Usefulness of Catechins and Caffeine Profiles to Determine Growing Areas of Green Tea Leaves of a Single Variety, Yabukita, in Japan

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Yabukita green tea leaves in Shizuoka, Kagoshima and Mie areas in Japan were analyzed for catechins and caffeine using 6-*O*-*α*-D-glucosyl-*β*-cyclodextrin-modified micellar electrokinetic chromatography (6G-*β*-CD-modified MEKC). Caffeine and seven catechins [(−)-catechin (C), (+)-C, (−)-epicatechin (EC), (−)-catechin gallate (CG), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epigallocatechin gallate (EGCG)], but not (+)-EC, were detected in all extracts. Linear discriminant analysis was applied to differentiate green tea samples according to their geographical origins. As a result, all samples were correctly classified, which represented a recognition ability of 100%. These results suggest that catechins and caffeine profiles of Yabukita green tea leaves could be useful to determine their growing areas in Japan.

Key words —— catechin, green tea, capillary electrophoresis, geographic origin, linear discriminant analysis

INTRODUCTION

Tea, which is one of the most widely consumed beverages in the world, is made from tender leaves of different varieties of *Camellia sinensis*. Many kinds of teas are produced, although these can be classified principally into three types: green (unfermented), oolong (semifermented) and black (fully fermented) teas. Labelling the country of origin on foods including green tea leaves was enacted by Japanese Agricultural Standards in October, 2006. Though the chemical composition of green tea leaves at different tea-producing areas is similar, it is possible to find parameters that may differentiate them. Agricultural conditions, such as climate, soil, water, fertilizers, *etc.*, can constitute important influences on the composition of the crops. A better discrimination may be obtained by using multivariate analysis in combination with some chemometric procedures. Inorganic element composition has been found to be adequate for distinguishing tea leaves cultivated in different countries of origin.[1–3)]

The main components of tea are polyphenolic compounds, commonly known as catechins, which are represented by a group of compounds having a flavan-3-olic structure and constitute up to 30% of the dry weight of the tea leaf. The six major tea catechins seem to be catechin (C), catechin gallate (CG), epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). Recently, Fernandez *et al.*[4)] determined the contents of catechins and xanthines in fermented and nonfermented tea samples and differentiated tea leaves according to geographic origin. They also found a certain separation between Japanese and Chinese green tea leaves. In Japan, green tea is the most popular of the three types of tea. The green tea-producing area of Yabukita, one of tea plant varieties, accounts for approximately 76% of all tea-producing areas in Japan. With encouragement from the tea industry, places where tea plants are cultivated have begun to turn their locations into brands. Thus, methods for differentiation of green teas according to local area of origin as well as country of origin are needed. However, to our knowledge, there is no report to determine

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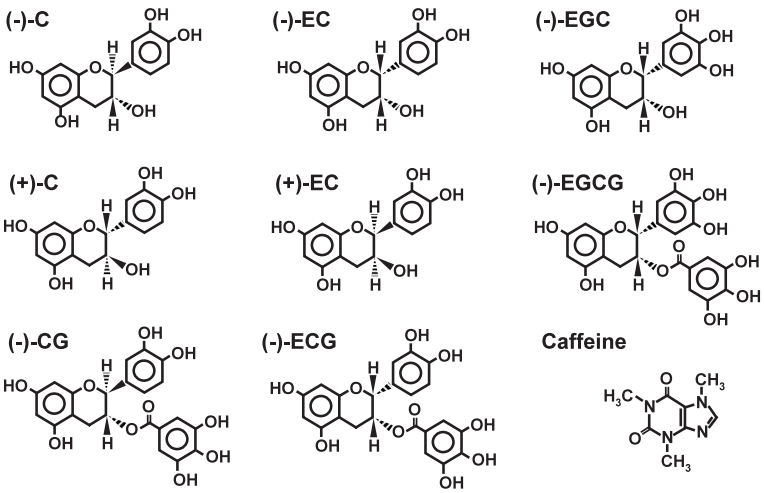

