JARQ 24, 49–53 (1990)

Determination of Chemical Constituents in Processed Green Tea by Near Infrared Analysis

Kenjiro IKEGAYA

Abstract

Applicability of the near infrared reflectance (NIR) spectroscopy method was examined to estimate total nitrogen, caffeine, total free amino acids, theanine and tannin (catechines) in processed green tea (Sencha in Japanese) as a substitute for the conventional time-consuming method commonly used in Japan. In was confirmed that these chemical constituents of green tea could be estimated accurately by the NIR spectroscopy method.

Discipline: Tea industry
Additional key words: calibration, near infrared, reflectance (NIR), spectroscopy

Introduction

Tea quality has been evaluated traditionally by an organoleptic test in Japan. In ensuring objectivity and consistency of the testing results, that method requires special skills of testers who should accumulate a great deal of experience in practice of testing. Another type of testing method based on chemical analyses to evaluate tea quality is generally complicated and not suited for practical use by manufactories. In the tea market, there is no rule and regulation enforcing to indicate contents of the chemical constituents of Japanese green tea in a label of package.

Use of the near infrared reflectance (NIR) spectroscopy was proposed by Ben-Gera[1] at the United States Department of Agriculture, Beltsvill Laboratory, where that method was first applied to analysis of soybean ingredients. Wider applications and practical uses have been developed in the United States and other countries as well to determine protein contents in wheat[3,13,14]. It is now recognized that the NIR spectroscopy is a rapid and non-chemically destructive method for quantitative analyses of food compositions.

Materials and method

1) Materials

In a series of the studies undertaken[6–10], 58 kinds of green tea with different tea qualities were subjected to test, among which half of the samples were selected for the calibration test to obtain data pertaining to actual levels and variations of the contents of total nitrogen (T–N), caffeine, total free amino acids (T–FAA), theanine and tannin (Table 1). The other half of the samples were used as "unknown samples" to evaluate the accuracy of the calibration curve obtained. Mean values and ranges of the contents of the chemical constituents of these samples are also shown in Table 1.

The contents of the constituents were determined as follows: Kjeldahl method for T–N; HPLC method for caffeine; an amino acids analyzer for T–FAA and theanine; and a colorimetric analysis for tannin content. Prior to the NIR measurement, the samples were ground into particles less than 0.5 mm in size with a cyclon-mill. A Neotec Research Composition Analyzer (Model 6350) was used for the NIR measurement. This instrument is equipped by a computer spectrophotometer, which operates in a single beam mode in the wavelength range from 1,100 to 2,500 nm.

Department of Tea Technology, National Research Institute of Vegetables, Ornamental Plants and Tea
(2769 Kanaya, Haibara, Shizuoka, 428 Japan)
Present address: Sakabe 2309-10, Haibara-cho, Shizuoka, 421-04 Japan

Table 1. Mean and range of chemical constituents of green tea samples

Constituents	Samples					
	For calibration			For prediction		
	n	Mean (%)	Range (%)	n	Mean (%)	Range (%)
T-N	29	5.23	4.18— 6.23	31	5.41	4.08— 6.47
Caffeine	30	2.94	1.94— 4.68	28	3.06	2.05— 4.41
T-FAA	29	2.91	0.86— 6.53	29	2.83	0.86— 6.45
Theanine	30	1.48	0.40— 3.15	28	1.41	0.41— 3.12
Tannin	29	12.41	9.00—18.50	29	13.77	9.60—17.10

An InfraAlyzer (Bran Lubbe Ltd.) which is a fixed filter instrument at 19 wavelengths was used to measure the contents of T-N, caffeine, T-FAA and theanine of green tea for the application of NIR method to tea quality control by a tea factory.

2) *Calibration and prediction*

Reflectance (R) spectra in the wavelength range of 1,100 to 2,500 nm were determined with 2 nm intervals in the NIR illuminated samples and their records were taken as the second derivative values of the original log (1/R) curve. A stepwise multiple-linear regression was employed in formulating prediction equations, for which values of the wavelength best correlated with the chemically determined contents were used.

The NIR spectra of the "unknown samples" were used to formulate NIR prediction equations for the contents of T-N, caffeine, T-FAA, theanine and tannin of the materials to be tested.

Results and discussion

An NIR spectrum of a processed green tea sample and its second derivative spectrum are shown in Fig. 1. In the region from 1,100 to 2,500 nm, absorption that can be assigned to NH combination group of protein[12] occurs at 1,980, 2,050 and 2,180 nm. In this connection, Krikorian and Mahpour[11] reported that the bands at 1,960, 2,000, 2,050, 2,100 and 2,150 nm were assigned to NH asymmetrical stretching (asym. str.) plus amide II; NH symmetrical stretching (sym. str.) plus amide II; NH asym. str. plus amide III; NH sym. str. plus amide III; and twice amide I plus amide III, respectively.

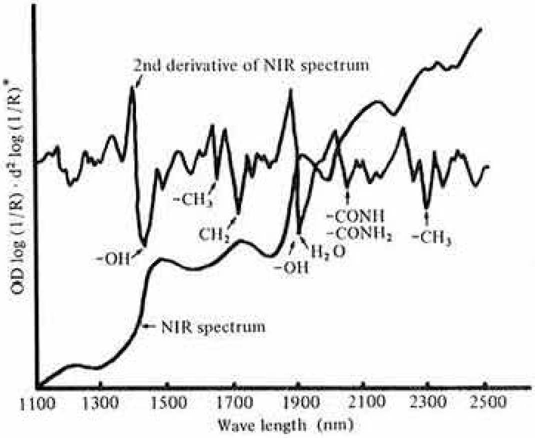

Fig. 1. Spectrum of pulverized green tea
* OD: Optical density

A methyl ($-CH_3$) group has absorption bands at 1,350, 1,690 and 2,260 nm[9], while the OH first overtone band in alchohol and phenols occurs in the region of 1,405—1,425 nm with a second overtone band between 945 and 985 nm[4]. The formation of hydrogen bands results in a displacement of the fundamental and hence overtone bands to 1,492 and 1,010 nm. For comparison, Curico and Petty[2] observed a band at 1,940 nm in the spectrum of liquid water, and Hecht and Wood[5] reported a band at 1,945 nm, which was in their view attributed to water bands.

Table 2 presents results of the predictions with multiple regression equations as well as related statistical parameters to indicate the accuracy of the determinations of the chemical constituents estimated by the NIR method. An equation to predict

Table 2. Values of constants, wavelength and statistics for equation[a] for predicting constituent contents of green tea with an NIR method

Equation	k0	k1	λ1	k2	λ2	k3	λ3	k4	λ4	k5	λ5	r[b]	d[c]	SEP(%)[d]	t
T-N (%)	5.428	−937.784	1978	209.759	2018	623.533	1442	753.317	1338	—	—	0.982	−0.050	0.146	1.895
Caffeine (%)	6.706	−170.187	1692	405.499	1990	−1631.893	2422	762.173	2074	780.390	2364	0.912	0.006	0.350	0.091
T-FAA (%)	7.001	−661.855	1988	1938.600	1668	−1546.216	1408	−1626.512	2130	—	—	0.947	−0.075	0.438	0.912
Teanine (%)	0.625	477.793	2162	−926.026	1992	−1123.977	2272	−1217.702	2336	−1792.781	1804	0.935	−0.035	0.282	0.657
Tannin (%)	0.283	587.225	1458	8774.270	1770	5594.531	1626	—	—	—	—	0.852	−0.105	1.022	0.555

a): All the equations are of the form: $A(\%) = k_0 + k_1 d^2 (\log I/R)\lambda_1 + k_2 d^2 (\log I/R)\lambda_2 + \cdots + k_n d^2 (\log I/R)\lambda_n$.
b): Correlation coefficient between the chemical values and the NIR values.
c): Mean difference between the chemically analyzed values and the NIR predicted values.
d): Standard error of prediction.

determinations of chemical constituents was formulated from the selection of calibration curve which showed a highest multiple correlation coefficient for the "unknown samples".

The least standard error and the bias in predicting constituents contents of the "unknown samples" were calculated on the basis of the calibrations of T-N, caffeine, T-FAA, theanine and tannin in the relevant stepwise regression analyses. Following is the equation formula employed:

$$A\% = k_0 + k_1 d^2 (\log I/R)\lambda_1 + k_2 d^2 (\log I/R)\lambda_2 + \cdots k_n d^2 (\log I/R)\lambda_n;$$

where A is chemical compound of the processed green tea, k_1, k_2, k_3 ··· are constants; and $d^2 (\log I/R)\lambda_1$ ··· are second derivative values of original $\log (I/R)$ at wavelengths of λ_1, λ_2, λ_3, ··· λ_n, respectively.

The first specific wavelengths for these calibrations were designated at 1,978 nm for estimating T-N contents; 1,692 nm for caffeine contents; 1,988 nm for T-FAA contents; 2,162 nm for theanine contents; and 1,458 nm for tannin contents in green tea, respectively. Those first wavelengths have a greater correlation coefficient with the contents of chemical constituents and also with absorbances presented in the second derivative spectrum.

