JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

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Characterization of Tea Cultivated at Four Different Altitudes Using ^{1}H NMR Analysis Coupled with Multivariate Statistics

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Supporting Information

ABSTRACT: The taste of black tea differs according to the different areas in which the tea is grown, even for the same species of tea. A combination of ^{1}H NMR spectroscopy and partial least-squares discriminate analysis (PLS-DA) was used to assess the quality differences of tea leaves from four cultivation areas with different elevations, RAN > 1800 m, UDA = 1200 m, MEDA = 600 m, and YATA < 300 m, in Sri Lanka. As a result of a statistical analysis, PLS-DA showed a separation between high- and low-quality black teas derived from the four different tea cultivation areas. RAN from the highest elevation showed characteristic trends in the levels of theaflavin and theaflavin 3,3′-digallate that were found only in RAN, and the levels of theanine and caffeine were higher, and the levels of thearubigins, especially thearubigin 3,3′-digallate, were lower in RAN than in UDA, MEDA, and YATA. The structures of these components were determined by 1D and 2D NMR analyses. These results demonstrate that this method can be used to evaluate black tea quality according to the chemical composition or metabolites, which are characteristic of the tea leaves cultivated in four regions with different elevations in Sri Lanka.

KEYWORDS: Black tea, quality, elevation, ^{1}H NMR, multivariate analysis, PLS-DA

INTRODUCTION

Tea is a beverage consumed widely throughout the world.[1,2] The four main marketed varieties of tea, white, green, oolong, and black, differ in their fermentation process; green tea is unfermented, white tea is lightly fermented, oolong tea is partially fermented, and black tea is fermented. All four teas are made from leaves of the same species of plant, *Camellia sinensis*. On the other hand, there are teas for which the origin is not the *C. sinensis* plant. For example, the term "herbal tea" usually refers to an infusion of other plant material such as leaves, flowers, fruits, or herbs, and the term "red tea" refers to tea made from the South African rooibos plant. According to recent studies, it has been reported that various elements that positively influence the health of people are found in teas.[3−10] Black tea has a mildly fragrant, slightly bitter, astringent flavor. Drinking black tea is associated with several health benefits, in particular related to a reduction of cardiovascular risk.[9,10] Black tea is more oxidized than the oolong, green, and white varieties, has a stronger flavor, and contains more caffeine than the less oxidized teas.[11] After tea leaves are hand-picked and dried, there is a process of mechanical kneading and crushing in the manufacture of black tea. During this manufacturing process, enzyme-catalyzed oxidation and partial polymerization of flavanols occur, and as a result, theaflavins (TFs) and thearubigins characteristic of the black tea taste and color are produced.[2] In addition, flavonoids constitute 10−12% of the dry leaf weight of black tea.[12] Although the tea leaves used to produce black tea are the same species of plant, the taste of teas differs according to differences in the growing environment. Therefore, it is possible that climate condition variations within the same country could result in differences in the chemical composition or metabolites of a tea as well as different tea tastes.

In Sri Lanka, tea is registered according to the different elevations in which the tea leaves are cultivated. There are six principal tea-planting regions: Nuwara Eliya (RAN) > 1900 m, Dimbula (UDA) = 1000−1500 m, Kandy (MEDA) = 600−1200 m, Uva = 914−1524 m, a low-grown area (YATA) < 600 m, and a high-grown area > 1200 m. Many factors including taste and color affect tea quality. Tea leaves are the most basic and important factor for making good quality tea. Although the quality of tea is generally assessed through its scent, flavor, and color, taste is the key evaluation criteria for tea quality. In general, it is difficult to accurately discriminate tea quality by this method, even though the classification of tea quality is mainly performed by professional tea tasters.[13] However, characterization of the differences in the quality of tea leaves according to the different cultivation area elevations in Sri Lanka has not yet been reported. Recently, analytical techniques such as mass spectrometry (MS),[14] nuclear magnetic resonance (NMR),[15] and gas chromatography/mass spectrometry (GC/MS)[16] coupled with multivariate analysis have been employed to evaluate the quality of foods and drugs and to evaluate drug toxicity.[17,18] Among multivariate statistical methods, principal component analysis (PCA) and partial least-squares discriminate analysis (PLS-DA) are often useful for profiling and classifying sample groups and for characterizing the most effective variables in separated compounds. In evaluation of tea quality, NMR can give much more structural information than other analytical techniques;

Received: October 5, 2010
Accepted: April 3, 2011
Revised: March 31, 2011
Published: April 03, 2011

