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Nondestructive Determination of Oil Content and Fatty Acid Composition in Perilla Seeds by Near-Infrared Spectroscopy

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Near-infrared reflectance spectroscopy (NIRS) was used as a rapid and nondestructive method to determine the oil content and fatty acid composition in intact seeds of perilla [*Perilla frutescens* var. *japonica* (Hassk.) Hara] germplasms in Korea. A total of 397 samples (about 2 g of intact seeds) were scanned in the reflectance mode of a scanning monochromator, and the reference values for the oil content and fatty acid composition were measured by gravimetric method and gas−liquid chromatography, respectively. Calibration equations for oil and individual fatty acids were developed using modified partial least-squares regression with internal cross validation ($n = 297$). The equations for oil and oleic and linolenic acid had lower standard errors of cross-validation (SECV), higher R^2 (coefficient of determination in calibration), and higher ratio of unexplained variance divided by variance (1-VR) values than those for palmitic, stearic, and linoleic acid. Prediction of an external validation set ($n = 100$) showed significant correlation between reference values and NIRS estimated values based on the standard error of prediction (SEP), r^2 (coefficient of determination in prediction), and the ratio of standard deviation (SD) of reference data to SEP. The models for oil content and major fatty acids, oleic and linolenic acid, had relatively higher values of SD/SEP(C) and r^2 (more than 3.0 and 0.9, respectively), thereby characterizing those equations as having good quantitative information, whereas those of palmitic, stearic, and linoleic acid had lower values (below 2.0 and 0.7, respectively), unsuitable for screening purposes. The results indicated that NIRS could be used to rapidly determine oil content and fatty acid composition (oleic and linolenic acid) in perilla seeds in the breeding programs for development of high-quality perilla oil.

KEYWORDS: Perilla seed; *Perilla frutescens* var. *japonica* (Hassk.) Hara; NIRS; perilla oil; fatty acid composition; oleic acid; linolenic acid; GLC

INTRODUCTION

Perilla [*Perilla frutescens* var. *japonica* (Hassk.) Hara] is one of the most important oilseed crops and is widely used as a source of edible vegetable oil and as a traditional health food in Asian countries such as Korea, China, and Japan. The traditional commercial products of perilla are seed oil as a spice or medicine and the leaf as a vegetable. Perilla oil, contributing approximately 40% of seed weight, is composed primarily of fatty acids such as palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic acid (18:3) (*1*, *2*). Linolenic acid (α-linolenic acid; ALA), an *n*-3 unsaturated fatty acid, is the most abundant, making up 60% of perilla oil, with oleic and linoleic acid the next most abundant. ALA is a precursor of long-chain *n*-3 polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6), and has beneficial effects in the control of chronic disease (*3*). Dietary intake of perilla oil containing a large amount of ALA provides various health benefits such as the lowering of the plasma lipid level and the increase in EPA and DHA in the hepatic membranes of rats (*4*, *5*).

Modification of the fatty acid composition in the oil, as well as an increase in the total oil content in the seeds, has been an important objective in the breeding for seed quality in perilla. These modifications require determination and analyses of the fatty acid composition of a large number of breeding lines. Oil content was measured using the gravimetric method following solvent extraction from pulverized seeds. Fatty acid composition was typically determined by gas−liquid chromatography and flame ionization detection (GLC-FID) with a capillary column. However, these methods require stepwise sample preparation such as saponification and esterification: fatty acids must be converted to fatty acid methyl esters (FAMEs) for GLC analysis. Although these analytical methods usually offer a high level of accuracy and precision, they are time-consuming, expensive, labor-intensive, and destructive and, therefore, not advantageous

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for the selection of superior lines of perilla. Thus, a rapid and nondestructive method for the evaluation of quality traits of seed oil is in high demand for the perilla breeding program.

Recently, the development of equipment featuring improved electronic and optical components, the improvement of computers capable of effectively processing information contained in spectra, and the development of powerful chemometric applications have facilitated the expansion of spectroscopic techniques in an increasing number of biological fields, allowing efficient management of spectra and chemical data obtained from the samples. The application of rapid analytical techniques such as near-infrared spectroscopy (NIRS) has many advantages compared to standard, traditional techniques such as the chromatographic method. NIRS analysis is performed with considerable reduction in analytical time and cost, and without using hazardous chemicals. In addition, samples can be analyzed in their natural form without destruction, and simultaneous analyses of numerous traits may be performed (*6*).

NIRS is known to play a fundamental role in the simplification of the analysis of chemical and physical properties without sample preparation: NIRS was used to analyze quality characteristics in food and agricultural commodities (*7*, *8*). NIRS has also been used for the determination of diverse compounds and classifications of numerous foods and industrial crops, such as sesame (*9*, *10*), soybean (*11*), peanut (*12*), sunflower (*13*), rice (*14*, *15*), maize (*16*, *17*), and sweet potato (*18*). However, application of the nondestructive NIRS to determine fatty acid composition in perilla seeds has not been previously reported, except for a report of the application of NIRS for perilla oil from ground seed samples (*12*).

The objectives of this study were to investigate NIRS application for predicting oil content and fatty acid composition and to develop a massive screening technique in intact seed samples for use with the perilla breeding program.

MATERIALS AND METHODS

Perilla Seed Samples. A total of 397 seed samples of perilla germplasm were obtained from National GeneBank, Rural Development Administration (Suwon, Korea), and were used to develop the NIRS prediction model for the determination of oil content and individual fatty acid composition. Samples were stored in desiccators prior to NIRS and GLC analysis.

Reference Analysis of Oil Content and Fatty Acid Composition. Approximately 2 g of each sample was homogenized using an Ultra-Turax T8 homogenizer (IKA-Werke GmbH & Co. KG, Staufen, Germany), extracted in 30 mL of *n*-hexane for 1 day by shaking at 100 rpm using a VS-8480 SRN horizontal shaker (Vison Co., Bucheon, Korea), and filtered with filter paper. The residues were extracted twice more, and the final volume of each extract solution was adjusted to 100 mL. The 50 mL of *n*-hexane extract was concentrated, dried for 6 h at 100 °C, weighed, and calculated for oil content, with data being doubled values. The total oil content was expressed as percentage of the dry seed weight.

For fatty acid analysis, a 1 mL aliquot of *n*-hexane extract was transferred into a reaction vial and concentrated under nitrogen flow at 70 °C. For the saponification of the oil, 0.5 mL of 0.5 N NaOH solution was added, allowed to react at 100 °C for 10 min, and then cooled. After 0.5 mL of 14% boron trifluoride (BF_3) solution was added, it was allowed to react at 100 °C for 10 min for the esterification of the fatty acids. After cooling, 1.5 mL of *n*-hexane and 1.0 mL of distilled water were mixed and partitioned in *n*-hexane, and the upper layer (containing fatty acid methyl esters in *n*-hexane extract) was transferred into a 2 mL autosampler vial for GC fatty acid analysis. The GLC system 6890N GC (Agilent Technologies Co., Palo Alto, CA), equipped with a flame ionization detector (FID) and a 30 m × 0.25 mm i.d., film = 0.25 μm, HP-Innowax capillary column (J&W Scientific, Agilent Technologies Co., Palo Alto, CA), was used. The oven temperature was raised from 160 °C (holding for 1 min) to 230 °C at a constant rate of 5 °C/min and held for 10 min. The injector and detector port temperatures were maintained at 230 and 250 °C, respectively. The carrier gas was nitrogen at a flow rate of 1.0 mL/min, and the split ratio at the injector port was 20:1. The running time was about 20 min per sample. Individual fatty acids were expressed as a percentage of total fatty acids.

Spectra Collection and Pretreatment. The NIR spectroscopic analysis was performed using a NIRSystem model 6500 near-infrared scanning monochromator (Foss NIRSystems Inc., Silver Spring, MD) in the reflectance mode. Intact seed samples (about 2 g) were placed in a standard ring cup and scanned. Reflectance energy readings were referenced to corresponding readings from an internal ceramic disk. Each spectrum was recorded from each sample, and the average of 32 successive scans was recorded. As a control, 16 scans over the standard ceramic disk were made before and after the samples. All spectral data were recorded as the logarithm of the reciprocal of reflectance (log 1/*R*) in the wavelength range from 400 to 2500 nm at 2 nm intervals to give a total of 1050 data points per sample. Absorption of radiation in the region of 400−2500 nm, the visible plus near-infrared region, was used to develop calibration equations related to sample properties. The scanning procedure could be completed in 1.5 min per sample, once the NIRS instrument was warmed up, and the stability of NIRS through photometric repeatability (noise test) and wavelength accuracy test was confirmed.

The NIRS manipulation for scanning, mathematical processing, and statistical analysis was performed with the WinISI II software (Windows version 1.60, Foss and Infrasoft International LLC, State College, PA). In WinISI software, the Score program was used to select samples for spectrum outliers and samples to represent the entire sample set (n = 397) before calibration and validation. The distance between a sample and its neighbor was measured as the *H* distance and was used as a criterion for selecting those samples representing the calibration and validation sets. The Score algorithm ranks spectra according to Mahalanobis distance (*H* distance) from the average spectrum, gives spectral boundaries to eliminate outliers with $H > 3.0$, and eliminates samples with similar spectra with $H < 0.6$ from their nearest neighboring samples for the development of an accurate and robust prediction equation (*19*). The final number of samples for calibration and validation was variable and based on the cutoff point of *H* distance, depending on the spectral and chemical variability of samples in the population used for NIRS estimation.

The samples (n = 397) were randomly split into two sets using the WinISI program. The calibration set (297 samples) was used to calibrate and cross-validate the derived equation, and the other 100 samples were used as an external validation set to test the fit of the developed equations.

Data Processing. The equations for NIRS prediction were developed using the Global program in WinISI software with modified partial least-squares (MPLS) regression using wavelengths of the entire visible (400−1100 nm) and near-infrared (1100−2500 nm) regions at every 8 nm. Besides MPLS, regression methods such as PLS (partial least-squares), principal component regression, and multiple linear regression were tested to develop calibration for fatty acid composition in perilla seeds. Various mathematical treatments using the raw optical spectrum (log 1/*R*), or first or second derivatives of the 1/*R* data, were applied for calibration equation development. For example, in 2,5,5,1, the first number indicates the order of the derivative (two is the second derivative of log 1/*R*), the second number is the gap in data points over which the derivative was calculated, and the third and fourth numbers represent the number of data points used in first and second smoothings, respectively (*20*). The application of the second-derivative algorithm to the raw spectra (log 1/*R*) resulted in an increase in the complexity of spectra and a clear separation between peaks, which overlapped in the raw spectra.

In addition to no scatter correction (log 1/*R*), scatter corrections using standard normal variate and detrending (SNVD) were evaluated for the calibration. The SNVD was designed to remove additive baseline and multiplicative signal effects resulting in a spectrum with zero mean and a variance equal to one. Application of SNVD transformation to

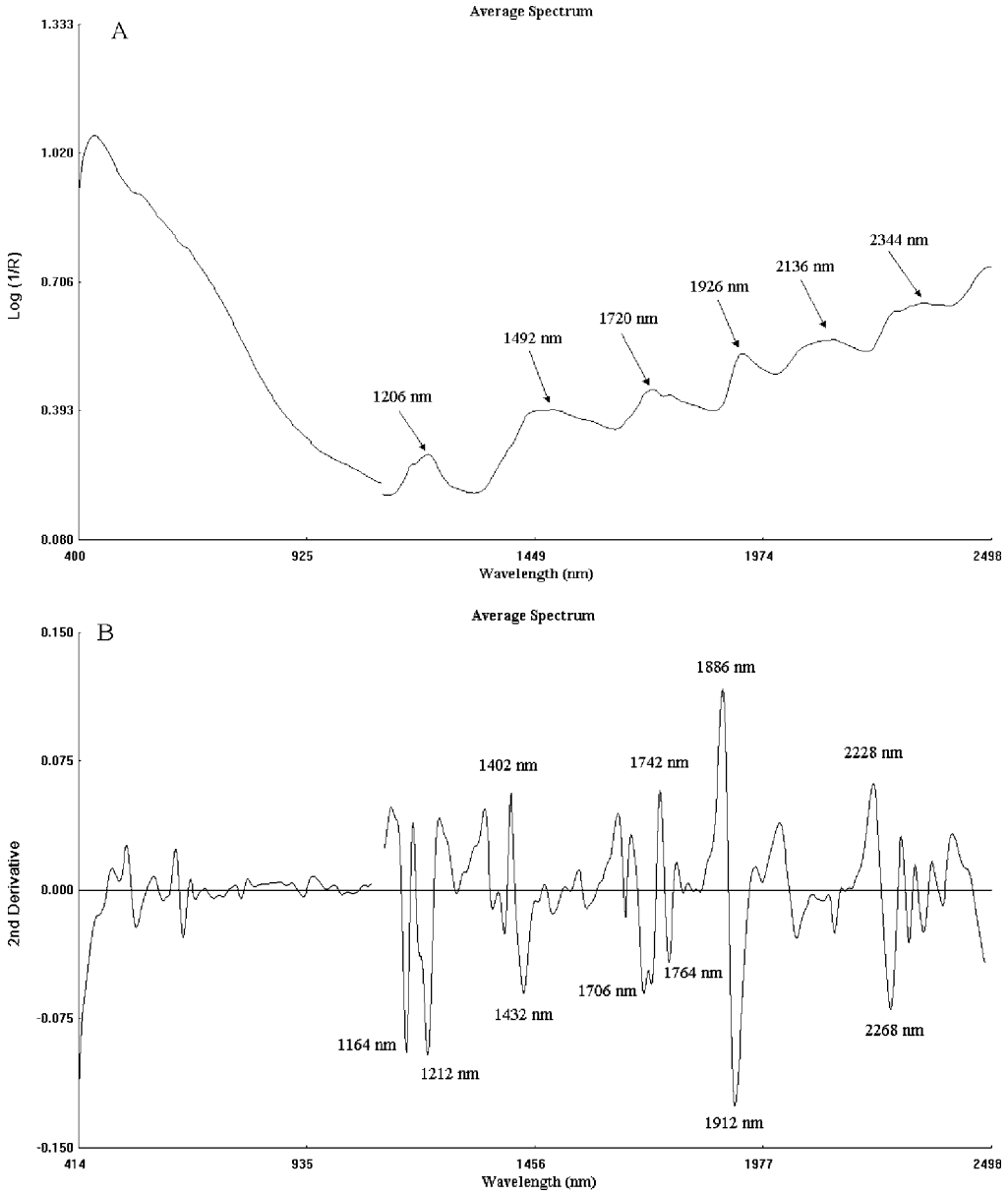

Figure 1. Raw spectrum (log 1/*R*; **A**) and second derivative (2,5,5,1 + SNVD; **B**) of NIRS average spectrum of intact perilla seeds.

raw spectral data reduces the differences in spectra related to physical characteristics such as particle size and path length of samples (*21*, *22*).

Calculated calibration statistics included the standard error of calibration (SEC), the coefficient of determination in calibration (R^2), and the standard error of cross-validation (SECV). The performance of the different equations obtained in the calibration were determined from cross-validation as an internal validation method. Internal cross-validation was used to avoid overfitting of the equations by selecting the minimum number of PLS terms in each model (*23*). The best-predicted equations for each chemical component were selected on the basis of minimizing SECV and increasing R^2 (*24*). Two passes to eliminate outliers were set by two outlier detection methods, *t* and *H* statistics (Mahalanobis distance) in WinISI software. The *t* statistics identified outliers having residuals from reference analysis of >2.5 times the SEC. Outliers indicated that their reference values were in doubt and that the samples were in different populations due to atypical spectra. The ratio (SD/SECV) of the standard deviation of reference data (SD) to SECV, designated RSC, was calculated as a criterion for evaluating the performance of calibrations (*25*).

After calibration, the developed regression equations allowed for accurate analysis of many other samples by prediction of data based on the spectra. In addition to the internal cross-validation, the external validations of calibration models were tested for the prediction capacity on the basis of the standard error of prediction (SEP) and the coefficient of determination in prediction (r^2). The ratio of SD for the validation samples to the corrected SEP (designated RSP) was also used as a criterion to evaluate the accuracy of the equations. This RSP value as cutoff point was 3.0 in this study, which is the value recommended for screening purposes (*25*). The validation sample set allowed the NIRS equation to be validated for prediction accuracy, using random samples not included in the calibration sample set (*24*). The equations selected for fatty acid composition in intact seeds of perilla were monitored with the Monitor program in WinISI software, using the validation set ($n = 100$).

RESULTS AND DISCUSSION

Spectroscopic Analysis. The raw NIR reflectance and second-derivative spectra of the intact seed samples of perilla are shown in **Figure 1**. The second derivative was calculated from the log(1/*R*) spectra at gaps of five data points (10 nm) and a smoothing over segments of five data points (2,5,5,1) with scatter correction (SNVD).

The main absorption bands are observed at 1720 nm related to C−O (oil) and C−H stretching first overtone (−CH_2), 2344

Table 1. Descriptive Statistics for Fatty Acid Composition and Oil Content in Intact Perilla Samples Used in both Calibration and Validation

	calibration ($n = 297$)			validation ($n = 100$)		
constituent	mean[a]	range	SD[b]	mean	range	SD
oil content	42.1	29.7–50.2	3.79	41.7	26.0–49.4	4.59
palmitic acid	6.48	5.7–7.5	0.26	6.50	5.9–7.4	0.29
stearic acid	2.11	1.4–3.4	0.31	2.13	1.4–2.7	0.29
oleic acid	14.8	8.4–21.0	2.16	14.9	10.8–19.8	2.12
linoleic acid	14.4	10.7–18.8	1.33	14.8	11.0–18.6	1.54
linolenic acid	62.2	56.1–69.8	2.48	61.7	56.0–66.5	2.30

[a] Expressed as percent of dry seed weight for oil content and as percent of individual fatty acid for total fatty acid composition. [b] SD, standard deviation of mean.

nm related to C−O (oil), 1206 nm related to $-CH_2$, 1492 nm related to −C═O and water, 1926 nm related to water, and 2136 nm attributed to oil. The information for the functional groups in the spectrum was determined using WinISI software. Compared with references, absorption bands at 1460 and 1930 nm are associated with water, the band at 1720 nm is associated with oil and fatty acids (*26*), and the band at 1708 nm is associated with linoleic acid (*9*), which were absorption bands appearing at similar wavelengths to our spectrum. The overall spectrum shows strong absorption bands related with oil and water and is similar to those of other oil crops such as soybean and sesame, especially in the near-infrared region (*10*, *12*). The second-derivative spectra had a trough corresponding to each peak in the original spectra, removing the overlapping peaks and baseline effects (*27*). The average spectrum of the second derivative showed absorption bands at 1212 nm related to C−H stretching second overtone ($-CH_2$), 1764 nm related to C−H stretching first overtone, 1164 nm related to −C═O, 1402 and 1706 nm related to hydrocarbons (−CH), and 1706 and 1764 nm also related to water in the NIR region. Most of the absorption bands at the second-derivative spectrum moved to the left somewhat as compared to the raw spectrum, but had a trough corresponding to each peak in the original spectra, showing absorbance peaks similar to those of the raw spectrum.

The NIRS method is based on the ability of several natural products to readily absorb NIR radiation at a specific region or wavelength, but it is so difficult to elucidate what wavelength or region at near-infrared spectrum was closely related with contents of natural compounds analyzed. Chemical information associated with each wavelength and region could be partially obtained from the reported references in edible fat and oil samples (*9*, *28*) and WinISI software, although the chemical interpretation for a specific wavelength would possibly be varied according to what experimental materials and chemical components were treated for NIR analysis. However, the NIR method has successfully been applied for estimating contents of chemical components in food and oil crops, even without chemical information related to the near-infrared spectrum.

Reference Analysis of Oil Content and Fatty Acid Composition. The descriptive statistics including mean, standard deviation (SD), and range for individual fatty acid composition of perilla seed samples used in the calibration and validation sets are shown in **Table 1**. Mean values of oil content and individual fatty acid composition were 42.1% oil, 6.5% palmitic acid, 2.1% stearic acid, 14.8% oleic acid, 14.4% linoleic acid, and 62.2% linolenic acid in the calibration set, values similar to those in the validation set. Each reference value of fatty acid composition in the validation sample set was similar to those in the calibration sample set. Also, the observed mean values are similar to previously reported results (*1*, *2*).

Calibration Model. From the development of NIRS models for the oil content and individual fatty acid composition, the statistics of calibrations and cross-validations are shown in **Table 2**. The MPLS regression model in the whole NIR spectra range (400−2500 nm) using the second-derivative transformation with scatter correction (SNVD) of raw reflectance spectra yielded the equations for oil and oleic and linolenic acid, showing higher R^2 (>0.8) and lower SEC in the calibrations and higher 1-VR and lower SECV values in the cross-validations than the different regression methods tested in this study. Using this whole vis−NIR range (400−2500 nm), higher R^2 and lower SEC values were obtained, better than the visible range (400−1100 nm) and the near-infrared range (1100−2500 nm). Optimum wavelengths for NIR analysis have generally relied on empirical calibrations to predict qualitative constituents for agricultural products because of the broad array of chemical compounds present in the samples, which lead to extensively overlapping and perturbed NIR absorption bands. The equations for each fatty acid composition using mathematical treatment 2,5,5,1 were selected with higher RSC (SD/SECV) values as the selection criteria of models, rather than the different mathematical treatments. The reliable equations for oil content and major fatty acids (oleic and linolenic acid) had high values of R^2 (0.917, 0.949, and 0.957, respectively) and RSC (2.97, 3.42, and 3.84, respectively), indicating a close relationship between reference values and NIRS estimated values. These equations for oleic and linoleic acid as major fatty acids had good accuracy, similar to those of other oilseed crops (*6*, *9*, *11*, *17*, *29*), with higher R^2 (>0.9) and lower SEC values. However, the models for palmitic, stearic, and linoleic acid did not have good statistics regarding the accuracy of the calibration models. These results may be due to the lower range and standard deviation for their content in the samples used in the calibration. The equation for linoleic acid, at a similar rate as oleic acid, also had relatively high R^2 (0.800) and low SECV, but the very low 1-VR value observed in the cross-validation indicated that it was not a strong model. In this study, 1-VR as a statistic for calibration was an important factor in addition to R^2. Even models having higher R^2 values could not show good correlation between reference values and NIRS estimated values if they did not have the high 1-VR value, closer to 1.0, as an explained variation. The best calibration models for oil content and oleic and linolenic acid were developed with the mathematical approach over the visible and near-infrared segment (400−2500 nm).

External Validation. The robustness of the calibration models developed by NIRS analysis was tested through external validation with 100 samples, which were not included in the calibration process. The statistics of external validation for oil and fatty acids in intact perilla seeds are shown in **Table 3** and include r^2, SEP(C) (the corrected standard error of prediction), and RSP [SD/SEP(C)] values, which were factors used to evaluate the reliability of the calibration model. On the basis of lower SEP(C) and higher r^2 and RSP values, an accurate prediction can be monitored with the reliability of the established calibration models. The r^2 and RSP values for palmitic, stearic, and linoleic acid were low, indicating poor correlation between reference values and NIRS estimated values, similar to the calibration models developed in this study. The predictions for oil content, oleic acid, and linolenic acid were confirmed by higher r^2 (0.932, 0.917, and 0.907, respectively) and RSP values (3.82, 3.48, and 3.28, respectively), indicating a good correlation

Table 2. Equation Development Statistics Using MPLS and Scatter Correction for the NIRS Prediction of Fatty Acid Composition and Oil Content in Intact Perilla Seeds

					calibration		cross-validation		
constituent	N [a]	mean	SD	terms[b]	SEC[c]	R^2 [d]	1-VR[e]	SECV[f]	RSC[g]
oil content	287	42.1	3.66	9	1.052	0.917	0.887	1.232	2.97
palmitic acid	288	6.49	0.24	9	0.148	0.616	0.502	0.168	1.42
stearic acid	290	2.10	0.30	6	0.155	0.725	0.686	0.165	1.78
oleic acid	289	14.8	2.11	14	0.476	0.949	0.914	0.617	3.42
linoleic acid	288	14.4	1.27	13	0.575	0.800	0.679	0.723	1.76
linolenic acid	290	62.2	2.45	14	0.507	0.957	0.932	0.638	3.84

[a] Samples used to develop the model. [b] Number of PLS loading factors in the regression model MPLS (modified partial least-squares). [c] SEC, standard error of calibration. [d] R^2, coefficient of determination of calibration. [e] 1-VR, one minus the ratio of unexplained variance divided by variance. [f] SECV, standard error of cross-validation. [g] RSC, SD/SECV, the ratio of SD (standard deviation of reference data) to SECV in the calibration set.

Table 3. Monitoring Statistics for Fatty Acid Composition and Oil Content in Intact Perilla Seeds

constituent	N[a]	mean	SD[b]	bias[c]	r^2 [d]	SEP(C)[e]	slope[f]	RSP[g]
oil content	95	41.8	4.31	0.416	0.932	1.128	1.019	3.82
palmitic acid	92	6.48	0.25	–0.001	0.451	0.186	0.899	1.34
stearic acid	98	2.14	0.29	0.005	0.686	0.163	0.923	1.78
oleic acid	94	15.0	2.08	–0.103	0.917	0.597	1.011	3.48
linoleic acid	92	14.6	1.42	0.188	0.677	0.808	1.044	1.76
linolenic acid	94	61.8	2.23	–0.159	0.907	0.680	0.972	3.28

[a] Samples used to monitor the model. [b] SD, standard deviation of mean. [c] Bias, average difference between reference and NIRS values. [d] r^2, coefficient of determination of cross-validation. [e] SEP(C), the corrected standard error of prediction. [f] Slope, the steepness of a straight line curve. [g] RSP, SD/SEP(C), the ratio of SD of reference data to SEP(C) in the external validation set.

between reference values and NIRS predicted values in the application of the calibration equations. In contrast, those for palmitic, stearic, and linoleic acid had low values of r^2 and RSP (below 0.7 and 2.0, respectively), unsuitable for screening purposes.

Palmitic and stearic acid in seed oil had minor composition in many oilseed crops (*6*, *9*, *11*, *17*, *29*), and calibrations for them did not show sufficient accuracy, with lower r^2 (below 0.8) and higher SEP(C) values, similar to the result obtained in this study. The calibration equation for stearic acid in sesame was reliable, with relatively higher r^2 value (0.869), using specific near-infrared region, although the equation for palmitic acid was not (*9*). Whereas validation for linoleic acid did not show the robustness of equation developed in this study, those in other crops were reliable with relatively high r^2 values such as 0.959 in sesame (*9*), 0.950 in soybean (*11*), 0.97 in Indian mustard (*6*), and 0.84 in single seed of maize (*17*). Our result for validation of the linolenic acid model showed better reliability with higher r^2 (0.907) in perilla than those (below 0.9) in other crops (*6*, *11*, *17*). Future studies require development of a new model for linoleic acid using more diverse germplasms differing from present samples, which should explain why our models did not predict the composition of linoleic acid with accuracy and reliability, in spite of the fact that samples used for this model had relatively high variation and linoleic acid was a major component contained at similar amount (about 14.0% in oil) to oleic acid in perilla.

Figure 2 represents laboratory reference values compared to NIRS predicted values in the validation set for oil content and oleic and linolenic acid, also showing the relationship between NIRS and reference. These results demonstrated the accurate prediction abilities of the calibration models for oil and oleic and linolenic acids using a nondestructive NIRS method in perilla seeds, and further application in the routine analysis of

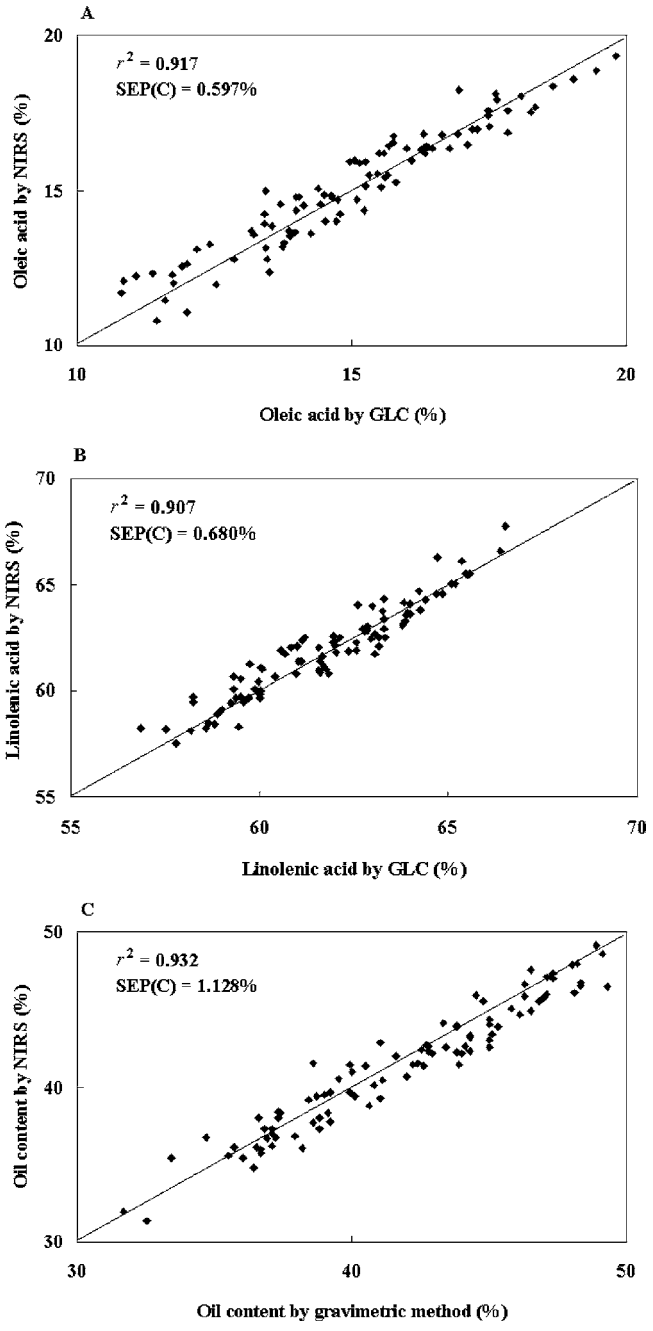

Figure 2. Scatter plots of NIRS versus reference values for oleic acid (**A**), linolenic acid (**B**), and oil content (**C**) in the validation set of intact perilla seeds.

calibration equations showed a high reliability for application in plant breeding. However, the reliability of the calibration models for the determination of palmitic, stearic, and linoleic acid was considerably lower and could not be considered adequate for use in routine analysis for massive screening of breeding lines in perilla.

It is concluded that the determination of oil content and fatty acid composition (oleic and linolenic acid) can be predicted with reliable accuracy using NIRS analysis of whole perilla seeds. Also, the future availability of perilla samples covering a wide range of reference values is required to obtain an accurate prediction of palmitic, stearic, and linoleic acid.

This nondestructive NIRS method could simplify the analysis of qualitative factors of interest because extraction steps with organic solvents were not required and whole samples were easily analyzed in a few minutes without grinding. Although the information from the NIR spectra tends to be somewhat complicated and difficult to interpret, each chemometric technique establishes a mathematical relationship between variations in the NIR spectra and parameters measured for each sample. This relationship can therefore be used to predict the parameter value in unknown samples. Consequently, it is a very reliable method for predicting samples similar to those used in the calibration. NIRS also has the main advantage that it may be performed simultaneously to the estimation of other quality components such as protein, water, and functional compounds, in addition to oil and individual fatty acids. These analytical characteristics were critical factors for the quality evaluation of nutritional food, the selection of superior breeding lines, and the identification of new germplasm. For the analysis of numerous samples, the NIRS method is capable of replacing the chromatographic analytical methods such as GLC. However, samples very different from the calibration samples are often not predicted reliably. This is likely to be due to different conditions such as species, cultivation area, drying and storage methods, and influences of genetic and environmental variation.

This is the first reported study of a NIRS calibration model developed for the estimation of fatty acid composition of perilla seed oil. The development of these NIR equations for individual fatty acids represents only a first step: although NIRS is a practical method, the equations should be updated, expanded, and improved with future samples from different environments and germplasms and covering a wider range of oil and fatty acid values of the samples used for the calibration models.

LITERATURE CITED

(1) Kwak, T. S. Major growth characters and fatty acid composition of Korean native perilla collections. *Korean J. Breed.* **1994**, *26*, 148−154.

(2) Lee, B. H.; Ryu, S. N.; Kwak, T. S. Current status and prospects of quality evaluation in perilla. *Korean J. Crop Sci.* **2002**, *47*, 150−162.

(3) Connor, W. E. Importance of n-3 fatty acids in health and disease. *Am. J. Clin. Nutr.* **2000**, *71* (1 Suppl.), 171−175.

(4) Kim, H. K.; Choi, H. Dietary α-linolenic acid lowers postprandial lipid levels with increase of eicosapentaenoic and docosahexaenoic acid contents in rat hepatic membrane. *Lipids* **2001**, *36*, 1331−1336.

(5) Kim, H. K.; Choi, S.; Choi, H. Suppression of hepatic fatty acid synthase by feeding α-linolenic acid rich perilla oil lowers plasma triacylglycerol level in rats. *J. Nutr. Biochem.* **2004**, *15*, 485−492.

(6) Font, R.; del Río-Celestino, M.; de Haro-Bailón, A. The use of near-infrared spectroscopy (NIRS) in the study of seed quality components in plant breeding programs. *Ind. Crop Prod.* **2006**, *24*, 304−313.

(7) Williams, P.; Norris, K. *Near Infrared Technology in the Agricultural and Food Industries*, 2nd ed.; American Association of Cereal Chemists: St. Paul, MN, 2001.

(8) Batten, G. D. Plant analysis using near infrared reflectance spectroscopy: the potential and the limitations. *Aust. J. Exp. Agric.* **1998**, *38*, 697−706.

(9) Sato, T.; Maw, A. A.; Katsuta, M. NIR reflectance spectroscopic analysis of the FA composition in sesame (*Sesamum indicum* L.) seeds. *J. Am. Oil. Chem. Soc.* **2003**, *80*, 1157−1162.

(10) Kim, K. S.; