Near Infrared Spectra of Caffeine and its Related Compounds and their Application to Determination of Caffeine Content in Green Tea

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Near infrared reflectance spectra of caffeine and its related compounds such as xanthine, theophylline and theobromine were studied in connection with their chemical structures. A major difference in the spectra was characterized with a difference in the number of CH3 groups contained in the compounds. It has been verified that the near infrared reflectance spectroscopy can be applied to the quantitative analysis of caffeine in green tea.

Lately caffeine in food especially in such favorite foods as tea and coffee has become a matter of concern for consumers. At present, there is no regulation of indicating caffeine content of Japanese green tea on a label; however, there may be some regulation in a near future.

A semi-micro Kjeldahl method has been used as a standard analytical method for caffeine content in the green tea1). In this method it takes 2~3 days to complete a cycle of analytical procedures including an extraction process of caffeine with a modified Soxhlet. One of authors had developed a more convenient method using a high performance liquid chromatography2). This HPLC method has capability to analyze about 20~30 samples in duplicate in a day, but the method still needs an extraction procedure and is not convenient enough for a large number of samples to be analyzed at a time.

In the present work, a near infrared reflectance (NIR) spectroscopy which has been highlighted as a rapid or nondestructive method for quantitative analysis of food compositions, was studied to ascertain its applicability to caffeine content in the green tea as a substitute for such time-consuming methods.

Materials and Methods

Materials

Samples used are the same as used in a previous study3). They consist of 58 kinds of green tea. Totally 30 green teas are selected as calibration samples so that a uniform distribution of caffeine content may be obtained in the range expected. The other 28 green teas are used as predicted or "unknown" samples to verify the accuracy of calibrations obtained. Values of mean and range of caffeine content for the samples are shown in Table 1. Analysis of caffeine content was carried out by the standard method using a semi-micro Kjeldahl method1).

All the samples were ground into 0.5 mm of particle size with a cyclone mill before the NIR measurement.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Mean and range of caffeine contents for samples used</th>
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</thead>
<tbody>
<tr>
<td>Samples</td>
<td>n</td>
</tr>
<tr>
<td>Calibration</td>
<td>30</td>
</tr>
<tr>
<td>Prediction</td>
<td>28</td>
</tr>
</tbody>
</table>
Instrumentation

A Neotec Research Composition Analyzer (Model 6350) was used for the NIR measurement. This instrument has a computer-controlled spectrophotometer which is operated in a single beam mode in the wavelength region of 1100 nm to 2500 nm. All the measuring procedures are similar to those in a previous paper.

Calibrations

Calibrations were performed by using second derivative spectra in the same procedure as described previously.

Results and Discussion

NIR spectra of caffeine and its related compounds

Spectra of caffeine and its related compounds are shown in Fig. 1, and their chemical structural formulae are shown in Fig. 2. Basically, the compounds are characterized by a purine structure in the formula. They can be classified individually according to the number and position of CH₃ groups in the structure. That is, theophylline is 1, 3-dimethyl-xanthine, theobromine is 3, 7-dimethyl-xanthine and caffeine is 1, 3, 7-trimethyl-xanthine.

In the region from 1100 nm to 2500 nm, absorptions which can be assigned to CH₃ group appear at 1350 nm, 1690 nm and 2260 nm. It is noted that absorbances at these wavelengths seem to be correlated to the number of CH₃ groups in the chemical structure. Another absorption at near 2240 nm also seems to have the same tendency.

As compared to the spectrum of unhydrated caffeine, that of hydrous one, shows some differences at several wavelength regions. Major differences are seen as a sharp absorption at 1962 nm as well as a dull one around 1460 nm. It has been reported that bound water tends to show its absorption at a higher wavelength than that of free water. According to the second derivative spectrum of hydrous caffeine at 1450 nm region as shown in Fig. 3, it is apparent that the spectrum consists of several small absorptions at 1434 nm, 1462 nm and 1500 nm. It is supposed that the absorption at 1434 nm is assigned to an intramolecular OH while the others are to OH group of water. An absorption band due to OH group absolutely free water is known to appear at 1410 nm region. Based on these
considerations, it is suggested that water in hydrous caffeine may be strongly bound in the molecule.

It is reliable that absorptions at 2358 nm, 1628 nm and 1226 nm in the spectrum of xanthine are assigned to a combination (2358 nm) and a first (1628 nm) and a second (1226 nm) overtone of fundamental vibrations for CH group. However, a reason why these bands can be clearly seen only in the spectrum of xanthine is not known at present. A very strong absorption at 2198 nm in xanthine is supposed to be associated with OH group which only this compound contains in the structure.

The spectrum of theophylline is basically similar to that of theobromine except wavelength regions such as 2450 nm and 2080 nm. A strong absorption at 2082 nm for theobromine may be assigned to a structure of CONH according to a study reported on the spectra of amides because only the theobromine has this structure.

In addition, a tendency that absorbances at wavelengths associated with CH₃ group may be correlated to the number of this group in the compounds encourages us to apply the NIR method to the quantitative analysis of caffeine content in green tea.

**Calibration for caffeine in green tea**

Fig. 4 shows a distribution of simple correlation coefficient of regression analysis between caffeine content and absorbance presented in the second derivative spectrum. Absolute values of the correlation coefficient bigger than 0.70 are seen at 13 wavelengths. At wavelength regions close to 2100 nm which may be assigned to OH group, the biggest positive values were obtained. Since the positive correlation coefficient indicates that near infrared reflectance increases in the intensity in parallel with caffeine content, there should be an indirect relationship between caffeine content and absorbances at the regions.

On the other hand, the biggest negative correlation coefficient was obtained at 1990 nm. At this wavelength in the second derivative spectrum of unhydrous caffeine, a relatively strong absorption is seen as shown in Fig. 4 as well as a weak absorption in that of green tea itself. This wavelength is not assignable exactly; however, it can be associated with N-H group according to a chart of spectra-structure correlations reported.

It is noted that big and negative correlation coefficients are obtained at wavelengths which have been assigned to CH₃ that is, they are −0.815 at 2244 nm and −0.726 at 1692 nm.

Table 2 shows calibrations obtained with a
Table 2 Results of calibration

<table>
<thead>
<tr>
<th>Wavelengths</th>
<th>CAL I</th>
<th>CAL II</th>
<th>CAL III</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_1 )</td>
<td>1.990</td>
<td>1.692</td>
<td>2.244</td>
</tr>
<tr>
<td>( \lambda_2 )</td>
<td>2.422</td>
<td>1.900</td>
<td>1.624</td>
</tr>
<tr>
<td>( \lambda_3 )</td>
<td>2.262</td>
<td>2.422</td>
<td>2.378</td>
</tr>
<tr>
<td>( \lambda_4 )</td>
<td>2.364</td>
<td>2.074</td>
<td>2.422</td>
</tr>
<tr>
<td>( \lambda_5 )</td>
<td>1.706</td>
<td>2.364</td>
<td>2.366</td>
</tr>
</tbody>
</table>

\( R^{1)} \) | 0.970 | 0.866 | 0.968 |
\( SEE(\%)^{2)} \) | 0.208 | 0.221 | 0.215 |

1) Multiple correlation coefficient between chemically determined caffeine content and absorbances at 5 specific wavelengths in the second derivative spectrum. All the wavelengths in CAL I were automatically selected in each step of the regression analysis performed with a computer programme, while wavelengths such as 1692 nm and 2244 nm in CAL II and CAL III, respectively, were designated as the first specific wavelength for the respective calibrations. Wavelengths next to the first one were selected automatically as well as in CAL I.

Prediction of unknown samples

Table 3 shows caffeine content predicted for 28 unknown samples by means of different calibrations shown in Table 2. The smallest standard error as well as bias in predicting the unknown samples were obtained with CAL II, suggesting that the CAL II in which 1692 nm was designated as the first wavelength might be the best calibration.

In order to verify the accuracy of prediction, a statistical analysis was carried out for evaluating mean differences between chemically analyzed and NIR predicted values by using a t-test as follows:

\[
t = \frac{d}{SEP/\sqrt{n}}
\]

where \( d \): mean difference between chemically analyzed and NIR predicted values

\( SEP \): standard error of prediction

\( n \): number of samples used

By substituting respective values of \( d = 0.006, SEP = 0.350 \) and \( n = 28 \), a t-value was calculated to be 0.091. As a result of comparison of this value to 2.77 which is a significant limit at 1% level, there is no significant difference in the mean difference between the both values.

In conclusion, it is verified that caffeine content in green teas can be determined accurately with the NIR method.

References

9) Iwamoto, M., Uozumi, J. and Nishinari, K.: In print
(Received Dec. 26, 1985)

近赤外分光法による緑茶中のカフェインの定量

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緑茶中のカフェインを迅速に定量するため近赤外分光法の適用を試みた。カフェインならびに関連物質として測定したキサンチン、テオフィリン、テオプロミンの近赤外スペクトルから、-CH₃に帰属される1692 nm 及び2244 nm にある吸収をキーバンドとすることにより、カフェインの定量が可能と考えられた。30 個の緑茶について 2 次微分スペクトルを用いて得たキャリプレーションをもとに、28 個の未知試料中のカフェインを定量した結果、1692 nm を第一波長に指定したキャリプレーションで最良の精度を得ることができ、近赤外分光法が実用的なことを確認した。