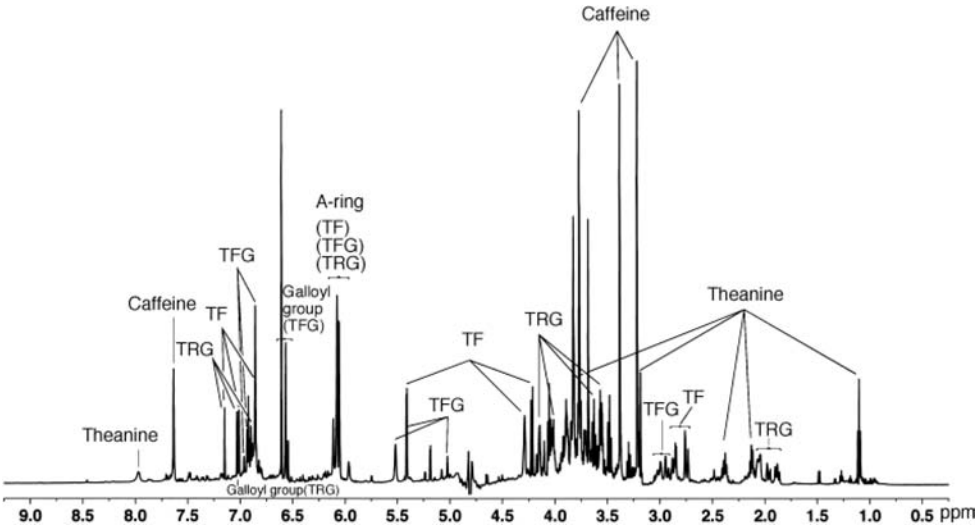

Figure 1. Representative ^{1}H NMR spectrum of black tea from RAN.

especially, the technique is favorable for obtaining an enormous amount of information about the molecular structure of tea components from one- and two-dimensional (1D and 2D) NMR spectra. In addition, by combination with multivariate statistics, the alternation in major components derived from the difference with tea qualities can be characterized.

The aim of this study was to evaluate tea quality according to the chemical composition or metabolites, which are characteristic of tea leaves cultivated in four regions with different elevations in Sri Lanka, using a combination of multivariate statistics with PLS-DA and ^{1}H NMR.

MATERIALS AND METHODS

Chemicals and Reagents. All reagents used for 1D and 2D NMR experiments were of analytical grade (purity >99%) and were used without further purification. Deuterium oxide (D_2O, isotopic purity 99.9%) and TSP [3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid, sodium salt, 98 atom % D] were purchased from Aldrich (St. Louis, MO). TSP was used as an internal standard with a chemical shift (δ) of 0.0 ppm for ^{1}H NMR measurements. Authentic materials for full NMR assignments of TF and theaflavin 3,3′-digallate (TFG) in tea extracts were purchased from Nagara Science (Gifu, Japan).

Origin of Samples. Tea leaf (*C. sinensis*) cultivars harvested from four geographical regions with different elevations in Sri Lanka, Nuwara Eliya (RAN) > 1800 m, Dimbula (UDA) = 1200 m, Kandy (MEDA) = 600 m, and low-grown area (YATA) < 300 m, were kindly supplied by Waltz Co., Ltd. (Toyohashi, Japan) .

Cultivation and Harvest Time and Harvest Portion of Tea Leaves. Tea is cultivated in Sri Lanka using the "contour planting" method, where tea bushes are planted in lines coordinated with the contours of the land, usually on slopes. Tea leaves are harvested throughout the year. Generally, two leaves and a bud, which gives flavor and aroma, are skillfully plucked. Next, the tea leaves are spread in boxes for a process known as withering, which removes excess moisture from the leaves. As a result, the withered tea leaves become rolled and twisted. Finally, the crude tea is made through a process of fermentation and drying. The fermentation conditions in each factory, which belonged to its own field, were almost identical.

Sample Preparation for ^{1}H NMR Spectroscopic Analysis. Two grams of dried tea leaves was added to 150 mL of boiled H_2O (MilliQ). The mixture was boiled in a microwave for 4 min and then was left at 25 °C for 10 min. After centrifugation (3000 rpm for 5 min) by centrifugal filter (Ultrafree-MC, 0.45 μm PVDF membranes, Millipore), 630 μL of filtrate was added by pipet to 70 μL of 5 mM TSP/D_2O to give a 700 μL of solution for the NMR measurement. All samples were freshly prepared just prior to ^{1}H NMR spectroscopic analysis. The sample was introduced into an NMR test tube, and nuclear Overhauser effect spectroscopy (^{1}H NOESY) and 1D and 2D ^{1}H NMR spectra were recorded at 25 °C using a Varian 600 MHz NMR spectrometer equipped with a coldprobe (Figures 1, 2, and 4). Thirty-two free induction decays (FIDs) with 77 K data points per FID were collected using a spectral width of 9615.4 Hz, an acquisition time of 4.00 s, and a total pulse recycle delay of 2.02 s. Water resonance was suppressed by presaturation during the first increment of the NOESY pulse sequence, with irradiation occurring during the 2.0 s relaxation delay and also during the 100 ms mixing time. Prior to Fourier transformation (FT), the FIDs were zero-filled to 128 K, and an exponential line-broadening factor of 0.5 Hz was applied.[19−21] Experiments were carried out in quadruplicate, and all peak intensities (in arbitrary units) were expressed as the means of four separate experiments (n = 4).

NMR Data Reduction and Preprocessing. All ^{1}H NMR spectra were phased, baseline corrected, and referenced to TSP at 0.0 ppm by Chenomx NMR Suite 5.0 software, professional edition (Chenomx Inc., Canada). Each ^{1}H NMR spectrum was subdivided into regions having an equal bin size of 0.04 ppm over a chemical shift range of 0.5−9.0 ppm (excluding the region around the water signal; 4.6−5.0 ppm), and the regions within each bin were integrated. The integrated intensities were then normalized to the total spectral area, and the data were converted from the Chenomx software format into Microsoft Excel format (*.xlt). The resultant data sets were then imported into SIMCA-P version 12.0 (Umetrics AB, Umeå, Sweden) for multivariate statistical analysis.

