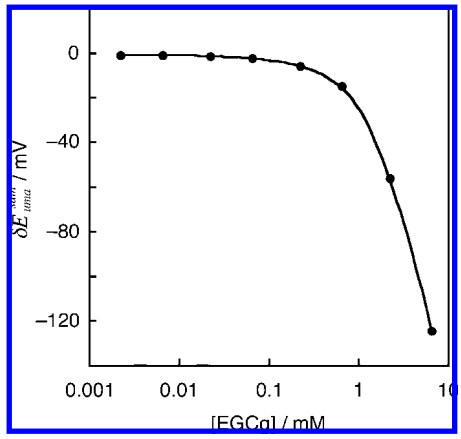

Figure 2. Response of the umami taste sensor for EGCg.

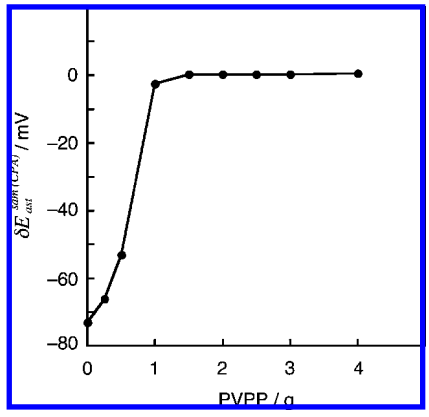

Figure 3. Addition experiment of PVPP into the EGCg aqueous solution: changes in the outputs of the astringent taste sensor ($\delta E_{ast}^{sam(CPA)}$).

of a sample solution was measured by the SA402B taste sensor system (Intelligent Sensor Technology, Inc., Kanagawa, Japan), fitted with a sensor probe for umami taste (SB2AAE) and a reference probe (**Figure 1**). The sensor probe consists of a lipid/polymer membrane, an Ag/AgCl electrode, and an internal cavity filled with a 3.3 M KCl aqueous solution saturated with AgCl. The lipid/polymer membrane is composed of lipids [methyltrioctylammonium chloride and di(2-ethylhexyl) phosphate], polymer [poly(vinyl chloride)], and dioctyl phenylphosphonate as a plasticizer. The reference probe consists of a liquid junction made with ceramics, an Ag/AgCl electrode, and an internal cavity filled with a 3.3 M KCl aqueous solution saturated with AgCl.

The sensor measurement was automatically carried out at 25 °C. The sensor probe and the reference probe were dipped into the sample solution or the standard solution for 30 s to detect the membrane potential change (δE_{uma}^{sam} or δE_{uma}^{std}), where δE_{uma}^{sam} and δE_{uma}^{std} are the membrane potential changes in the sample and in the standard solutions, respectively. The probes were then washed in acidic aqueous ethanol (30% aqueous ethanol including 0.1 M aq HCl) for 90 s, followed by washing in 30 mM KCl aqueous solution including 0.30 mM tartaric acid for 120 s two times. The measurements were performed in the

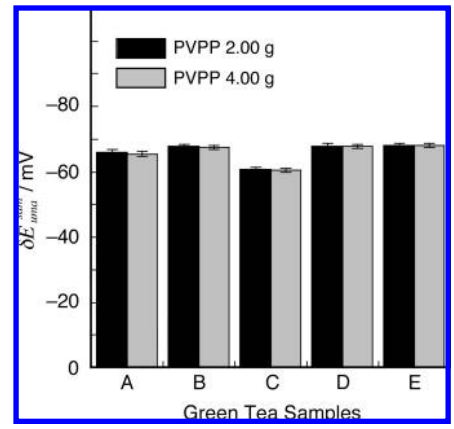

Figure 4. Addition experiments of PVPP into the green tea infusions (100 mL): comparison with the outputs of the umami taste sensor (δE_{uma}^{sam}) between addition of 2.00 and 4.00 g. The error bars indicate standard deviations.

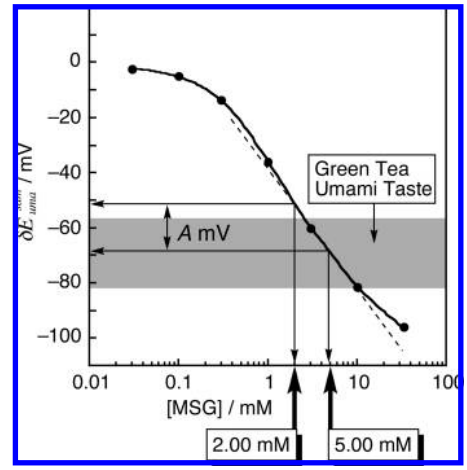

Figure 5. Relationship between the δE_{uma}^{sam} value and the concentration of glutamic acid monosodium salt.

following order: the standard solution [5.00 mM glutamic acid monosodium salt (MSG) aqueous solution including 30 mM KCl and 0.30 mM tartaric acid], 2.00 mM MSG aqueous solution including 30 mM KCl and 0.30 mM tartaric acid (for calculating the EIT_{uma} values), then sample solutions.

