Application of Near-Infrared Reflectance Spectroscopy to the Simultaneous Prediction of Alkaloids and Phenolic Substances in Green Tea Leaves

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A near-infrared reflectance spectroscopic (NIRS) method for the prediction of polyphenol and alkaloid compounds in the leaves of green tea [*Camellia sinensis* (L.) O. Kuntze] was developed. Reference measurements of the individual catechins, gallic acid, caffeine, and theobromine were performed by reversed-phase HPLC. The total polyphenols were determined according to the colorimetric Folin−Ciocalteu assay. Using the partial least-squares algorithm, very good calibration statistics were obtained for the prediction of gallic acid, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate, (−)-epigallocatechin gallate, caffeine, and theobromine ($R^2 > 0.85$) with standard deviation/standard error of cross-validation (SD/SECV) ratio ranging from 2.00 to 6.27. Simultaneously, the dry matter content of the tea leaves can be analyzed very precisely ($R^2 = 0.94$; SD/SECV = 4.12). Furthermore, it is possible to discriminate tea leaves of different age by principal component analysis on the basis of the received NIR spectra. Prediction of the total polyphenol content is performed with a lower accuracy, which might be due to the lack of specificity in the colorimetric reference method. The study demonstrates that NIRS technology can be successfully applied as a rapid method not only for breeding and cultivation purposes but also to estimate the quality and taste of green tea and to control industrial processes, for example, decaffeination.

Keywords: *Green tea; polyphenols; catechins; alkaloids; caffeine; HPLC; NIRS*

INTRODUCTION

Catechins are present in green tea leaves in relatively high amounts (up to 30% of dry matter). These compounds are mainly responsible for the characteristic astringent and bitter taste of black tea brews (Zhang et al., 1992; Kiehne, 1996). Recently the catechins have attracted much attention in relation to their antimutagenic and antitumorigenic activities (Wang et al., 1989; Zhu and Xiao, 1991). In contrast, Bu-Abbas (1997) stated that the contribution of flavanols to the antimutagenic activity of green tea is limited. The catechins have strong antioxidative properties as illustrated by their ability to scavenge oxygen radicals and chelate metal ions (Shahidi et al., 1993; Wanasundara and Shahidi, 1994; Chen and Ho, 1995). It has been stated that (−)-epigallocatechin gallate (EGCG) and (−)-epicatechin gallate (ECG) possess the strongest antioxidative effect in meat lipids and marine oils (Wanasundara and Shahidi, 1996). Epidemiological studies have also shown that tea polyphenols are effective in the chemoprevention of colon cancer (Kim et al., 1994). The health properties of tea flavonoids have been recently extensively reviewed (Tijburg et al., 1997; Hollman et al., 1997).

It is known today that the individual catechins have different antioxidative and health properties. This is why there is a need for a rapid and simple method to determine individual catechins in tea leaves, tea beverages, or instant teas.

In addition, the two plant alkaloids, caffeine and theobromine, known for their stimulatory effect, have to be recognized as important quality factors in green tea leaves. In contrast to the catechins, the content of the alkaloids is little or not affected by the fermentation process. In the past few years different methods of analysis have been employed to determine the contents of the compounds in question. Several catechins and alkaloids were separated by HPLC [e.g., Kuhr and Engelhardt (1991) and Goto et al. (1996)]. Some approaches to estimate the content of catechins and alkaloids using capillary electrophoretic techniques have also been described (Hoehne and Engelhardt, 1996; Horie et al., 1997; Larger et al., 1998). However, all of the methods mentioned above are time-consuming and require sophisticated equipment.

Near-infrared reflectance spectroscopy (NIRS) has already been used, for example, by Hall et al. (1988), for measuring the theaflavin and moisture contents as well as for the prediction of black tea quality as assessed by tea tasters. Attempts have been made also to describe the quality as defined by tasters of Chinese tea or Japanese tea by NIRS prediction of total polyphenols, total nitrogen, tannin, and total free amino acid content (Yan et al., 1990; Ikegaya, 1990; Goto et al., 1991). On the basis of a photometric reference method, a calibration for caffeine in green and black tea was also established by using four selected wavelengths (1470, 1646, 2084, and 2344 nm) and the full wavelength range from 1100 to 2500 nm, respectively.

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The aim of the present study was to examine the potential of NIRS as a rapid method to determine simultaneously the most relevant nonvolatile components in green tea leaves.

MATERIALS AND METHODS

Samples. One part (52) of the investigated green tea samples was supplied by various German tea packers. The other samples (43) were collected in the northern part of Thailand (Mae Chedi). The fresh leaves were immediately dried after harvest in a microwave-oven (at 100 W for 40−60 s) to inhibit uncontrolled enzymatic reactions. The average dry matter content of the green tea samples was 94%.

The catechin, gallic acid, and caffeine standards were kindly provided by Unilever (U.K.).

Pure theobromine was received from Roth (Germany). The theogalline content was determined using a gallic acid correlation plot corrected by molecular mass ratio.

The other reagents were of analytical grade and used without any further purification.

Cleanup. Green tea leaves were crushed by a beater mill (Janke & Kunkel, Germany) at 2000 rpm and sieved with a 0.5 mm screen. Approximately 0.2 g of the powdered material, accurately weighed, was extracted twice with 5 mL of 70% aqueous methanol each for 10 min at a temperature of 70 °C. After cooling, the extracts were centrifuged at 3000 rpm for 10 min. The liquid phases of both extracts were collected in a 10 mL volumetric flask and made up to volume by 70% aqueous methanol. The final tea extract was diluted 5-fold with the mobile phase prior to HPLC analysis.

HPLC Parameters. Equipment included a System Gold solvent delivery module M 126 (Beckman Instruments, San Ramon, CA), an autosampler 502 with a 20 μL sample loop, and a diode array detector (DAD; Beckman Instruments detector module 168) with IBM PS/2 PC and System Gold software.

Separation Conditions. The column used was a Nucleosil 100 C18 column (250 × 4.6 i.d.). The column was kept at 35 °C using a column oven. Eluents were (A) water/acetonitrile/acetic acid (88:10:2, v/v/v) and (B) acetonitrile. The gradient used was as follows: 100% A for 15 min, linear to 32% B in 10 min; isocratic for 7 min; in 1 min to 100% A; reset to the initial conditions, 20 min isocratic. Flow rate was 1 mL/min. DAD setting was 278 nm. Spectra were recorded in the range 190−400 nm.

Colorimetric Measurements. Total phenolics were estimated on the same extracts by a photometric Folin−Ciocalteu assay according to a proposed international standard method (ISO, 1994). The calibration standard was gallic acid.

NIRS Measurements. The NIRS analyses were carried out on a dispersive near-infrared NIRSystems 5000 (Foss Instruments Inc., Rodgau, Germany) in the reflectance mode. A commercial spectral analysis program (NIRS2, Infrasoft International Inc., Port Matilda, USA) was used to process the data and to develop the most appropriate chemometric methods. The spectral data were pretreated with weighted multiple scatter correction (WMCS) to eliminate interferences of scatter and were transformed individually with first- to third-derivative processing. The calibration program was set up with a wavelength range from 1108 to 2490 nm using the partial least-squares (PLS) algorithm. The optimum number of PLS factors used for the individual prediction was determined by cross-validation (Martens and Naes, 1989). The accuracy of the calibration models is described by the multiple coefficient of determination (R^2) and the overall error between modeled and reference values [standard error of cross validation (SECV)].

RESULTS AND DISCUSSION

The HPLC separation of a green tea sample is shown in Figure 1. The quality of the separation has frequently been checked by its spectra recorded using the DAD.

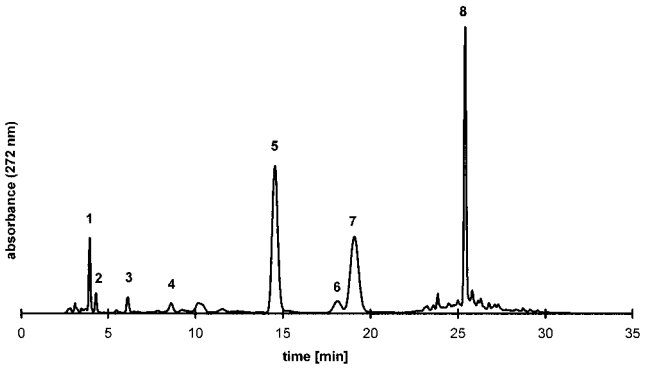

Figure 1. HPLC separation of catechins and alkaloids: (1) theogallin; (2) gallic acid; (3) theobromine; (4) epigallocatechin; (5) caffeine; (6) epicatechin; (7) epigallocatechin gallate; (8) epicatechin gallate. Conditions are given in the text.

Table 1. Calibration and Prediction Results Obtained for 95 Green Tea Samples by Applying NIRS in the Reflectance Mode

parameter	unit	ref measurements			NIR calibration stats		
		range	mean	SD	SECV	SD/SECV	R^2
DL	%	4.5−7.8	6.1	0.7	0.2	4.12	0.94
TPP	g kg^{-1}	60.8−199.8	151.0	33.6	19.3	1.74	0.67
CAF	g kg^{-1}	3.3−50.5	34.9	9.1	1.7	5.35	0.97
TB	g kg^{-1}	0.2−4.0	1.4	1.1	0.4	2.75	0.86
TG	g kg^{-1}	0.5−23.2	8.2	5.1	1.3	3.92	0.94
GA	g kg^{-1}	0.1−1.8	0.6	0.4	0.2	2.00	0.89
EC	g kg^{-1}	4.3−59.6	21.0	16.3	2.6	6.27	0.97
EGC	g kg^{-1}	10.9−45.1	28.7	8.0	3.1	2.58	0.85
EGCG	g kg^{-1}	10.2−122.1	68.1	26.0	6.9	3.77	0.93
ECG	g kg^{-1}	7.8−64.8	32.5	18.9	4.1	4.61	0.95

[a] Abbreviations: SECV, standard error of calibration; SD/SECV, ratio of the SECV to the standard deviation of the reference data; DL, drying loss; TPP, total polyphenols; TG, theogalline; GA, gallic acid; CAF, caffeine; TB, theobromine; EGC, epigallocatechin; EC, epicatechin; EGCG, epigallocatechin gallate; ECG, epicatechin gallate.

Individual NIR calibrations were developed by combining all 95 tea leaf samples in one common set. Table 1 shows the mean concentrations, standard deviations, and ranges of the different quality parameters analyzed by the reference methods (HPLC, Folin−Ciocalteu). It can be seen from Table 1 that the variation data are good for most of the criteria under investigation. Furthermore, it summarizes also the statistic parameters obtained in the development of the calibration equations. A high correlation between NIRS-predicted and HPLC values for the major catechins [(−)-epicatechin (EC), ECG, and EGCG] and for caffeine is obtained. The reason the NIRS determination of (−)-epigallocatechin (EGC) presents somewhat less prediction quality is probably referred to the applied HPLC reference procedure, which has been performed according to the described ISO method. In this method EGC is not detected at the absorption maximum. Taking into account the SD/SECV ratio of the investigated quality parameters, almost all of the developed models can be used for analytical purposes. The low SD/SECV ratio for total phenolics (1.74) is attributed to the limited selectivity of the applied photometric reference method.

Because cross-validation is a very useful tool to estimate the predictive ability of the calibration equations, no attempts have been made to perform additional external validation.

In Figures 2, 3, and 4 the correlation between the actual values calculated from HPLC analyses and the

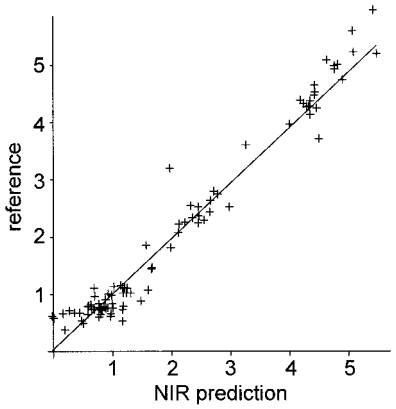

Figure 2. Reference determination versus NIR prediction of epicatechin in dried green tea leaves ($n = 95$). $R^2 = 0.97$; SECV = 0.26.

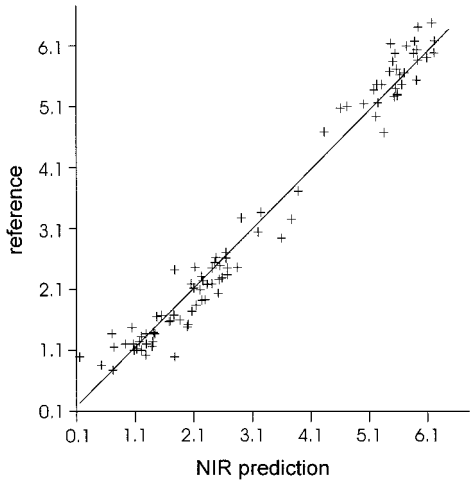

Figure 3. Reference determination versus NIR prediction of epicatechin gallate in dried green tea leaves ($n = 95$). $R^2 = 0.95$; SECV = 0.41.

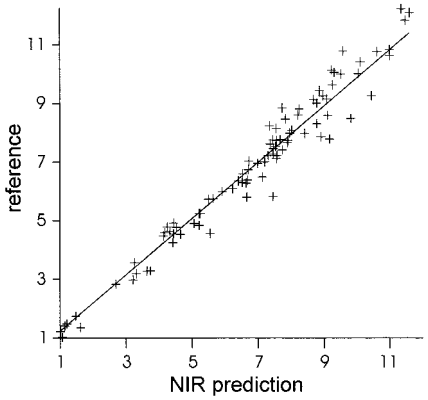

Figure 4. Reference determination versus NIR prediction of epigallocatechin gallate in dried green tea leaves ($n = 95$). $R^2 = 0.93$; SECV = 0.69.

predicted values received from the NIRS calibration set is shown for EC, ECG, and EGCG, respectively.

Pretreatment of the NIR spectra led to some improvement of final calibration results. The best results were achieved with the first or second derivatives, which were used to glean additional information and to avoid some band interference or overlap between different compounds. Furthermore, WMCS transformation, in which each sample's spectrum was corrected according to the major absorption bands, resulted in a similar scatter level.

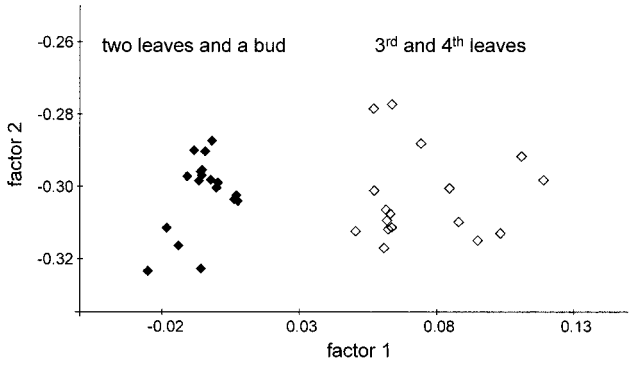

Figure 5. Discrimination of different aged green tea leaves by PCA.

To discriminate green tea leaves of different age, principal component analysis (PCA) was applied in the same way on the NIRS database. Five PCA axes are required to explain 97.5% of the spectral variation. Using the first two PCA axes (90.6% of the spectral variation explained) is sufficient to discriminate between the population groups "two leaves and a bud" and "third and fourth leaves" (Figure 5). This very distinctive discrimination is probably due to the fact that young sprouting parts of the tea shrub do contain more EGCG and ECG but less EC in comparison to older leaves.

It can be concluded that the described NIRS technique has high potential to estimate in a nondestructive way and with a high degree of accuracy the catechin and alkaloid composition in green tea leaves. These results have special significance because NIRS is a multitrait technique. Important quality parameters such as total phenolics, individual catechins, caffeine, theobromine, and dry matter contents may be determined simultaneously by one measurement in <1 min. Therefore, a simple, rapid, and reliable overall characterization of green tea quality may be obtained at a low cost. This is advantageous when large numbers of samples have to be analyzed, for example, in breeding programs or cultivation of tea plantations (harvest time). There also might be an application for quality control purposes and process control in the industry (instant and decaffeinated tea products).

In comparison to existing, time-consuming chromatographic methods, the results obtained in this feasibility study represent a considerable improvement in the estimate of quality parameters of green tea leaves by NIRS. Up to now, it was not possible to determine simultaneously all relevant catechins and alkaloids in green tea leaves without performing any cleanup procedures. The calibration equations developed in this study are based on wide ranges of the determined components.

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