Multivariate Data Analysis. To maximize the separation between samples, PLS-DA was applied. The PLS-DA can be described as the regression extension of PCA, an unsupervised pattern recognition method,[22,23] giving the maximum covariance between the measured data (X) and the response variable (Y). PLS-DA models were calculated to compare the quality of tea leaves cultivated at four different elevations in Sri Lanka (Figures 5 and 6). The four data points of each sample groups in Figure 6 represent individual experiment. The quality of the PLS-DA models was described by the total variance of PLS1and PLS2 at a confidence level of 95%.

RESULTS AND DISCUSSION

Tea Leaves from Four Different Regions. The black tea in Sri Lanka is famous for its high quality, and as the fourth largest

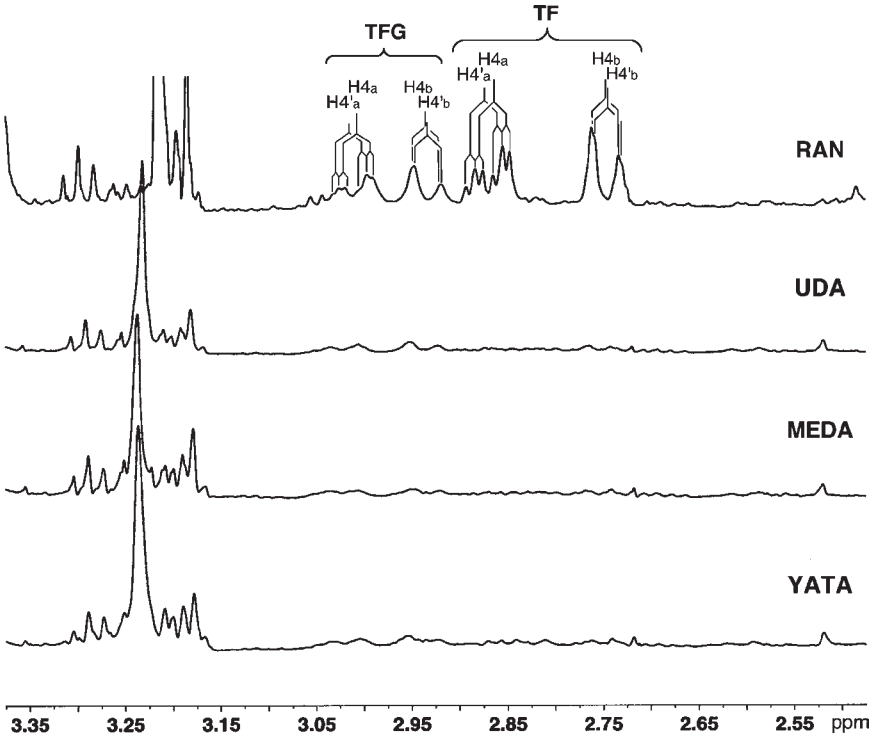

Figure 2. ^{1}H NMR spectrum expansion (2.55−3.35 ppm) of RAN, UDA, MEDA, and YATA.

tea-producing country after China, India, and Kenya, it has a production share of 9% of the international tea market. Four cultivation areas for black tea in Sri Lanka, Nuwara Eliya (RAN), Dimbula (UDA), Kandy (MEDA), and low-grown area (YATA), are studied in this work. These four areas can be distinguished by their difference in elevation. RAN is an oval-shaped plateau over 1800 m of elevation, and the tea produced in RAN has a unique flavor. UDA is one of the first cultivation areas at 1200 m of elevation, planted in the 1870s, and the Southwestern monsoon rains and cold weather from January to March are one of the determining factors for its tea flavor. MEDA is famous for medium grown (midgrown) tea at elevations between 600 and 1200 m and is the area where the first tea plantations were started. YATA is mainly grown in southern Sri Lanka. YATA teas are grown at an elevation of 600 m and thrive in fertile soils and warm conditions.

The differences in climate conditions of the tea-growing areas, temperature, light, soil, and water, might be sufficient to affect the chemical composition or metabolites of the teas and consequently might be responsible for the unique taste of each black tea.

^{1}H NMR Spectroscopy of Dried Black Tea Leaves. Aqueous solutions of tea, extracted from the dried tea leaves with boiling water for 4 min, were used for 1D and 2D NMR analyses. A representative ^{1}H NMR spectrum of tea extracted from tea leaves harvested in RAN is shown in Figure 1. The signal assignments of the components have been carried out on the basis of analysis of 2D NMR spectra or by comparison with authentic material or published data.[24] Although some chemical shifts were slightly different from those assigned from authentic material due to differences of measurement solvent conditions, the pattern of the corresponding signals was in agreement with the authentic material. As shown in Figure 1, theanine (δ 1.10, 2.13, 2.37, 3.19, and 7.97 ppm), caffeine (δ 3.22, 3.38, 3.77, and 7.63 ppm), TF (δ 2.74, 2.75, 2.86, 2.87, 4.21, 4.29, 5.41, 6.01−6.13, 6.86, 7.02, and 7.15 ppm), TFG (δ 2.93, 2.94, 3.00, 3.01, 5.03, 5.42, 5.52, 6.04−6.13, 6.58, 6.61, 6.86, 6.92, and 6.94 ppm), and thearubigin 3,3′-digallate (TRG) (δ 1.88, 1.98, 2.05, 2.08, 3.54, 3.62, 4.02, 4.14, 6.04−6.13, 6.85, 7.03, and 7.16 ppm) were the main tea components identified in the ^{1}H NMR spectra (Figures 1, 2, and 4). Furthermore, Figure 2 shows the results of a more detailed analysis of TF and TFG. The two compounds, TF and TFG, were clearly observed only in RAN, and the peak assignments for these components were deduced from the COSY and TOCSY data and by comparison of the chemical shifts with authentic materials. The chemical shifts of TF were assigned in the ^{1}H NMR spectrum: H4a at 2.86 ppm (dd, J = 17.0, 4.5 Hz), H4b at 2.75 ppm (d, J = 17.0 Hz), H4′a at 2.87 ppm (dd, J = 17.1, 5.9 Hz), and H4′b at 2.74 ppm (d, J = 17.1 Hz).

