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Prediction of Japanese Green Tea Ranking by Fourier Transform Near-Infrared Reflectance Spectroscopy

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A rapid and easy determination method of green tea's quality was developed by using Fourier transform near-infrared (FT-NIR) reflectance spectroscopy and metabolomics techniques. The method is applied to an online measurement and an online prediction of green tea's quality. FT-NIR was employed to measure green tea metabolites' alteration affected by green tea varieties and manufacturing processes. A set of ranked green tea samples from a Japanese commercial tea contest was analyzed to create a reliable quality-prediction model. As multivariate analyses, principal component analysis (PCA) and partial least-squares projections to latent structures (PLS) were used. It was indicated that the wavenumber region from 5500 to 5200 cm^{-1} had high correlation with the quality of the tea. In this study, a reliable quality-prediction model of green tea has been achieved.

KEYWORDS: Metabolomics; metabolic fingerprinting; FT-NIR; quality evaluation; PLS

INTRODUCTION

Tea is the most popular beverage, and it is made from the leaves of the *Camellia sinensis* plant. There are three main types of tea: green tea, which is unfermented; oolong tea, which is semifermented; and black tea, which is fermented. Fermentation is an oxidation process. In green tea, enzymes are inactivated when heating begins to prevent oxidation of the leaf polyphenols. Tea originated in southern China and is mostly consumed in East and Southeast Asia countries. In these areas, green tea is very popular, not only as a medical drink but also as a tasty beverage (*1*). The taste of green tea is determined by several factors: kind of tea tree, plucking season, cultivation method, and processing. The tea is evaluated by professional tea tasters (*2*). Tea tasters are highly trained specialists who evaluate product quality on the basis of the leaves' appearance and the aroma, color, and taste of the brew. Because it takes years of experience to acquire these skills, it would be advantageous to determine product quality by some form of nonhuman measurement. In our previous research, we addressed the development of a reliable method for estimating the quality of green tea by chemical analysis. We made a prediction model of Japanese green tea ranking by gas chromatography and mass spectrometry (GC-MS), using a metabolic fingerprinting technique (*3*). However, our model did not have high enough accuracy, due to experimental errors. Furthermore, application of the technique to green tea manufacturing is difficult due to its complicated

Table 1. Comparison between Variance of Powder Samples and That of Paste Samples[a]

	sample no.				
sample type	10	20	30	40	50
powder	76.36	205.55	31.46	95.32	158.54
paste	5.12	53.10	11.87	39.60	6.25

[a] The variance was calculated on the basis of triplicate determination in the analysis step.

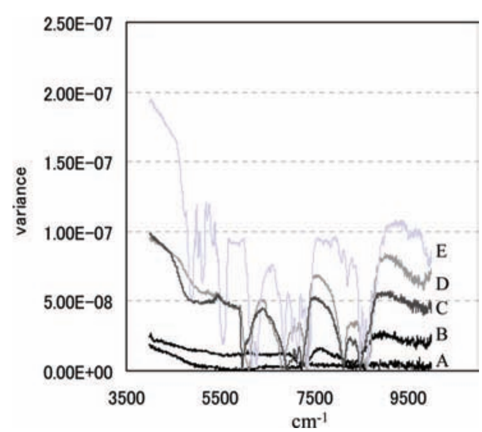

Figure 1. Variance of FT-NIR spectra of several solvents: (**A**) blank; (**B**) glycerol; (**C**) mineral oil; (**D**) hexane; (**E**) acetonitrile.

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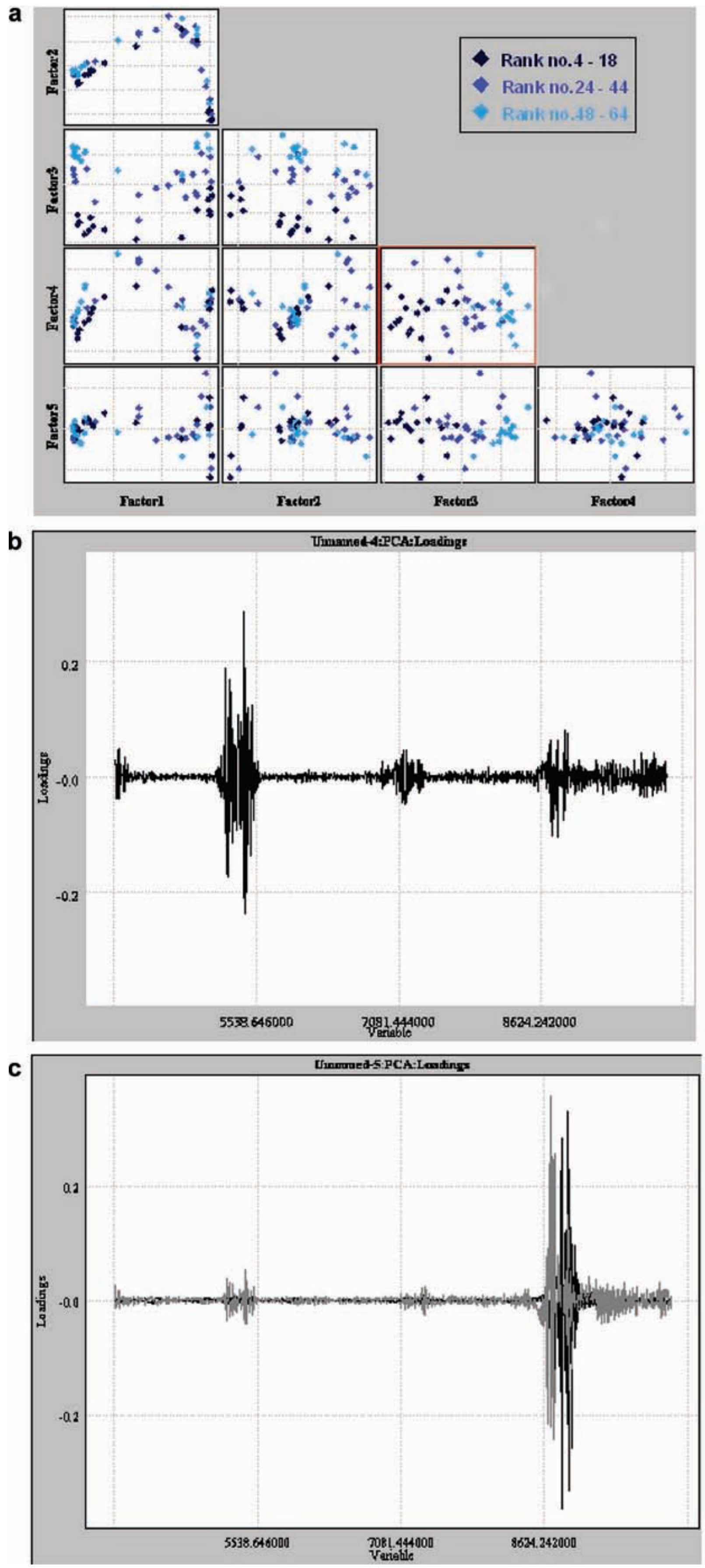

Figure 2. PCA: (**a**) score plot of 13 ranked samples (data were preprocessed with mean-center, second derivative, and SNV; color spots are graded from dark blue to lighter blue in parallel with their ranks varying from higher ranked to lower ranked samples); (**b**) loading plot of factor 3 (the *x*-axis represents "cm^{-1}"); (**c**) loading plot of factors 1 (black line) and 2 (gray line).

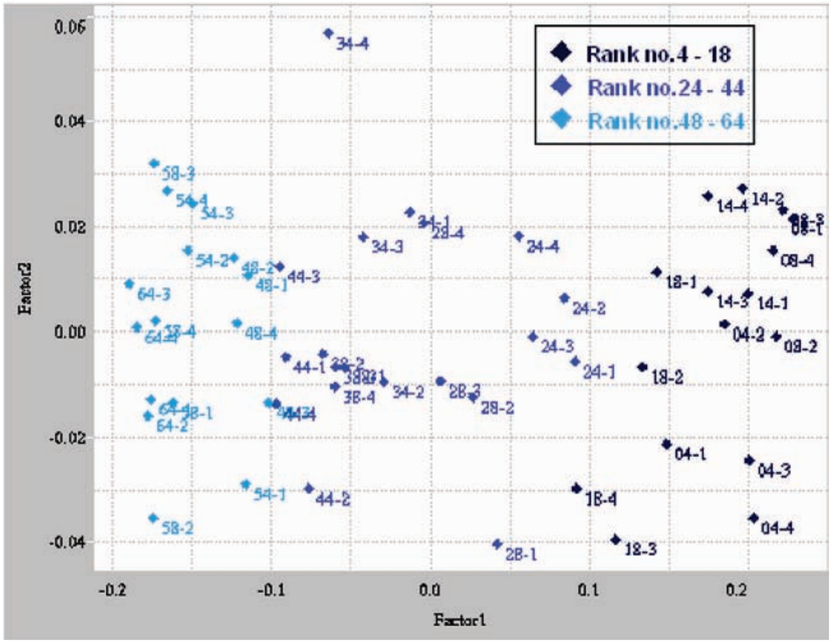

Figure 3. PCA score plot of the 5500–5200 cm^{-1} spectra. Data were preprocessed with mean-center, second derivative, and SNV. Color spots are graded from dark blue to lighter blue in parallel with their ranks varying from higher ranked to lower ranked samples. Data point labels show ranking number (left of "-") and trial number (right of "-").

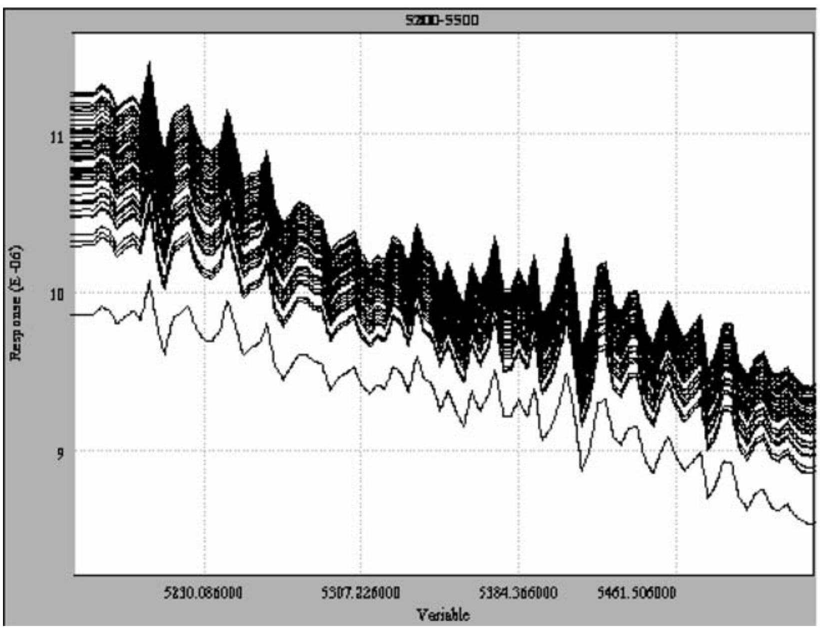

Figure 4. FT-NIR spectra of all samples in the wavenumber region from 5500 to 5200 cm^{-1}. The *x*-axis represents "cm^{-1}".