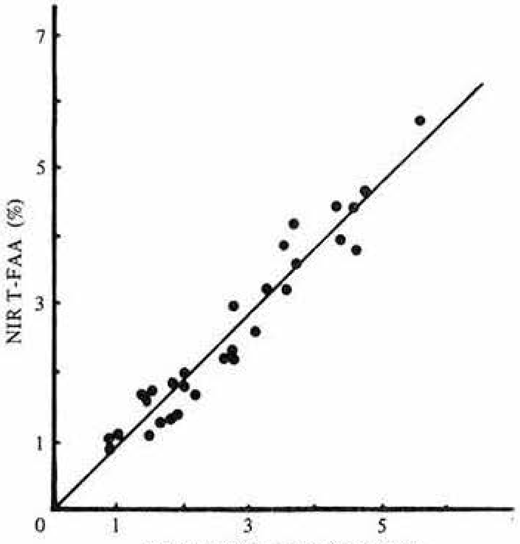

Fig. 2. NIR predicted T-N content plotted against the chemical values in green tea

The absorption bands may be assigned as follows: at 1,978 nm (for T–N) to NH asym. str. plus amide II; at 1,692 nm (for caffeine) to C–H str. first overtone of $-CH_3$ group; at 1,988 nm (for T–FAA) to NH asym. str. plus amide II; at 2,162 nm (for theanine) to 2 × amide I plus amide II; and at 1,458 nm (for tannin) to OH 1st overtone.

The predicted contents of the component constituents estimated from the above calibrations for the "unknown samples" are shown in Figs. 2–6.

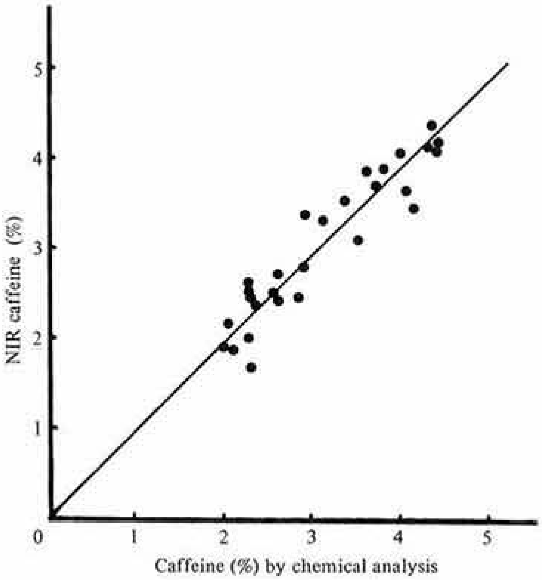

Fig. 3. NIR predicted caffeine content plotted against the chemical values in green tea

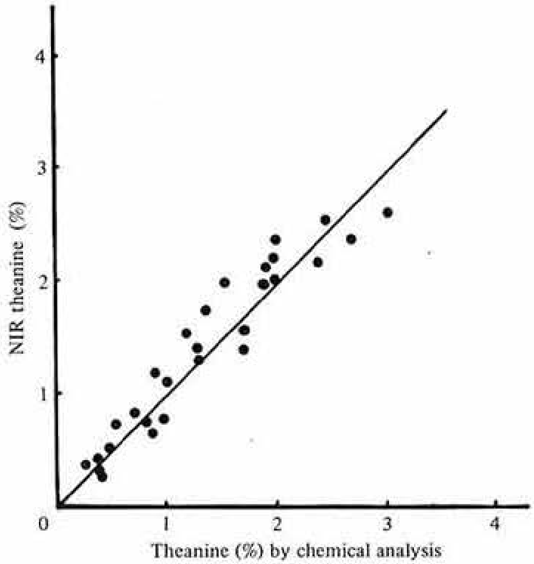

Fig. 5. NIR predicted theanine content plotted against the chemical values in green tea

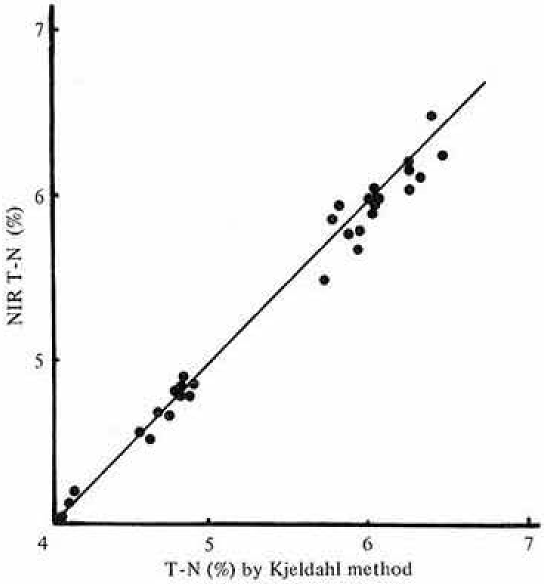

Fig. 4. NIR predicted T–FAA content plotted against the chemical values in green tea

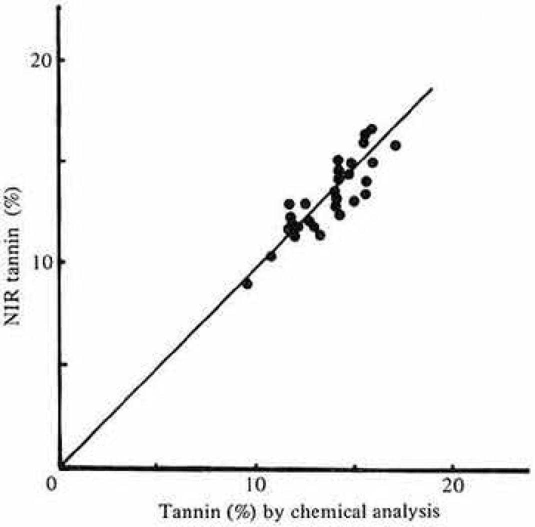

Fig. 6. NIR predicted tannin content plotted against the chemical values in green tea

In these figures, the NIR prediction values are plotted against the actual contents of chemical constituents. The standard errors of prediction (SEP), correlation coefficients between the chemical values and the NIR values (r) and mean differences between the actual contents and the NIR predicted values (d) are as follows:
for T–N, SEP = 0.146%, r = 0.982, d = 0.050;
for caffeine, SEP = 0.350%, r = 0.912, d = 0.006;
for T–FAA, SEP = 0.438%, r = 0.947, d = −0.075;
for theanine, SEP = 0.282%, r = 0.935, d = −0.035;
and for tannin, SEP = 1.022%, r = 0.852, d = −0.105, respectively. In order to evaluate the accuracy of prediction, a statistical analysis by a t-test was undertaken regarding the mean differences between the actual contents and the NIR predicted values. The t-values of the predicted equations are given in Table 2. Since the significant limit at 1% level for n = 27 and 30 is 2.77 and 2.75, respectively, there is no significant difference in the mean values.

From the above result, it is concluded that contents of T–N, caffeine, T–FAA, theanine and tannin of green tea can be estimated accurately with the NIR method.

References

1) Ben-Gera, I. & Norris, K. H. (1986): Determination of moisture content of soybeans by direct spectrophotometry. *Isurael J. Agr. Res.*, **18**, 125–132.
2) Curico, J. A. & Petty, C. C. (1951): The near infrared absorption spectrum of liquid water. *J. Optical Soc. Amer.*, **41**, 302–304.
3) Downey, G. & Byrne, S. (1983): Determination of protein and moisture in ground wheat by near infrared reflectance spectroscopy. *Isurael J. Food Sci. Technol.*, **7**, 135–146.
4) Goddu, R. F. (1960): Near-infrared spectrophotometry. *In* Advances in analytical chemistry and instrumentation I, 347–417.
5) Hecht, K. T. & Wood, D. L. (1956): The near infrared spectrum of the peptide group. *Proc. Royal Soc.*, **235**, 174–188.
6) Ikegaya, K. et al. (1985): Near infrared spectrophotometric analysis of total nitrogen content in green tea. *Nippon Shokuhin Kogyo Gakkaishi*, **32**, 553–559 [In Japanese with English summary].
7) Ikegaya, K. et al. (1985): Near infrared spectrophotometric analysis of total free amino acids in green tea. *In* Abstract of 61st Ann. Meet. Jpn. Soc. of Soil Sci. and Plant Nutr., 6 [In Japanese].
8) Ikegaya, K. et al. (1986): Near infrared spectrophotometric analysis of theanine in green tea. *In* Abstract of 63rd Ann. Meet. of Jpn. Tea-Technician's Assoc. *Tea Res. J.*, **63**, 75–76 [In Japanese].
9) Ikegaya K. et al. (1987): Near infrared spectra of caffeine and its related compound and their application to determination of caffeine content in green tea. *Nippon Shokuhin Kogyo Gakkaishi*, **34**, 254-258.
10) Ikegaya, K. et al. (1987): Near infrared spectrophotometric analysis of tannin. *In* Abstract of 65th Ann. Meet. of Jpn. Tea-Technician's Assoc. *Tea Res. J.*, **65**, 147 [In Japanese].
11) Krikorian, S. E. & Mahpour, M. (1973): The identification and origin of N-H overtone and combination bands in the near-infrared spectra of simple primary and secondary amides. *Spectrochimica Acta.*, **29A**, 1233–1246.
12) Law, D. P. & Tkachuk, R. (1977): Near infrared diffuse reflectance spectra of wheat and wheat components. *Cereal Chem.*, **54**, 256–265.
13) Osborne, B. G. et al. (1982): The development of universal calibrations for measurement of protein and moisture in UK home-grown wheat by near-infrared reflection analysis. *J. Sci. Food Agr.*, **33**, 736–740.
14) Williams, P. C. (1979): Screening wheat for protein and hardness by near infrared spectroscopy. *Cereal Chem.*, **56**, 169–172.

(Received for publication, Feb. 14, 1989)