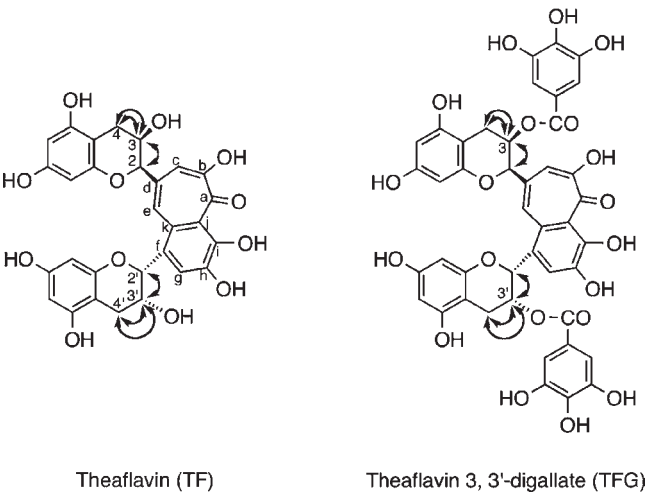

Figure 3. COSY and TOCSY (H ↔ H) correlations for TF and TFG.

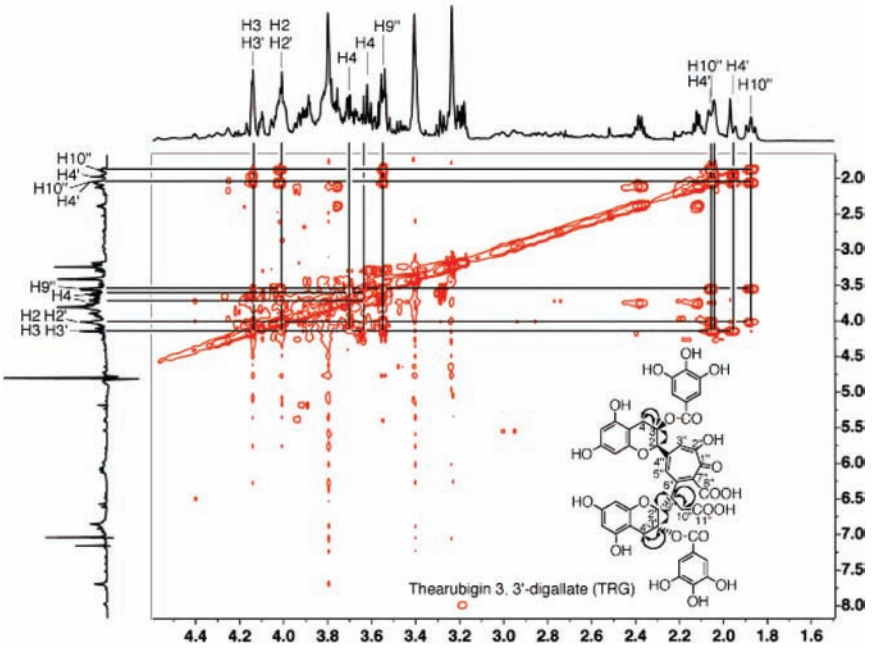

Figure 4. TOCSY (H ↔ H) correlations from the characteristic region of TRG in YATA.

The COSY spectrum of TF indicated correlation peaks between H2 and H3, H3 and H4, H2′ and H3′, and H3′ and H4′, respectively (Figure 3). The TOCSY spectrum of TF showed consecutive correlations from H2′ to H4′ and from H2 to H4 (Figure 3). These correlations in COSY and TOCSY spectra were in agreement with those for authentic samples of TF and TFG. The chemical shifts for TFG were assigned in the ^{1}H NMR spectrum: H4a at 3.00 ppm (dd, J = 17.5, 3.5 Hz), H4b at 2.94 ppm (d, J = 17.5 Hz), H4′a at 3.01 ppm (dd, J = 16.9, 4.3 Hz), and H4′b at 2.93 ppm (d, J = 16.9 Hz). The COSY spectrum of TFG indicated correlation peaks between H2 and H3, H3 and H4, H2′ and H3′, and H3′ and H4′, respectively (Figure 3). The TOCSY spectrum of TFG showed consecutive correlations from H2′ to H4′ and from H2 to H4 (Figure 3).