Fig. 1. Structures of Catechins and Caffeine

Fig. 2. Geographic Origins of Green Tea Leaves Used in This Study

growing areas of green tea leaves of a single variety. Recently, we reported that caffeine and 8 catechins (Fig. 1) including optical isomers of C and EC in tea samples were successfully determined by 6-*O*-α-D-glucosyl-β-cyclodextrin-modified micellar electrokinetic chromatography (6G-β-CD-modified MEKC).[5] The aim of the present study was to use a chemometrics approach to determine whether levels of catechins and caffeine are adequate chemical descriptors to differentiate Yabukita green tea samples according to growing areas in Japan. Green tea samples were obtained from Shizuoka, Kagoshima and Mie prefectures (Fig. 2), which are the first, second and third, biggest producers of Yabukita tea in Japan, respectively. Together, these areas accounts for 78% of the total yield of the Yabukita green tea leaves in Japan.

MATERIALS AND METHODS

Chemicals —— (±)-C, (+)-EC, (−)-EC, (−)-CG, (−)-EGC, (−)-EGCG, and (−)-ECG were obtained from Sigma (St. Louis, MO, U.S.A.). Caffeine, 6-*O*-α-D-glucosyl-β-cyclodextrin (6G-β-CD) and other chemicals (analytical grade) were obtained from Wako Pure Chemicals (Osaka, Japan).

Apparatus for Capillary Electrophoresis —— Electrophoretic experiments were carried out using a Capillary Electrophoresis System (Agilent Technologies, Waldbronn, Germany). Separation was performed in a fused-silica bubble cell capillary of 64.5 cm (effective length 56 cm) × 50 μm ID (Agilent Technologies). At the beginning of each day, the capillary was flushed with the background electrolyte (BGE) for 10 min. And then, water, sample solution, water and the BGE were injected at pressure of 50 mbar for 2, 2, 2 and 3 sec, respectively. Between runs, the capillary was flushed with 200 mM borate-20 mM phosphate buffer (pH 6.4) for 2 min and then with the BGE for 10 min. The capillary was kept at 20°C. The analytes were detected at 210 nm. The power supply was operated in the constant-voltage mode, at +25 kV, and the substances migrated towards the negative pole. The resulting current was approximately 85 μA.

Buffer and Sample Preparation for CE —— The BGE in the electrophoretic experiments was 200 mM borate-20 mM phosphate buffer (pH 6.4)

containing 240 mM sodium dodecyl sulfate (SDS) and 25 mM 6G-β-CD, and was filtered with a 0.2 μm filter before use. Purified water was obtained from a Toray (Mishima, Japan) Ultra Pure Water System. Stock solutions of 400 mg/l (±)-C, 400 mg/l (−)-CG, 1000 mg/l (−)-EC, 1000 mg/l (+)-EC, 1000 mg/l (−)-EGC, 1000 mg/l (−)-ECG, 1000 mg/l (−)-EGCG and 1000 mg/l caffeine were separately prepared in water and stored at −20°C. Internal standard solution was composed of 10 mM nicotineamide, 10 mM of *p*-hydroxyphenyl acetic acid and 10 mM of *p*-toluenesulfonic acid. A 20 mg/l standard mixture was prepared by mixing each single stock solution and internal standard solution, having 20 mg/l each of catechins and caffeine and 1 mM each of internal standards.

Green tea samples of Yabukita cultivated in Shizuoka, Kagoshima and Mie prefectures in Japan were used. In each area, four green tea samples grown at different points were obtained from local tea-manufacturers. The tea samples were extracted according to the following procedure: 100 mg of tea sample was extracted with 10 ml of 75% (v/v) ethanol solution at 60°C for 30 min. The extract was cooled and filtered with a 0.2 μm filter. Aliquot of 0.1 ml filtrate was mixed with 0.1 ml of internal standard solution and 0.8 ml of purified water, and was immediately analyzed by 6G-β-CD-modified MEKC.

