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ARTICLES

Quantitation of Curcuminoids in Curcuma Rhizome by Near-infrared Spectroscopic Analysis

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This study investigated a nondestructive and rapid quantitation method for the curcuminoids, including curcumin, demethoxycurcumin, and bisdemethoxycurcumin, present in turmeric using near-infrared (NIR) spectroscopy and multivariate statistics. In the second derivatives of the NIR spectra of turmeric samples, two characteristic absorptions of curcuminoids were detected around 1700 and 2300−2320 nm. Partial least-squares regression (PLS-R) analysis was applied to the NIR spectra obtained from 34 turmeric samples, and PLS models for the quantitation of curcumin, demethoxycurcumin, bisdemethoxycurcumin, and total curcuminoid contents in the pulverized turmeric samples were constructed. Combination usage of the Standard Normal Variate (SNV) and second derivatives was obviously superior to other preprocessing methods. The lowest root mean squared error of cross-validation (RMSECV) values were detected at 6, 6, 6, and 6 PLS factors, for the quantitative subjects curcumin, demethoxycurcumin, bisdemethoxycurcumin, and total curcuminoid contents. It was clarified that the prediction of the composition by PLS-R analysis showed high correlation with the results of HPLC quantitations.

KEYWORDS: Curcumin; demethoxycurcumin; bisdemethoxycurcumin; *Curcuma longa*; near-infrared spectroscopy; partial least-squares regression

INTRODUCTION

Curcuma longa (turmeric) is a perennial native plant of southern Asia, where its rhizome is used as a spice (main ingredient of curry), a pigment dye of textiles, and in traditional medicine. There are several reports on a variety of pharmacological activities of turmeric, such as its anti-inflammatory, antimicrobial, antioxidant, antiparasitic, antimutagenic, and anticancer properties (*1, 2*). It is also efficient in the treatment of liver diseases and dermatological disorders (*3, 4*). Compounds belonging to two groups of natural products, the diarylheptanoids such as curcuminoids and the sesquiterpenoids, are considered to be responsible for much of the biological activity of turmeric (*5*). In particular, the curcuminoids including curcumin, demethoxycurcumin, and bisdemethoxycurcumin have been shown to contribute to many of the pharmacological activities of turmeric (*6, 7*).

A variety of methods, including high performance liquid chromatography (HPLC) and its coupling to mass spectrometry (LC-MS), thin layer chromatography (TLC), and capillary electrophoresis (CE) have been used to analyze the curcuminoids

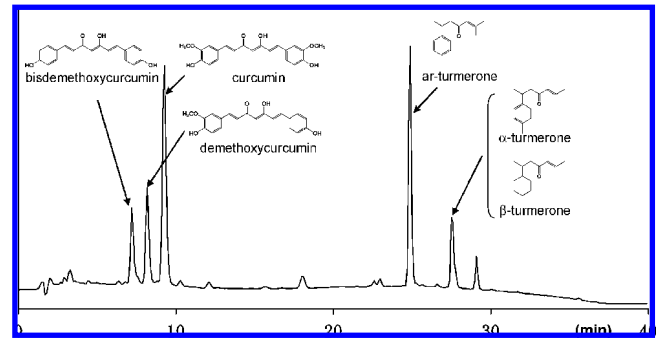

Figure 1. HPLC chromatograms of the extract of turmeric (rhizome of *Curcuma longa*) in 1% acetic acid/methanol solution (TMPW No. 20478).

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Table 1. Materials Used in This Study and Curcuminoid Content

name of crude drug and botanical source	production area	purchase from	collected date	TMPW[a] No.	curcuminoid content[b] (%) curcumin	demethoxy curcumin	bisdemethoxy curcumin	total culucuminoids
		Calibration Set						
Ukon	India	Tochimoto Tenkaido Co., Ltd., Osaka, Japan	1999.9	19148	0.29	0.11	0.05	0.45
	Guangdong, China	Uchida Wakanyaku Co. Ltd., Nigata, Japan	2000.4	19923	0.99	0.57	0.31	1.87
		Tochimoto Tenkaido, Osaka, Janan	2001.6	20283	0.14	0.04	0.00	0.18
	Viet Nam	Hino Pharmaceutical Co. Ltd., Osaka, Japan	2002.2	24978	2.15	0.57	0.37	3.09
	Guangdong, China	Hino Pharmaceutical Co. Ltd., Osaka, Japan	2002.4	24976	0.86	0.32	0.31	1.49
	Sichuan, China	Hino Pharmaceutical Co. Ltd., Osaka, Japan	2002.9	24977	1.03	0.41	0.23	1.67
	Viet Nam	Hino Pharmaceutical Co. Ltd., Osaka, Japan	2002.9	24979	2.42	0.68	0.45	3.55
	India	Uchida Wakanyaku Co. Ltd., Tokyo, Japan	2005.9	24455	2.14	1.00	0.70	3.84
	Sichuan, China	House Foods corporation, Tokyo, Japan	2007.10	25850	1.18	0.51	0.33	2.02
Turmeric alleppy finger	Alleppy, India	Tochimoto-Tenkaido, Osaka, Janan	2000.6	19962	1.42	0.54	0.30	2.26
Manjal	Tamil Nadu, India	R. D. Drug House, India	2000.12	20479	1.71	0.64	0.41	2.76
	Trivandrum, India	Perumal Pilla Drugs, India	2000.12	20480	1.45	0.54	0.42	2.41
	Trivanorum, India	Perumal Pilla Drugs, India	2000.12	20481	0.89	0.25	0.20	1.34
Ami Haldi	Vijayawada, India	Laladawasaz Priuate Ltd., India	2000.12	20487	0.45	0.16	0.00	0.61
Pasappu	Andhra Pradesh, India	Private Ltd., India	2000.12	20486	1.52	0.41	0.30	2.23
	Andhra Pradesh, India	Amrutha Company, India	2000.12	20494	1.66	0.36	0.22	2.24
	Andhra Pradesh, India	Amrutha Company, India	2000.12	20496	1.08	0.47	0.38	1.93
	Andhra Pradesh, India	Amrutha Company, India	2000.12	20498	1.12	0.54	0.31	1.97
Jianghuang	Sichuan, China	Qiwmingshi, Yunnan, China	1998.9	21654	0.06	0.01	0.00	0.07
	Yunnan, China	Qiwmingshi, Yunnan, China	1998.9	21657	0.37	0.18	0.19	0.74
	Sichuan, China	Jianweicounty Xinmin hospital, China	2000.8	20210	1.24	0.39	0.28	1.91
	Sichuan, China	Xinminzhen clinic, Jianwei, Sichuan, China	2000.8	20208	1.35	0.45	0.32	2.12
	Sichuan, China	Shuang Giucoiunty TCM Company, China	2000.8	20240	0.74	0.24	0.21	1.19
	Guangdong, China	Qingping Traditional Medicine Store, China	2001.9	21021	1.72	0.74	0.68	3.14
	Guangdong, China	Sihui Traditional Medicine Company, China	2001.9	21033	1.82	0.70	0.51	3.03
		Jiudetang, Jinshi City, Zhejiang, China	2001.9	21085	0.53	0.19	0.14	0.86
Kunir		Pasar Sentral Sorong, Irian Jaya, Indonesia	1997.11	17683	1.62	0.60	0.44	2.66
		Test Set						
Ukon		Uchida Wakanyaku Co., Ltd., Niigata, Japan	2000.6	19965	0.53	0.17	0.09	0.79
	Guangdong, China	House Foods corporation, Tokyo, Japan	2007.10	25851	0.76	0.34	0.37	1.47
Turmeric ganter finger	Gunter, India	Tochimoto Tenkaido, Osaka, Janan	2001.6	19964	1.21	0.33	0.19	1.73
Jianghuang	Yunnan, China	Qiwmingshi, Yunnan, China	1998.9	21656	1.19	0.41	0.34	1.94
	Yunnan, China	Hehachi Market, Sichuan, China	2000.8	20259	1.05	0.33	0.30	1.68
Kunir		Nyonya Meneer (Museum), Indonesia	1994.2	14131	1.36	0.48	0.54	2.38
		Akar Sari, Indonesia	1994.2	14208	1.38	0.43	0.39	2.20

[a] The sample voucher number of the Museum of Materia Medica, Institute of Natural Medicine, University of Toyama (TMPW). [b] Curcuminoid contents were calculated as the average of triplicate quantitations.

Table 2. Linearity of the Calibration Curve, Accuracy and Precision of Developed Analytical Method[a]

	r^2	range (mg/mL)	recovery (%)	R. S. D. (%) intraday variability	interday variability
curcumin	0.999	0.01−0.1	101.10 ± 1.63	1.02	0.89
demethoxycurcumin	0.999	0.01−0.1	101.14 ± 1.78	0.91	1.35
bisdemethoxycurcumin	0.999	0.01−0.1	100.22 ± 2.20	1.64	1.72

[a] R. S. D., relative standard deviation.

Table 3. RMSECV, SEP, and Correlation Coefficient between the Measured and Predicted Values of Three Curcuminoids and Total Curcuminoid Content[a]

	factor	RMSECV	SEP	r calibration set	r test set
curcumin	6	0.402	0.117	0.994	0.975
demethoxycurcumin	6	0.147	0.061	0.989	0.925
bisdemethoxycurcumin	6	0.103	0.070	0.991	0.978
total curcuminoids	6	0.573	0.174	0.994	0.988

[a] RMSECV, root mean squared error of cross validation; SEP, standard error of prediction; *r*, correlation coefficient.

in various turmeric samples (*8*−*12*). However, such chromatographic methods are time-consuming, require experienced personnel to perform the analysis, and are destructive.

Near-infrared (NIR) spectroscopy has been developed and proved to be a powerful tool for qualitative and quantitative analyses of constituents in food, agricultural, and pharmaceutical production control (*13, 14*). This technique has some advantages compared to traditional chemical and chromatographic methods. It provides a rapid, nondestructive method and requires minimal or no sample preparation. Furthermore, it is a multianalytical technique in which several determinations including physical properties and content of chemical constituents can be made simultaneously.

This article reports results from a feasibility study on the application of NIR in quantitative analysis of curcuminoids in turmeric powder.

MATERIALS AND METHODS

Materials and NIR Analytical Sample Preparation. Thirty-four crude drug samples were purchased from pharmaceutical companies or markets (**Table 1**). The botanical sources of samples were carefully identified through nucleotide sequence analysis of the *trn*K gene (*15*). All samples were deposited in the Museum of Materia Medica, Institute of Natural Medicine, University of Toyama (TMPW).

A sample was pulverized, and the powder was screened through 150 μm sieves. One gram of the fine powder was accurately weighed and placed in a glass vial (10 mL, 2 cm i.d., AS ONE Co. Ltd., Tokyo, Japan).

Standard Samples and Reagents. Curucmin, demethoxycurcumin, and bisdemethoxycurucmin were isolated from the methanol extract of turmeric or rhizome of *C. longa* (TMPW No. 24455) by column chromatography and preparative TLC (developing solvent, chloroform−

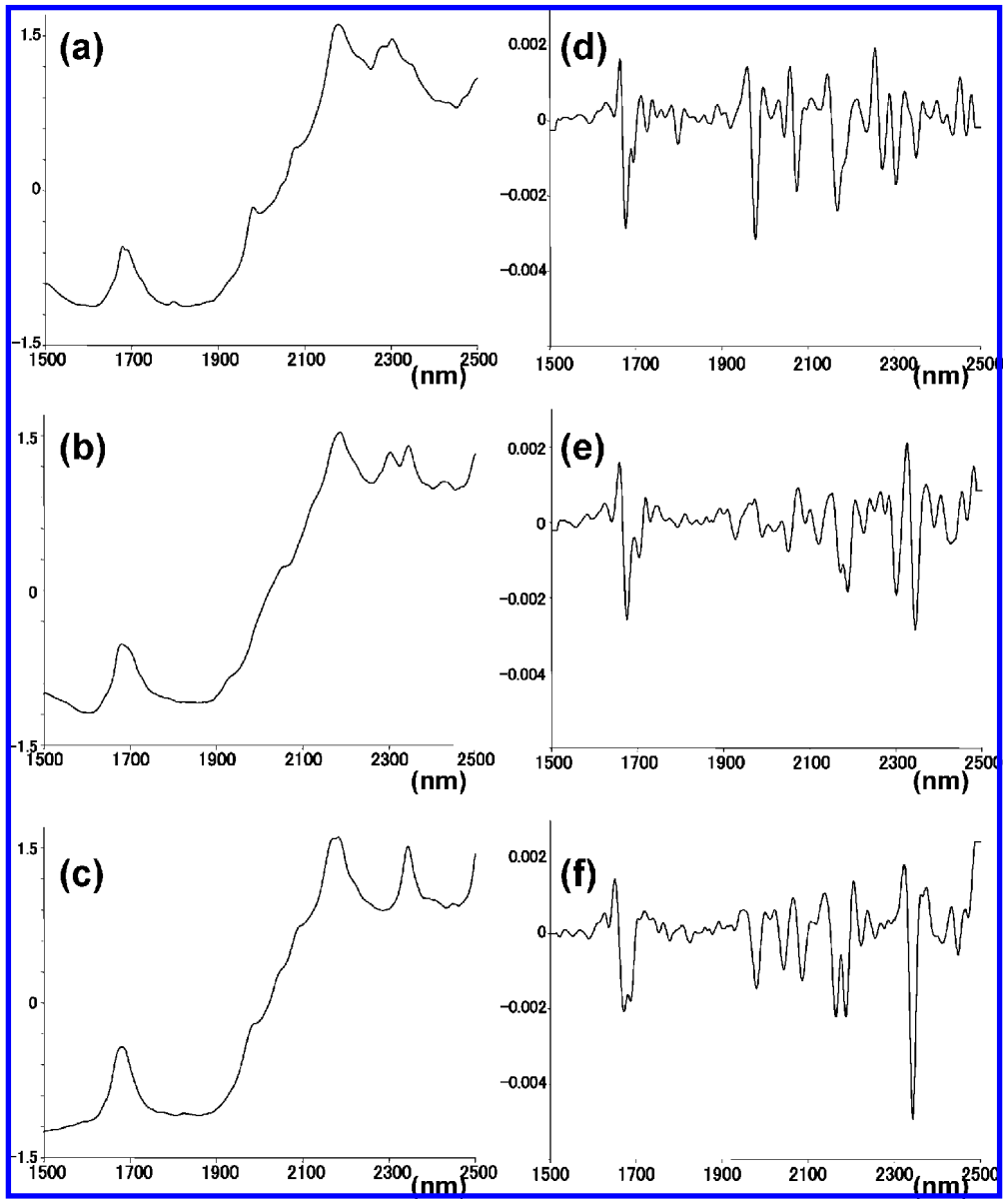

Figure 2. Standard normal variate (SNV) averaged NIR spectra of curcuminoids: (**a**) curcumin, (**b**) demethoxycurcumin, (**c**) bisdemethoxycurcumin, and 2nd derivatives of their spectra (**d**) curcumin, (**e**) demethoxycurcumin, and (**f**) bisdemethoxycurcumin.

methanol (9:1, v/v)). All isolated compounds were identified by comparison of their NMR and mass spectral data with those reported (*12, 16*).

All chemicals were of analytical grade, and chromatographic solvents were of HPLC grade.

Quantitation of Curcuminoids by HPLC Analysis. Pulverized samples were extracted two times with 25 mL of methanol−acetic acid solution (99:1, v/v) by shaking vigorously for 20 min. The organic solvents were combined and the volume adjusted to exactly 50 mL by adding methanol.

LC analyses were performed using a Shimadzu LC-10 LC system equipped with a Shimadzu SPD-10A UV detector. A Waters Symmetry C_{18} column (4.6 mm i.d. × 150 mm, 5 μm) was used. The column temperature was set at 40 °C and eluted compounds were detected by monitoring the UV absorbance at 254 nm. The mobile phase was a binary eluent of (A) 1% acetic acid solution and (B) CH_3CN under flowing gradient conditions 0−12 min isocratic at 45% B, 12−32 min linear gradient from 45% to 100% B, and 32−40 min isocratic at 100% B. Flow rate was 1.0 mL/min. Data analyses were carried out using chromatographic data processing software (TS Laboratory Inc., Tokyo, Japan).

Calibration Curves. Standard solutions of three curcuminoids were prepared and diluted to appropriate concentrations for the construction of calibration curves. Three concentrations of the curcuminoids were analyzed in three replicate injections. The calibration curves were constructed by plotting the peak areas versus the actual concentration of each curcuminoid.

Precision and Accuracy of Curcuminoid Quantitation by HPLC. Quantitation of intra- and interday variability was utilized to determine the precision and accuracy of the extraction and HPLC analytical methods for the curcuminoids. The intraday variability was examined on three replicate extractions of the turmeric powder (TMPW No. 20259) and analyses within 1 day; and the interday repeatability was determined for 3 consecutive days. The relative standard deviation (R. S. D.) was calculated as a measurement of extraction and HPLC analytical method repeatability. In order to examine the recovery of the extraction method, accurate amounts of the three standard curcuminoids were added to 100 mg of turmeric powder sample (TMPW No. 20259) and then extracted and analyzed. The recoveries were calculated by the formula: recovery (%) = (amount found − original amount)/amount spiked × 100%.

NIR Spectroscopic Analysis. NIR spectra for each powder sample were collected in triplicate using an acoustic optic tunable filter type portable NIR spectrometer (Systems Engineering Inc., Tokyo, Japan), by scanning directly through the base of the glass sample vials. NIR spectral acquisition was performed using GRAMS software (Thermo

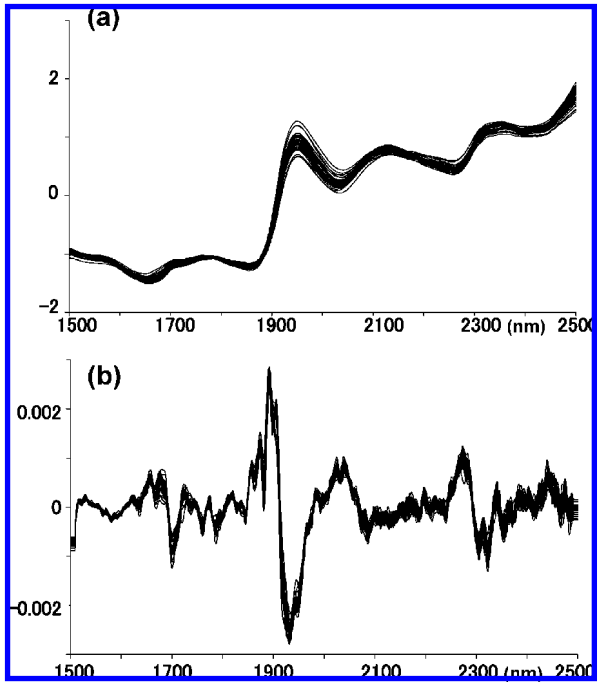

Figure 3. (**a**) Standard normal variate (SNV) averaged NIR spectra of 34 turmeric samples (**Table 1**) and (**b**) 2nd derivatives of their spectra.

Scientific Co. Ltd., Waltham, MA). To compensate for the effects of the OH group in the glass, the background was measured using a Spectralon 99% Reflective Standard (Labsphere, North Sutton, NH) through the base of the glass sample vials. NIR spectra were measured by scanning from 1500 to 2500 nm in the diffuse reflection mode. Individual sample vials were rotated 120° between triplicate scans, and respective spectra were recorded separately. To remove the baseline shifts in the spectra caused by particle size and packing differences, standard normal variate (SNV) transform was applied.

Multivariate Data Analysis. Before statistical analysis of the data by means of multivariate methods, NIR spectral data were converted to ASCII format using GRAMS software. All statistical analyses were carried out using Piroruet software (GL Science Inc., Tokyo).

If a sample's NIR spectrum differs greatly from the average training set NIR spectrum, it will have a great influence on the model, drawing the model closer to its location in factor space. A sample's influence is quantified by its *leverage*. In addition, if a sample's curcuminoid content (y) value is extreme, it has a greater influence on the model than a sample close to an average y value ($\hat{y}$). The extreme sample pulls the model toward it. Therefore, leverage objects and outliers have to be removed prior to constructing the calibration model. After SNV processing of the NIR spectra, examination of the presence of high leverage samples and the outlying observations in the data of spectra and contents of curcuminoid values were initially carried out. In this study, no data of the sample was removed as an outlier.

The optimal model complexity, i.e., the number of latent factors in the PLS model, was determined by the leave-two-out cross validation procedure. The root mean squared error of cross validation (RMSECV) is computed for PLS models with different numbers of latent factors:

$$\mathrm{RMSECV}(f) = (\Sigma(y\mathrm{cv}_i - y_i)^2/N)^{1/2}$$

where y_i is the measured response of the ith sample, $y\mathrm{cv}_i$ is a predicted response from a calibration equation obtained for the data without the ith sample, N is the number of calibration samples, and f denotes number of latent factors. The optimal complexity of the PLS model corresponds to the number of latent factors resulting in the lowest RMSECV.

RESULTS AND DISCUSSION

Quantitation of Curcuminoids by HPLC Analysis. Figure 1 shows the HPLC chromatogram of the extract of turmeric in 1% acetic acid−methanol solution. In comparative studies on the extraction procedures of curcuminoids, it was confirmed that the extraction efficiency of 1% acetic acid–methanol solution was higher than methanol alone, and almost complete extraction could be achieved. Three curcuminoids, curcumin, demethoxycurcumin, and bisdemethoxycurcumin, were completely separated and detected at retention times (*Rt*) 7.2 min (bisdemethoxycurcumin), 8.2 min (demethoxycurcumin), and 9.2 min (curcumin). In addition, several sesquiterpenoids, such as arturmerone (Rt = 24.8 min), α- and β-turmerone (Rt = 27.5 min), were detected. This indicated that present HPLC conditions could be utilized for simultaneous analysis of two types of major active constituents of turmeric. The linearity of the calibration curve, accuracy, and precision of the developed analytical method are presented in **Table 2**. All of the calibration curves of the three curcuminoids showed good linearity within the test range. In addition, the present method exhibited good accuracy with recovery 101.10 ± 1.63% (curcumin, n = 5), 101.14 ± 1.78% (demethoxycurcumin, n = 5), and 100.22 ± 2.20% (bisdemethoxycurcumin, n = 5). The intra- and interday variabilities were less than 5% for all curcuminoids. The average curcuminoid content in the turmeric analyzed in this study was 0.30% (bisdemethoxycurcumin), 0.42% (demethoxycurcumin), 1.16% (curcumin), and total curcuminoids was 1.88%.

NIR Spectra of Curcuminoids and Turmeric. The standard normal variate (SNV) averaged NIR spectra of curcuminoids are shown in **Figure 2a−c**. It is well known that the NIR spectral baseline level shifts depending on the particle size of the analyte; generally, the larger the particle the greater the baseline offset. To remove such a change in the baseline offset (*15*), SNV transformations were applied to the original spectra. The main differences between the spectra were observed in the positions of the inflection points of the absorptions around 1620−1740 and 2100−2550 nm. The absorptions around 1620−1740 nm are assignable to C−H stretching vibrations, and the differences of the spectra of respective curcuminoids are clearly indicated in their second derivatives as shown in **Figure 2d−f**. Generally, substitution on the benzene ring reduces the symmetry of the molecule and allows additional vibrational peaks in the first combination bands region (2100−2550 nm). In the NIR spectrum of bisdemethoxycurcumin, a strong absorption band is observed at 2345nm, whereas demethoxycurcumin showed additional absorption at 2300 nm. In the NIR spectra of curcumin, additional absorption at 2280 nm is observed.

The NIR spectra of turmeric and their second derivatives are shown in **Figure 3**. In general, it is difficult to find specific bands in NIR spectra on the basis of the species present since there are many components in herbal medicines, and NIR bands are composed of overtones and combinations of fundamental vibrations of functional groups. However, in the second derivatives of the spectra (**Figure 3b**), two characteristic absorptions of curcuminoids were detected around 1700 and 2300−2320 nm. The strong absorption around 1900 nm is assignable as that of water in the sample. The extremely strong absorption of water has a significant influence on the PLS model created. Therefore, exclusion of water absorption bands in the NIR spectra for PLS regression becomes a required general procedure for quantitative analysis. In this study, the absorption bands at 1850−2040 nm were removed prior to construction of the calibration model.

Quantification of Three Curcuminoids and Total Curcuminoid Content by PLS Regression Analysis. Partial least squares (PLS) regression is a method for constructing predictive models and is especially useful in quite common cases where

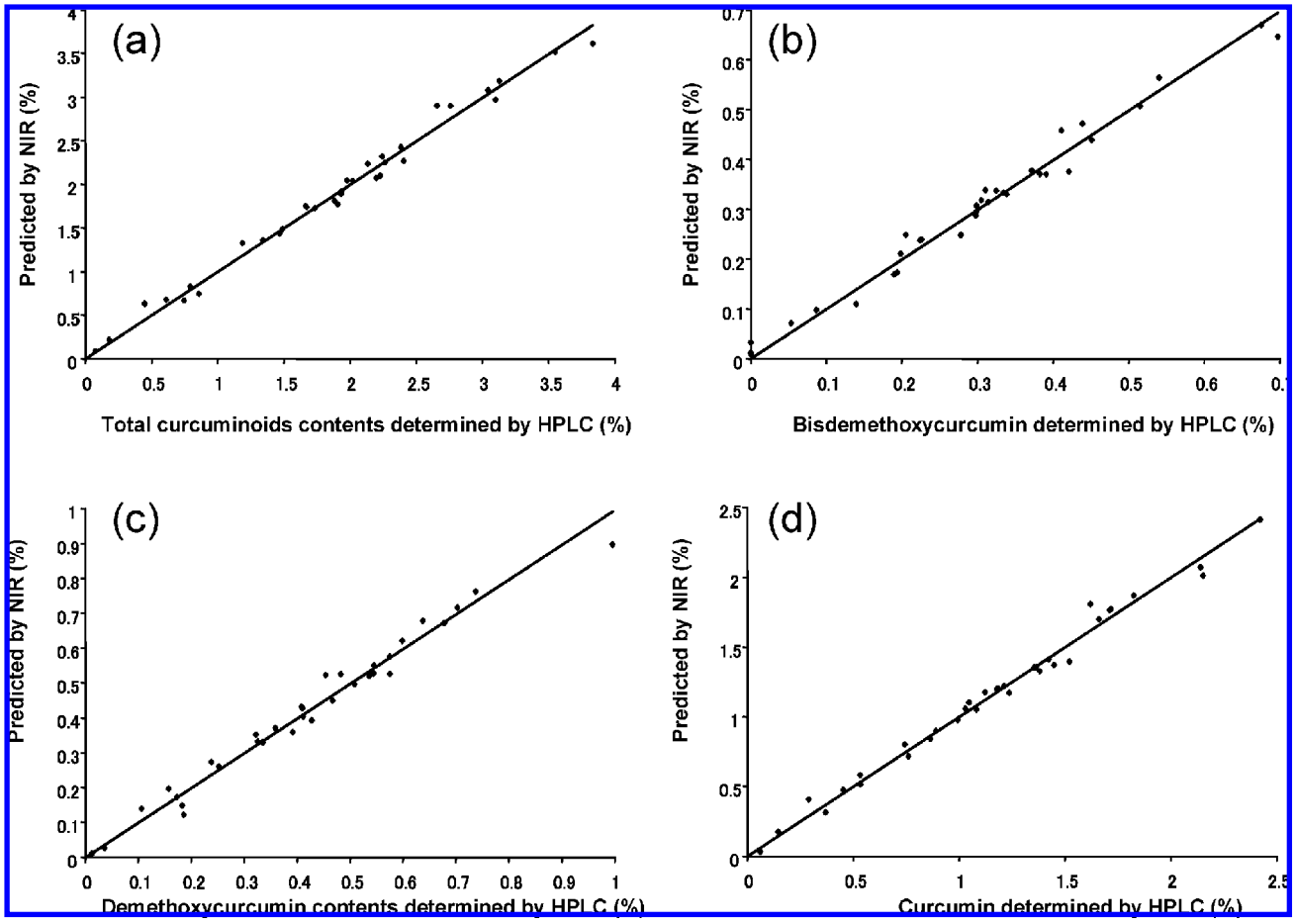

Figure 4. Score plots of prediction value and HPLC quantitated value of (**a**) total curcuminoids, (**b**) bisdemethoxycurcumin, (**c**) demethoxycurcumin, and (**d**) curcumin in 34 turmeric samples.

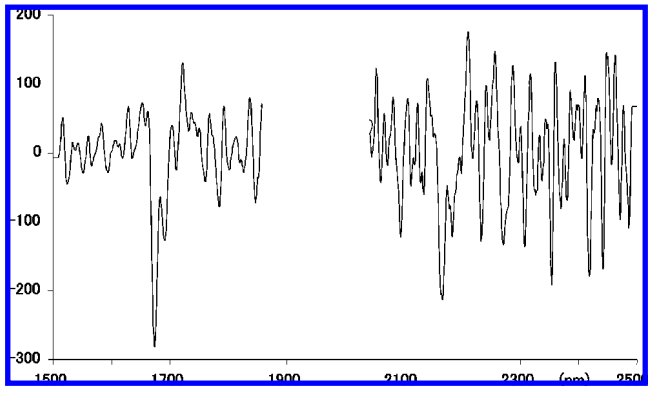

Figure 5. Regression vector of the PLS model for total curcuminoid content in turmeric. The region of absorption by water (1860−2040 nm) was removed.

the other factors leading to correlations between variables exist (*17*). However, spectroscopic analysis generates a variable-rich data set compared to the number of samples. In such circumstances, overfitting becomes the particular problem in PLS model creation. Specific potential information associated with the calibration data set is included in the model, but when unknown samples are predicted by this model, the information will mislead the prediction results. In order to validate the regression model, all 34 samples were divided into two groups: a calibration data set and a test set as shown in **Table 1**. The samples containing extremely low or high total curcuminoid content were included in the calibration data set, and other samples were randomly divided into two groups; 7 samples (20% of total sample number) were selected as the test data set.

A PLS model for quantitative analysis was established for curcumin, demethoxycurcumin, bisdemethoxycurcumin, and total curcuminoid content in turmeric using only the calibration data set. In PLS regression analysis, it is generally known that the spectral preprocessing and the number of PLS factors are critical parameters. The optimum number of latent factors of the PLS model can be determined by the lowest root mean squared error of cross-validation (RMSECV) in the leave-two-out cross validation procedure. Comparison of RMSECV values plotted as a function of PLS factors for three curcuminoids and total curcuminoids for different spectral processing, such as SNV with second derivatives and SNV alone and no-processing, indicated that the combination of SNV and second derivatives was obviously superior to other preprocessing methods. In the SNV and second derivatives preprocessed data set of the whole NIR spectral range (1500−2500 nm) without water absorption bands (1850−2040 nm), the lowest RMSECV values were detected at 6, 6, 6, and 6 PLS factors, for the quantitative subjects curcumin, demethoxycurcumin, bisdemethoxycurcumin, and total curcuminoid content. **Table 3** shows the RMSECV, standard error of prediction (SEP), and correlation coefficient between the measured and predicted values of the three curcuminoids and total curcuminoid content for the calibration and the test data set. The SEP values of curcumin, demethoxycurcumin, bisdemethoxycurcumin, and total curcuminoids were 0.117, 0.061, 0.070, and 0.174, respectively, and good correlation coefficients for the three curcuminoids and total curcumi-

noids were obtained. These results indicated that the present PLS models for the prediction of three curcuminoids and total curcuminoid content had high reliability.

In **Figure 4**, scatter plots showing the correlation between NIR prediction values and quantitated values by HPLC analysis for curcumin, demethoxycurcumin, bisdemethoxycurcumin, and total curcuminoid content in the 34 turmeric samples are presented. The calibration and prediction data indicated good correlation with those of the HPLC analysis, and many data points are on or close to the unity line. The correlation coefficients were 0.99, 0.98, 0.98, and 0.99 for curcumin, demethoxycurcumin, bisdemethoxycurcumin, and total curcuminoid content, respectively.

In the regression vector, it can be considered that the positive or negative peaks make strong contributions to the prediction of the contents by PLS regression analysis. **Figure 5** shows the regression vector of the PLS model for total curcuminoid content in turmeric. It shows the strong contributions of the absorption bands around 1650−1780 nm, and the absorption band was coincident with the characteristic absorption band of curcuminoids.

In conclusion, we verify the potential use of PLS-R models for the quantitation of curcumin, demethoxycurcumin, bisdemethoxycurcumin, and total curcuminoid content in pulverized turmeric samples. It was clarified that the prediction of the contents of the compounds by PLS regression analysis showed high correlation with the results of HPLC quantitations. Though a large set of samples from different sources is required to create a robust PLS model, NIR spectroscopic analysis for the quantitation of chemical constituents in herbal medicines provides the advantages of being a nondestructive and rapid measurement technique.

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