The umami taste intensity was defined as the difference (ΔE_{uma}^{sam}) between δE_{uma}^{sam} and δE_{uma}^{std}. The ΔE_{uma}^{sam} value of each sample solution was calculated by the average of three measurements.

Sensory Test. The 12 green tea infusions for the sensory test were prepared by the following procedure. Green tea leaves (5.00 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 (25 °C). 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 stirring. The infusion was cooled to ambient temparature in an ice−water bath.

Sensory tests were performed with nine healthy, trained, and expert panelists, who graded the green tea samples according to relative intensity of umami taste.

Validation. The repeatability and reproducibility of the sensor measurements were assessed by the method of Mizukami et al. (*11*). To investigate the intraday variation of the δE_{uma}^{sam} values, the tea infusion was prepared in triplicate, and each aliquot was measured in triplicate on the same day. The relative standard deviation (RSD %) was calculated on the basis of the nine measurements. An interday variation in the δE_{uma}^{sam} values was assessed by performing the measurements on three consecutive days with freshly prepared tea infusions (in triplicate). The nine measurements obtained on each day were treated as single data points; the RSD was calculated between days.

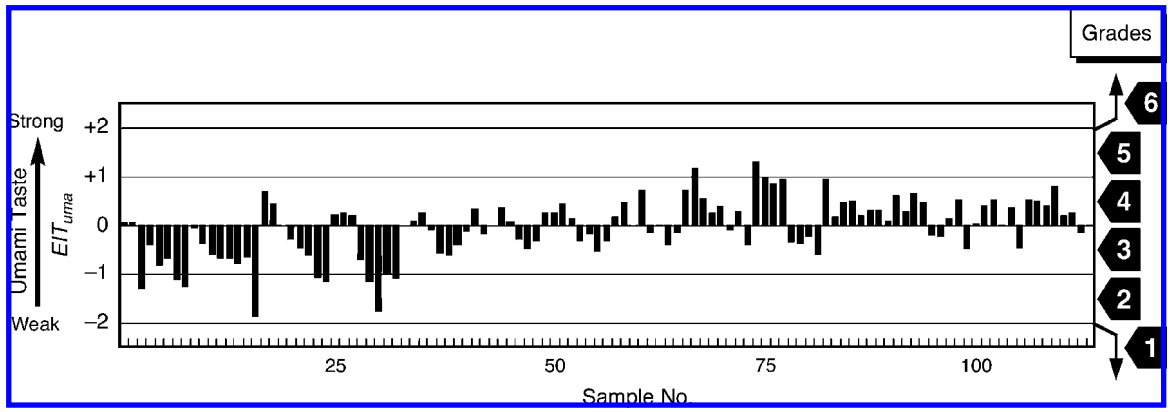

Figure 6. EIT_{uma} values of 111 green tea infusions and the grading system.

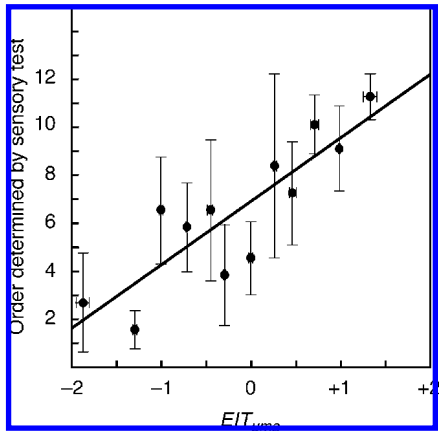

Figure 7. Relationship between the EIT_{uma} value and the human gustatory sense. The error bars indicate standard deviations.

Response of the Umami Sensor for EGCg. EGCg aqueous solutions of the following concentrations including 30 mM KCl and 0.30 mM tartaric acid were prepared: 2.20 μM, 6.50 μM, 22.0 μM, 65.0 μM, 0.220 mM, 0.650 mM, 2.20 mM, and 6.50 mM. The δE_{uma}^{sam} values for each solution were recorded on the basis of the 30 mM KCl aqueous solutions, which included 0.30 mM tartaric acid.