process (extraction and derivatization), analysis time, and running cost. Thus, we used Fourier transform near-infrared (FT-NIR) reflectance spectroscopy, which is a rapid and easy analytical technique. Near-infrared spectroscopy (NIR) is widely used for rapid and nondestructive analysis in industries such as agriculture, food, pharmaceutical, and polymer production (*4*, *5*). NIR has several advantages in these fields: samples can be analyzed with a photometer without pretreatment (extraction and derivatization) and both liquid and solid samples can be analyzed. It is a big advantage to have an online measurement using fiber optics in the manufacturing process. NIR is also used to measure the concentration of several compounds in samples. In such cases, principal component regression (PCR) and partial least-squares (PLS) are applied to make calibration curves (*4–6*).

This research is aimed to develop a precise and reliable prediction model to determine the quality of green tea samples. Known ranked samples from the Kansai tea contest held in Japan were used as samples for making the prediction model. As data-mining methods, principal component analysis (PCA) and PLS were performed. PCA is known as the most common technique used for exploratory multivariate analysis (*7*, *8*). PCA was used for investigation of data preprocessing and optimal wavenumber range. For actual application to the online measurement and online prediction, narrowing the range of wavenumbers is important to reduce the calculation time and database size. Finally, we made an accurate prediction model to estimate the quality of green tea samples using PLS.

MATERIALS AND METHODS

Materials. Thirteen different types of dried leaves selected from 64 ranked first-crop tea samples from the 2005 contest were analyzed.

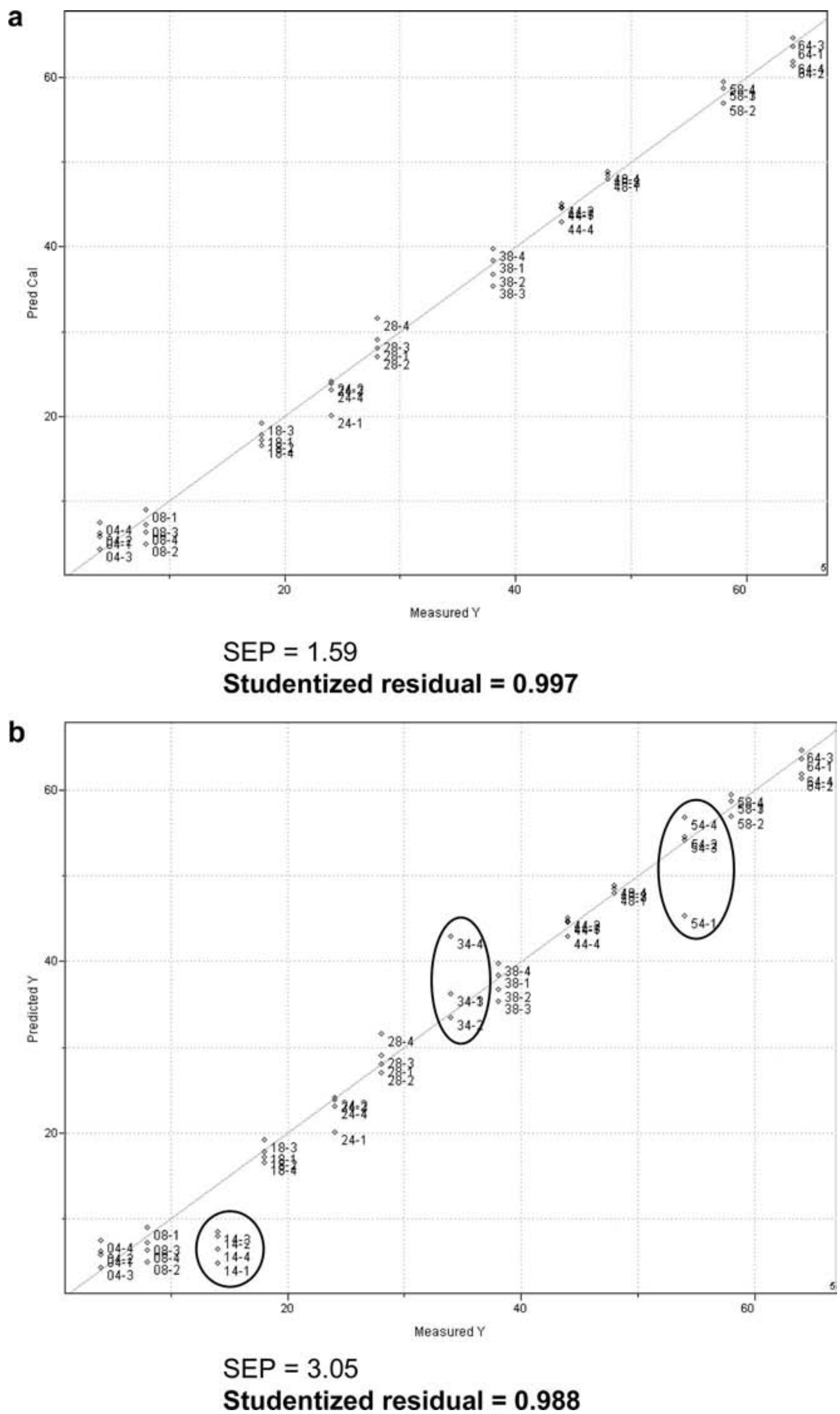

Figure 5. Relationship between measured and predicted green tea quality (ranking) of PLS model: (**a**) 10 green tea samples as a training set; (**b**) all 13 ranks of both testing (marked by circle) and training sets.

These tea samples have 4, 8, 14, 18, 24, 28, 34, 38, 44, 48, 54, 58, and 64 ranking numbers. The tea samples were obtained from the tea branch of the Nara Prefecture Agricultural Experiment Station. Professional tea tasters determined the ranking of teas based on the total scores (200 points full marks) of the sensory tests: leaf appearance (30 points), smell (70 points), color of the brew (30 points), and its taste (70 points). First, the leaf appearance was evaluated by 41 tea tasters. After that, the smell, color of the brew, and its taste were evaluated by different tea tasters at the same time. The smell was evaluated by 10 tea tasters. The color was evaluated by 9 tea tasters. The taste was evaluated by 9 tea tasters.

Sample Preparation for FT-NIR Analysis. Dried tea leaves (200 mg) in 2 mL Eppendorf tubes were ground with a Retsch ball mill (20 Hz, 10 min), and glycerol was added (600 μL). Glycerol was purchased from Wako (Osaka, Japan). The mixture was homogenized with a ball mill again (20 Hz, 10 min). Subsequently, samples were transferred to individual 2 mL glass vials.

FT-NIR Measurements. Diffuse reflectance spectra of tea samples were measured using a Nicolet 6700 FT-IR (Thermo Electron K.K., Kanagawa, Japan) equipped with a Smart Near-IR UpDRIFT, a CaF2 beamsplitter, and a cooled InGaAs detector. On each sample, a diffuse reflectance spectrum was measured three times. As the blank sample, an empty glass vial was also measured three times. The FT-NIR spectra were recorded from 10000 to 4000 cm^{-1} at intervals of 3.857 cm^{-1}. The mirror velocity was 1.2659 cm s^{-1}, and the resolution was 8 cm^{-1}. The total number of data points was 1557 for each spectrum.

Multivariate Analysis. Initially, each set of data, that is, the measurements, was averaged and multiplied by 100000 to reduce the variance of three times measurements and match the digit. As preprocessing, all data were transformed by mean-center, second derivative, and standard normal variate (SNV). Principal component analysis (PCA) was performed with Pirouette (Informatrix, Inc.). By PCA, important ranges for separation of tea samples were selected.

Partial least-squares (PLS) was applied to create a prediction model. PLS finds a relationship between two sets of variables: observations and responses.

RESULTS AND DISCUSSION

Investigation of Measurement Condition. When 200 mg of tea leaves, homogenized by ball mill, was measured by FT-NIR, the variance of measurement data was quite large (**Table 1**). In this method, each set of data was differentiated by changes in the measurement angle. This variance was thought to be caused by heterogeneity of powder samples. Thus, we tried to reduce the variance by adding some solvent to the powder samples to make paste samples. We investigated four solvents: glycerol, hexane, acetonitrile, and mineral oil. These solvents were transferred to 2 mL glass vials and measured by FT-NIR. Although the FT-NIR spectra were almost the same, the variance of glycerol's spectrum was the smallest (**Figure 1**). We made the paste samples by adding 600 μL of glycerol to 200 mg powder samples. These paste samples were measured by FT-NIR. The variance of measurements was small enough (**Table 1**) to use these data for data mining.

Metabolic Fingerprinting with PCA. In factor 3 of the PCA, the 13 ranked tea samples were divided into three groups according to their grades (**Figure 2a**). The variance of factor 3 was 2.38% of total variance, which was quite low. In the loading of factor 3, it was suggested that the wavenumber region from 5500 to 5200 cm^{-1} had a high contribution ratio (**Figure 2b**). We considered that much noise, having no correlation with tea quality, was included in the wavenumber region from 9000 to 8000 cm^{-1} because the loadings of factors 1 and 2 had high contribution ratios in that region (**Figure 2c**). As a result, we expected that the 5500–5200 cm^{-1} spectral range had a high correlation with the tea grades. In the results of PCA using the data of the 5500–5200 cm^{-1} spectral region, the tea samples were arranged by grade in factor 1 (**Figure 3**). The variance of factor 1 was 92.74% of the total variance.

These findings suggest that the wavenumber region from 5500 to 5200 cm^{-1} had a high correlation with tea ranking in the data of FT-NIR measurements. The FT-NIR spectra of all samples in the wavenumber region from 5500 to 5200 cm^{-1} are shown in **Figure 4**.

Prediction of Tea Ranking by PLS. A prediction model to estimate quality of green tea samples, based on correlation between tea ranking and FT-NIR spectra, was made. PLS is suitable for this approach. To perform PLS, samples were divided into groups of training and testing sets. The samples ranked 14th, 34th, and 54th were excluded for the model validation. In data preprocessing, the 5500–5200 cm^{-1} spectral range from FT-NIR data was used to perform the second derivative and SNV. The number of latent factors in the PLS model was determined by cross-validation. The optimum number was five in the model of training set. The standard error of prediction (SEP) of the training set was 1.59, and the SEP of the test set was 3.05 (**Figure 5**). The Studentized residual of the training set was 0.997, and that of the test set was 0.988. These numeric values show accuracy of the prediction model; when the value is closer to 1, the result is said to be accurate. These facts suggest that our prediction model is quite accurate. From the result in the test sets, this model was accurate in predicting low-quality samples, but not so accurate in predicting high-quality samples (**Figure 5b**). This result suggests that when the tea quality is higher, prediction becomes more difficult, because the difference in the quality is smaller.

This research showed that quality prediction of tea by metabolic fingerprinting using FT-NIR was accurate and realistic. Of course, we cannot note that we got the quality-prediction model of all green tea for the following reasons. Overall, the samples used in this study were of very high quality and the same cultivar and from the same production area, because these samples were the samples that had been exhibited in the contest. However, we consider that this study is useful enough because quality estimation of tea leaves that are of the same cultivar and from the same production area is important for tea manufacturing. In addition, we have succeeded in making uniform samples and suppressing variation by making paste samples using glycerol. The results of PCA and PLS indicate that the wavenumber region from 5500 to 5200 cm^{-1} had a high correlation with the quality of tea. Thus, the online measurement and the online quality prediction will be attained by accumulating the FT-NIR data of the 5500–5200 cm^{-1} spectral region of tea in a database.

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