Data Analysis —— Linear discriminant analysis (LDA)[6] model (SPSS for Windows 13.0J, SPSS Japan Inc., Tokyo, Japan) was adopted to classify all tea samples into the groups of known geographical origin.

RESULTS AND DISCUSSION

Contents of Catechins and Caffeine

The amounts of catechins and caffeine extracted from tea leaves were previously shown to be significantly affected by the composition of extracted solution and temperature.[7] We reported that higher temperature caused an increase in epimerization of (−)-EC and (−)-ECG to (−)-C and (−)-CG, respectively.[8] As a condition giving both high extraction efficiency and low epimerization, eight catechins, (−)-C, (+)-C, (+)-EC, (−)-EC, (−)-CG, (−)-EGC, (−)-EGCG, and (−)-ECG, and caffeine in green tea leaves were extracted with 75% (v/v) ethanol solution at 60°C for 30 min. After extraction, catechins and caffeine were immediately analyzed by 6G-β-CD-modified MEKC (Table 1). A representative electropherogram is shown in Fig. 3. (−)-EGCG in all green tea samples, except for one sample (Mie area No. 3), was present in the highest level, but (+)-EC was not detected in all samples.

Geographical Classification of Tea Samples

The data set consisted of 12 samples. Three categories were considered; Shizuoka, Kagoshima and Mie teas. By using LDA, suitable classification rules for assigning categories to samples can be calculated. The so-called discriminant functions are obtained which best separate the classes according to the maximization of the F-ratio between

Table 1. Contents (mg/kg) of Catechins and Caffeine in Extracts of Yabukita Green Tea Leaves

Samples	(−)-C	(+)-C	CG	EGCG	ECG	EGC	(+)-EC	(−)-EC	CAF	Total
Shizuoka area										
1	11.1	10.3	7.27	781	158	485	N.D.	108	356	1920
2	11.4	6.60	8.86	677	134	687	N.D.	115	339	1980
3	9.38	5.98	11.9	679	128	505	N.D.	98.7	340	1780
4	8.05	8.40	2.57	681	144	435	N.D.	104	340	1720
Kagoshima area										
1	4.54	7.23	5.65	705	149	310	N.D.	68.8	416	1670
2	6.90	7.71	3.39	553	126	385	N.D.	78.4	374	1530
3	4.55	6.54	2.64	720	144	234	N.D.	55.1	455	1620
4	4.05	6.37	2.20	586	135	349	N.D.	73.9	371	1530
Mie area										
1	10.8	7.86	11.9	652	124	499	N.D.	85.0	315	1710
2	9.06	6.90	5.02	596	106	589	N.D.	84.1	245	1640
3	11.2	7.30	9.82	559	106	572	N.D.	91.6	279	1640
4	10.5	8.60	10.8	765	143	436	N.D.	80.7	309	1760

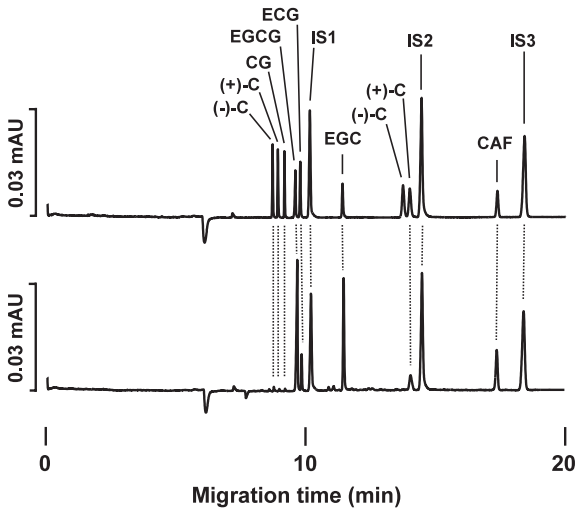

Fig. 3. Electropherograms of a Standard Solution (upper) and a Green Tea Sample (lower)

IS1, 2 and 3 represent nicotineamide, *p*-hydroxyphenyl acetic acid and *p*-toluenesulfonic acid, respectively.

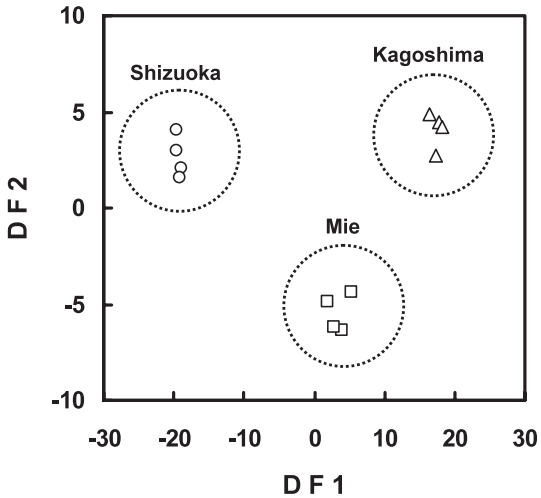

Fig. 4. Scatter Plot of the Two Discriminant Functions for Tea Samples with Known Geographic Origin: Shizuoka, Kagoshima and Mie Areas in Japan

classes sum of squares and within classes sum of squares.[4, 9] After applying LDA to the data in Table 1, two discriminant functions, DF1 and DF2, were calculated as follows;

$$\begin{aligned}\mathrm{DF1} = {} & -0.102\times[(-)\text{-C}]+2.529\times[(+)\text{-C}]+0.602\\ & \times[(-)\text{-CG}]-0.175\times[(-)\text{-EGCG}]\\ & +0.837\times[(-)\text{-ECG}]+0.096\times[(-)\text{-EGC}]\\ & -1.394\times[(-)\text{-EC}]-0.004\times[\text{Caffeine}]\\ & +61.649\end{aligned}$$

$$\begin{aligned}\mathrm{DF2} = {} & -1.164\times[(-)\text{-C}]+0.145\times[(+)\text{-C}]-0.023\\ & \times[(-)\text{-CG}]-0.001\times[(-)\text{-EGCG}]-0.04\\ & \times[(-)\text{-ECG}]+0.002\times[(-)\text{-EC}]+0.219\\ & \times[(-)\text{-EC}]+0.068\times[\text{Caffeine}]-28.085\end{aligned}$$

Figure 4 shows a scatter plot of samples obtained when using DF1 and DF2 as axes. All samples were correctly classified, which indicates a recognition ability of 100%. There is a clear separation among the three teas, which appear as three well-differentiated clusters.

The above equations were calculated on the basis of this particular data set. Thus, two discriminant functions, DF1 and DF2, described above must be newly calculated, when catechins and caffeine in green tea leaves from much more samples and/or tea producing areas are analyzed. Moreover, it is well known that the composition of tea varies with season and age of leaf other than climate and horticultural practices.[10] The storage conditions may also affect the composition of green tea leaves. Therefore, many more samples would be needed to evaluate the prediction ability of the contents of catechins and caffeine in relation to the geographical origins. Nevertheless, it is interesting that green tea leaves of a single variety, Yabukita, could be differentiated according to growing areas in Japan, though the smaller numbers of green tea samples in this particular set.

As concluding remarks, the contents of catechins and caffeine in Yabukita green tea leaves from Shizuoka, Kagoshima and Mie areas in Japan were analyzed by 6G-β-CD-modified MEKC. And then, LDA was applied to differentiate green tea samples according to their geographical origins. Green tea leaves of a single variety, Yabukita, could be differentiated according to growing areas in Japan. Further study using greater numbers of samples from many tea-producing areas is necessary to confirm the differentiation of green tea leaves according to geographical origin.

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