Addition Experiment of PVPP. For eight aliquots (100 mL) of 4.00 mM EGCg aqueous solution including 30 mM KCl and 0.30 mM tartaric acid, 0.250, 0.500, 1.00, 1.50, 2.00, 2.50, 3.00, and 4.00 g of PVPP were added. The resulting mixtures were shaken every 10 min at 25 °C for 1 h, and filtered through filter paper (Advantec, no. 2, Toyo Roshi, Tokyo, Japan). The filtrates and the 4.00 mM EGCg aqueous solution without pretreatment with PVPP were used for measurement of the astringent taste intensity, which followed the procedure in our previous report (*8*). $\delta E_{ast}^{sam(CPA)}$ values for each solution were recorded on the basis of the 30 mM KCl aqueous solutions, which included 0.30 mM tartaric acid, where the $\delta E_{ast}^{sam(CPA)}$ is an electrical potential change detected by a sensor probe for astringent taste (SB2AE1).

Response of the Umami Sensor for MSG. MSG aqueous solutions of the following concentrations including 30 mM KCl and 0.30 mM tartaric acid were prepared: 0.0300, 0.100, 0.300, 1.00, 3.00, 10.0, and 33.3 mM. The δE_{uma}^{sam} values for each solution were recorded on the basis of the 30 mM KCl aqueous solutions, which included 0.30 mM tartaric acid.

RESULTS AND DISCUSSION

Catechin Removal Conditions. We proposed that the discord between the umami sensor output and the sensory test was caused by the catechins that are astringent substances in the green tea infusion. The lipid in the lipid/polymer membrane of the astringent taste sensor is tetradodecylammonium bromide. On the other hand, a quaternary ammonium salt (methyltrioctylammonium chloride) is also used as one of the lipids in the membrane of the umami taste sensor. Although the sensitivity of the umami taste sensor is lowered for the astringent taste substances, there is a possibility that the catechins in the green tea infusion may affect the output of the umami taste sensor, because the green tea infusion contains a very large amount of catechins as compared to other foods. Therefore, the umami taste sensor output for EGCg, which is the major catechin in green tea, was investigated. **Figure 2** shows the relationship between the sensor output (δE_{uma}^{sam}) and the concentration of EGCg. As expected, the umami sensor responded to the catechin. The umami sensor indicated about −30 mV for the 1.3 mM EGCg solution (according to the analysis in ref *8*, EGCg concentrations in the astringent green tea infusions are about 1.3 mM under the present tea extraction conditions). This amount of the sensor output is not negligible, when the range of the umami sensor output for the green tea infusion, which is described below, is taken into account. Accordingly, removal of the catechins from the infusion was attempted with PVPP, which is often used as a polyphenol scavenger (*12*). To estimate the amount of PVPP needed for removing the catechins, an addition experiment of PVPP against 100 mL of 4.00 mM EGCg solution was carried out. According to the analysis in ref *8*, it is presumed that the total catechin concentration does not exceed 4.00 mM under the present tea extraction conditions, no matter how astringent the green tea infusion is. **Figure 3** shows changes in outputs of the astringent taste sensor ($\delta E_{ast}^{sam(CPA)}$) against the added amount of PVPP, revealing that EGCg can be removed with at least 1.50 g of PVPP. Therefore, a catechin-removing process in which the tea infusion (100 mL) is treated with 2.00 g of PVPP was used. In fact, the umami sensor outputs (δE_{uma}^{sam}) did not change between the green tea samples treated with 2.00 g of PVPP and the green tea samples treated with 4.00 g of PVPP (**Figure 4**). This result confirms that 2.00 g of PVPP is adequate to remove the catechins in the samples.

Standard Solution. A standard solution for the taste sensor experiments is required, which possesses sensor output and chemical behavior similar to those of the samples to obtain more accurate data (*8*). An aqueous solution of MSG was considered to be the best candidate, because glutamate is one of the umami substances in the green tea infusion. The diagram in **Figure 5** shows the relationship between the concentration of MSG and the corresponding δE_{uma}^{sam} value on the basis of the 30 mM KCl−0.30 mM tartaric acid aqueous solution. On the other hand, the taste sensor experiments of the 100 sample solutions revealed that the δE_{uma}^{sam} values of the green tea infusion are approximately within the range of −81 to −57 mV shown as a shaded zone in **Figure 5**. The sensor output of the standard solution should

be set around a midpoint in this output range of the tea infusion (*8*). Therefore, a 5.00 mM MSG standard solution was used.

Estimated Intensity of Taste for Umami Taste (EIT_{uma}) of Green Tea. To utilize the ΔE_{uma}^{sam} value for a more practical evaluation of the umami taste intensity, it was converted into an EIT_{uma} value in a way similar to that of the astringent taste. One unit on the EIT_{uma} scale was defined as the amount of the ΔE_{uma}^{sam} corresponding to a difference in 1.2 times the concentration of the standard substance (in this case, MSG). This concept is based on reports that the strength of basic taste is proportional to the logarithm of the concentration of the taste substances (*13*), and human beings can generally distinguish a difference in 1.2 times the concentration of taste substances (*14*). This EIT_{uma} value should be calculated from an electrical potential difference between two points on the linear portion of the curve in **Figure 5** (*8*). Fortunately, the relationship between the δE_{uma}^{sam} value and the concentration of MSG is linear within the sensor output range of green tea. The 5.00 mM (the standard solution) and 2.00 mM MSG solutions including 30 mM KCl and 0.30 mM tartaric acid were selected as these two points. Consequently, the EIT_{uma} value was calculated by $\Delta E_{uma}^{sam} \times (-5.03/A)$, where "*A*" is the electrical potential difference between the 5.00 mM MSG and the 2.00 mM MSG aqueous solutions, and "5.03" is used because 5.00 mM is 2.5 ($=1.2^{5.03}$) times as much as 2.00 mM. The EIT_{uma} value becomes larger with the increase in the strength of umami taste.

Grading of Umami Taste Intensity of Green Tea. The infusions of 111 green tea samples were measured by the taste sensor. The EIT_{uma} values ranged from about -2 to about $+2$, as shown in **Figure 6**. It was thought that the EIT_{uma} value of every green tea infusion would not deviate far from this range, because these samples were composed of infusions prepared from tea leaves with as many properties as possible, which were derived from the cultivar, the cultivation, manufacturing, etc. If the samples whose EIT_{uma} values are more than $+2$ or less than -2 are detected, they can be classified into the extra strong umami taste or the extra weak umami taste, respectively, because further grading in these extreme areas appears to be meaningless in practice. Accordingly, the umami taste intensity of green tea was classified into six grades by the EIT_{uma} value: in order of less intensity, level 1 ($EIT_{uma} < -2$), level 2 ($-2 \leq EIT_{uma} < -1$), level 3 ($-1 \leq EIT_{uma} < 0$), level 4 ($0 \leq EIT_{uma} < +1$), level 5 ($+1 \leq EIT_{uma} < +2$), and level 6 ($+2 \leq EIT_{uma}$).

Relationship between the EIT_{uma} Value and the Human Gustatory Sense. Twelve of the samples used in **Figure 6** were graded organoleptically according to the relative intensity of the umami taste. **Figure 7** shows a plot of the EIT_{uma} value against the relative order determined by the sensory test. A larger number in this order represents stronger umami taste. The EIT_{uma} value correlated well with the human gustatory sense (linear correlation coefficient = 0.86). This result supports our view that the EIT_{uma} value correctly evaluates the umami taste intensity of the green tea infusion. Therefore, it is concluded that the six-step grading system for the umami taste reflected the human gustatory sense and is an appropriate evaluating method.

Validation. The repeatability and reproducibility of the sensor measurements were assessed by the RSD of the δE_{uma}^{sam} values. The RSD values were low both in the intra- and in the interdays, and no significant difference was observed between the intra- and the interday RSD values, 0.39 and 0.50, respectively. These low RSD values, despite including the brewing process in the experimental procedure, show the high repeatability and reproducibility of the present method.

In conclusion, we established a method to evaluate the umami taste intensity of green tea using the taste sensor system. The key was the removal of the interference by the catechins from the tea infusion. For this purpose, pretreatment of the tea infusion with PVPP was effective. Strength of the umami taste was classified into six grades according to the EIT_{uma} value, which was highly correlated with the human gustatory sense. When these data are added to the data of astringent taste obtained by the method in our previous report (*8*), a more particular evaluation of the taste of green tea is possible. Much utilization of these methods is expected at the various scenes where the evaluation of the taste of green tea is required.

ABBREVIATIONS USED

PVPP, poly(vinylpolypyrrolidone); EGCg, (−)-epigallocatechin-3-*O*-gallate; MSG, glutamic acid monosodium salt; RSD, relative standard deviation.

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