The major TFs in black tea are TF, theaflavin-3-gallate, theaflavin-3′-gallate, and TFG. TFs are formed from dimerization of catechins at the fermentation stage in the manufacturing of black tea.[25] TFs contribute to the characteristic bright orange-red color and taste of black tea and account for 1−2% of the total dry weight of black tea. However, among four different tea samples, it was difficult to observe TFs in ^{1}H NMR spectra of UDA, MEDA, and YATA, while TF and TFG are major constituents in chemical compositions from RAN. Therefore, these results suggest that the two compounds, TF and TFG, give RAN its characteristic color and taste.

Although thearubigins are polymeric materials in black tea, their structures have not yet been fully elucidated. The marked acidity and water solubility are characteristic of thearubigins that are derived by oxidatively induced ring-opening of one or more aromatic rings in the phenolic substrates, generating one or more carboxyl groups in the process. The chemical structure of thearubigins has been reported by N. Kuhnert[26] and is in agreement with the assumed structure in this study. The TRG in Figure 4 shows a common structure for thearubigins, and the assignment was deduced from the COSY and TOCSY data. In the COSY spectrum, the H2 of TRG was correlated to H3, H3 to H4, H4′ to H3′, H3′ to H2′, H2′ to H9″, and H9″ to H10″, respectively. The TOCSY spectrum of TRG showed consecutive correlations from H4′ to H10′ and from H2 to H4, consisting of a typical structure for thearubigins. In addition, the aromatic protons of the galloyl group were assigned to the peak at 7.03 ppm.

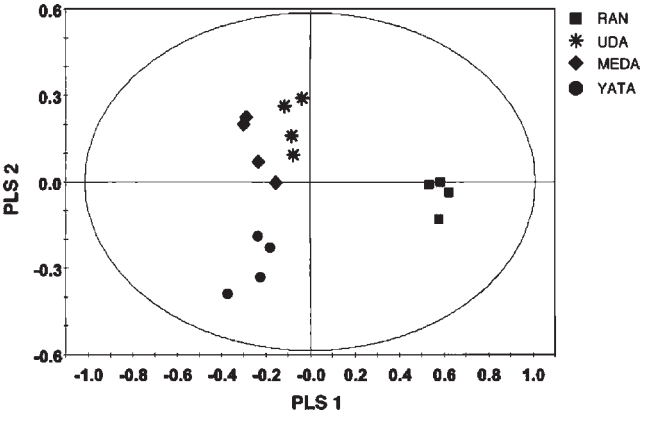

Figure 5. PLS-DA score plot derived from the ^{1}H NMR spectra of black teas from RAN (square), UDA (star), MEDA (diamond), and YATA (circle).

Thearubigins account for about 10−20% of the dry weight of black tea.[2] Thearubigins in black tea contribute to the characteristic reddish brown or coppery color and the astringent and bitter taste[27] and have a wide range of molecular weights.[24] It is generally known that thearubigins are produced by enzymatic oxidation of epigallocatechin or epicatechin via formation of TF intermediates.[28−30] The structures of TFs have a characteristic hydroxy-substituted benzotropolone ring containing a pyrocatechol substructure. It has been reported that pyrocatechol can be oxidatively cleaved in two different ways by enzymes: either between the hydroxyl groups (*ortho* cleavage) to give a *cis,cis*-

muconic acid or adjacent to the hydroxyl groups (*meta* cleavage).[31] The oxidative ortho cleavage of the C═C bond of the TF pyrocatechol moiety might be derived from enzymes in tea leaves.

Multivariate Statistical Analysis of Black Tea by ^{1}H NMR Spectroscopy. To see the difference of chemical components in tea leaves with respect to different cultivation area elevations, four different tea samples were analyzed. Although a PCA score plot was first employed to identify the different components in four groups, to clearly find out the significantly correlated components on effect of cultivation altitude, PLS-DA modeling was applied to the ^{1}H NMR spectra data sets from the four different tea samples (n = 4). The PLS-DA model was used to maximize covariance between measured data (X) and the response variable (Y) and the goodness of prediction parameter Q^2. Figure 5 shows clear separations among the RAN, UDA, MEDA, and YATA teas with total variance of 56%. The PLS-DA modeling revealed R_x^2, R_Y^2, and Q^2 values of 0.42, 0.33, and 0.29 for PLS1, indicating 42% of variance contribution rate and 29% predictability in the multidimensional space, respectively. On the other hand, PLS2 gave a low contribution ratio of 14%. This result suggests a high contribution rate for PLS1, indicating that the spectrum collected from RAN is considerably different from the other spectra collected from UDA, MEDA, and YATA. The differences of the data points for PLS1 likely reflect the difference of chemical compositions from RAN and the other three teas.

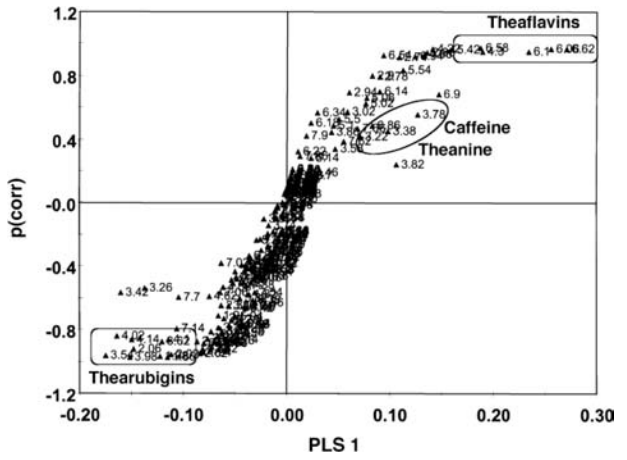

Figure 6. PLS-DA loading S-plot derived from the ^{1}H NMR spectra of black tea from RAN, UDA, MEDA, and YATA.

Potential components separated by difference of elevation were subsequently identified using S-plots, which were represented with covariance (p) against correlation ($p$corr)[32] (Figure 6). S-plots of the PLS-DA were proposed for identification of potential components for separation of the tea groups. As shown in Figure 6, the S-plots for PLS1 show the most relevant variables for the differentiation of two samples, RAN and three

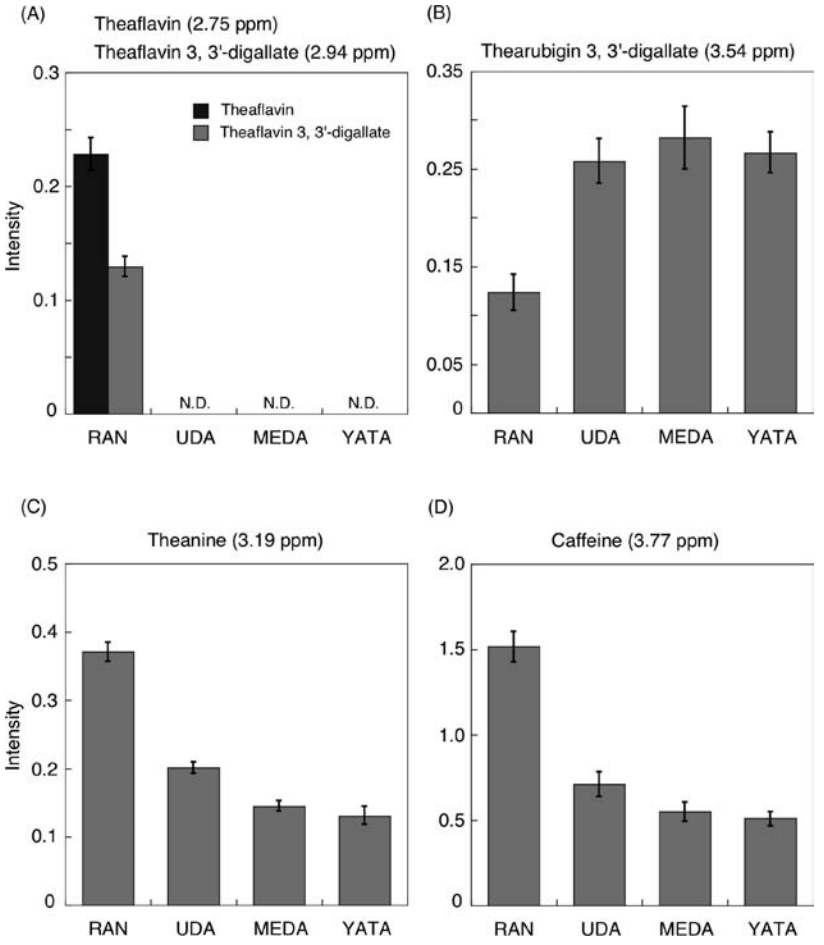

Figure 7. Different components from RAN, UDA, MEDA, and YATA. These components, identified from ^{1}H NMR spectra, are responsible for the differentiation in the PLS-DA model. (A) TF (2.75 ppm), TFG (2.94 ppm), (B) TRG (3.54 ppm), theanine (3.19 ppm), and caffeine (3.77 ppm).

others, and reveal the contributions of particular variables (integral regions, in this study) toward either an increase or a decrease in integration intensities. The four typical components, TFs (5.42, 4.3, 6.58, 6.1, 6.06, and 6.62), caffeine (3.22, 3.38, 3.78, and 7.66), theanine (1.10, 2.12, 2.38, and 3.1), and thearubigins (1.86, 2.06, 3.54, 3.62, 4.02, 4.14, and 7.14) were analyzed as variables of the S-plot (Figure 6). Each variable is represented as a peak with a particular chemical shift in the ^{1}H NMR spectral region as shown in Figure 1. The components responsible for increases or decreases could be identified, and the variables of the chemical shifts associated with the largest changes in integration intensity are indicated farther away from the center of the PLS1 coordinate axis. Thus, as a result of the S-plots analysis, the two components, TFs and thearubigins, contribute the most to the difference between RAN and three other teas in PLS1.

The integration intensities associated with these component variables are shown in Figure 7. The intensity of the variables of TF at 2.75 ppm and TFG at 2.94 ppm was observed only in RAN (Figure 7A). In addition, the intensity of the variables of theanine at 3.19 ppm and caffeine at 3.77 ppm in RAN showed higher levels as compared with UDA, MEDA, and YATA (Figure 7C, D). The amount of theanine and caffeine was clearly correlated with cultivated altitudes, showing that these levels were decreased at lower elevations. On the other hand, the intensity of the variable of TRG at 3.54 ppm in UDA, MEDA, and YATA showed higher levels relative to RAN (Figure 7B). Therefore, these results show that the separation between RAN and three other teas by PLS1 in the PLS-DA score plot is mainly attributable to the two components, TF and TFG, and the separation among UDA, MEDA, and YATA by PLS2 in the PLS-DA score plot is contributed by three components, TRG, caffeine, and theanine (further detailed in the Supporting Information, Figure S1).

In conclusion, the change in the principal components of the four tea groups was influenced by differences in the elevations of the cultivation areas. The TF, TFG, theanine, caffeine, and TRG compositions changed significantly with the different elevations of the harvested tea leaves (Figure 7). The components that were found in higher levels only in RAN were TF and TFG, formed from polymerization of catechins at the fermentation stage. Among four cultivation areas, the amounts of TRG in UDA, MEDA, and YATA were higher than that in RAN, while TF and TFG were only found in RAN (Figure 7A). Although the fermentation process in each factory, which belonged to its own field, were almost identical, some factors arising from the different elevations, such as oxygen levels, humidity, and temperature, might affect the fermentation condition, resulting in differentiation of the composition in black tea. Therefore, TRG in UDA, MEDA, and YATA is thought to result from the enzyme-catalyzed oxidation of catechin via the formation of TF or TFG. In RUN manufacturing, the insufficient oxidation process at the highest altitude (>1900 m) may result in the formation of TF and TFG concomitantly with the low amount of TRG. In addition to the condition of fermentation process, it also should be considered that the components of raw leaves characterize chemical constituents of black tea. Component analysis of tea leaves cultivated at the different altitudes is now underway to evaluate the effect of constituents produced by way of fermentation processes. Finally, we have demonstrated that this method of analyzing the component pattern of black teas is a useful tool in evaluating tea quality from tea leaves cultivated at different elevations in Sri Lanka.

■ ASSOCIATED CONTENT

Supporting Information. PLS-DA loading plot derived from the ^{1}H NMR spectra of black tea from RAN, UDA, MEDA, and YATA. This material is available free of charge via the Internet at http://pubs.acs.org.

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Funding Sources

This work was supported by a Grant-in-Aid for Young Scientists (B) (No. 22790126) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan, and by a Grant-in-Aid for Scientific Research (B) (No. 20390038) from the Japan Society for the Promotion of Science (JSPS).

■ ACKNOWLEDGMENT

We thank Waltz Co., Ltd (Toyohashi, Japan) for generously donating the tea leaves.

■ REFERENCES

(1) Wiseman, S. A.; Balentine, D. A.; Frei, B. Antioxidants in tea. *Crit. Rev. Food Sci. Nutr.* **1997**, *37* (8), 705–718.

(2) Balentine, D. A.; Wiseman, S. A.; Bouwens, L. C. The chemistry of tea flavonoids. *Crit. Rev. Food Sci. Nutr.* **1997**, *37* (8), 693–704.

(3) Frei, B.; Higdon, J. V. Antioxidant activity of tea polyphenols in vivo: Evidence from animal studies. *J. Nutr.* **2003**, *133* (10), 3275S–3284S.

(4) Lamoral-Theys, D.; Pottier, L.; Dufrasne, F.; Neve, J.; Dubois, J.; Kornienko, A.; Kiss, R.; Ingrassia, L. Natural polyphenols that display anticancer properties through inhibition of kinase activity. *Curr. Med. Chem.* **2010**, *17* (9), 812–825.

(5) Kuzuhara, T.; Iwai, Y.; Takahashi, H.; Hatakeyama, D.; Echigo, N. Green tea catechins inhibit the endonuclease activity of influenza A virus RNA polymerase. *PLoS Curr. Influenza* **2009**, RRN1052.

(6) Yang, C. S.; Lambert, J. D.; Ju, J.; Lu, G.; Sang, S. Tea and cancer prevention: Molecular mechanisms and human relevance. *Toxicol. Appl. Pharmacol.* **2007**, *224* (3), 265–273.

(7) Song, J. M.; Lee, K. H.; Seong, B. L. Antiviral effect of catechins in green tea on influenza virus. *Antiviral Res.* **2005**, *68* (2), 66–74.

(8) Hsu, S. Green tea and the skin. *J. Am. Acad. Dermatol.* **2005**, *52* (6), 1049–1059.

(9) Fujita, H.; Yamagami, T. Antihypercholesterolemic effect of Chinese black tea extract in human subjects with borderline hypercholesterolemia. *Nutr. Res. (N.Y.)* **2008**, *28* (7), 450–456.

(10) Davies, M. J.; Judd, J. T.; Baer, D. J.; Clevidence, B. A.; Paul, D. R.; Edwards, A. J.; Wiseman, S. A.; Muesing, R. A.; Chen, S. C. Black tea consumption reduces total and LDL cholesterol in mildly hypercholesterolemic adults. *J. Nutr.* **2003**, *133* (10), 3298S–3302S.

(11) Fujiwara, M.; Ando, I.; Arifuku, K. Multivariate analysis for 1H-NMR spectra of two hundred kinds of tea in the world. *Anal. Sci.* **2006**, *22* (10), 1307–1314.

(12) Van Dorsten, F. A.; Daykin, C. A.; Mulder, T. P.; Van Duynhoven, J. P. Metabonomics approach to determine metabolic differences between green tea and black tea consumption. *J. Agric. Food Chem.* **2006**, *54* (18), 6929–6938.

(13) Nakagawa, M. Chemical Components and Taste of Green Tea. *Jpn. Agric. Res. Q.* **1975**, *9*, 156–160.

(14) Crockford, D. J.; Holmes, E.; Lindon, J. C.; Plumb, R. S.; Zirah, S.; Bruce, S. J.; Rainville, P.; Stumpf, C. L.; Nicholson, J. K. Statistical

heterospectroscopy, an approach to the integrated analysis of NMR and UPLC-MS data sets: Application in metabonomic toxicology studies. *Anal. Chem.* **2006**, *78* (2), 363–371.

(15) Ohno, A.; Kawasaki, N.; Fukuhara, K.; Okuda, H.; Yamaguchi, T. Time-dependent changes of oxytocin using (1)H-NMR coupled with multivariate analysis: A new approach for quality evaluation of protein/peptide biologic drugs. *Chem. Pharm. Bull. (Tokyo)* **2009**, *57* (12), 1396–1399.

(16) Pongsuwan, W.; Bamba, T.; Yonetani, T.; Kobayashi, A.; Fukusaki, E. Quality prediction of Japanese green tea using pyrolyzer coupled GC/MS based metabolic fingerprinting. *J. Agric. Food Chem.* **2008**, *56* (3), 744–750.

(17) Clayton, T. A.; Lindon, J. C.; Cloarec, O.; Antti, H.; Charuel, C.; Hanton, G.; Provost, J. P.; Le Net, J. L.; Baker, D.; Walley, R. J.; Everett, J. R.; Nicholson, J. K. Pharmaco-metabonomic phenotyping and personalized drug treatment. *Nature* **2006**, *440* (7087), 1073–1077.

(18) Clayton, T. A.; Baker, D.; Lindon, J. C.; Everett, J. R.; Nicholson, J. K. Pharmacometabonomic identification of a significant host-microbiome metabolic interaction affecting human drug metabolism. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106* (34), 14728–14733.

(19) Ohno, A.; Kawasaki, N.; Fukuhara, K.; Okuda, H.; Yamaguchi, T. Complete NMR analysis of oxytocin in phosphate buffer. *Magn. Reson. Chem.* **2010**, *48* (2), 168–172.

(20) Barton, R. H.; Nicholson, J. K.; Elliott, P.; Holmes, E. High-throughput 1H NMR-based metabolic analysis of human serum and urine for large-scale epidemiological studies: Validation study. *Int. J. Epidemiol.* **2008**, *37* (Suppl. 1), i31–i40.

(21) Nicholson, J. K.; Foxall, P. J.; Spraul, M.; Farrant, R. D.; Lindon, J. C. 750 MHz 1H and 1H-13C NMR spectroscopy of human blood plasma. *Anal. Chem.* **1995**, *67* (5), 793–811.

(22) Eriksson, L.; Johansson, E.; Kettaneh-Wold, N.; Wold, S. *Multi- and Megavariate Data Analysis*; Umetrics Academy: Umeå, 2001.

(23) Wold, S.; C., A.; Dunn, W. J.; Edlund, U.; Esbensen, K.; Geladi, P.; Hellburg, S.; Johansson, E.; Lindberg, W.; Sjostrom, M. *Chemometrics: Mathematics and Statistics in Chemistry*; D. Reidel Publishing Company: Dordrecht, 1984.

(24) Sang, S.; Tian, S.; Stark, R. E.; Yang, C. S.; Ho, C. T. New dibenzotropolone derivatives characterized from black tea using LC/MS/MS. *Bioorg. Med. Chem.* **2004**, *12* (11), 3009–3017.

(25) Drynan, J. W.; Clifford, M. N.; Obuchowicz, J.; Kuhnert, N. The chemistry of low molecular weight black tea polyphenols. *Nat. Prod. Rep.* **2010**, *27*, 417–462.

(26) Kuhnert, N. Unraveling the structure of the black tea thearubigins. *Arch. Biochem. Biophys.* **2010**, *501* (1), 37–51.

(27) Ho, C. T.; Sang, S.; Jhoo, J. W. *Chemistry of Theaflavins: The Astringent Taste Compounds of Black Tea*; Americal Chemical Society: Washington, DC, 2003; Vol. 867, pp 125−138.

(28) Yan Li, A. S.; Yosuke, M.; Takashi, T.; Isao, K. Reaction of the Black Tea Pigment Theaflavin during Enzymatic Oxidation of Tea Catechins. *J. Nat. Prod.* **2010**, *73*, 33–39.

(29) Subramanian, N.; Venkatesh, P.; Ganguli, S.; Sinkar, V. P. Role of polyphenol oxidase and peroxidase in the generation of black tea theaflavins. *J. Agric. Food Chem.* **1999**, *47* (7), 2571–2578.

(30) Alastair Robertson, D. S. B. Production and HPLC analysis of black tea theaflavins and thearubigins during in vitro oxidation. *Phytochemistry* **1983**, *22* (4), 883–887.

(31) Rudolf Müller, F. L. Oxidative Cleavage of Pyrocatechol at the meta-Position; A Model Reaction for Cleavage by Pyrocatechol-2,3-dioxygenase? *Angew. Chem.* **1984**, *23* (1), 79–80.

(32) Ku, K. M.; Choi, J. N.; Kim, J.; Kim, J. K.; Yoo, L. G.; Lee, S. J.; Hong, Y. S.; Lee, C. H. Metabolomics analysis reveals the compositional differences of shade grown tea (*Camellia sinensis* L.). *J. Agric. Food Chem.* **2010**, *58* (1), 418–426.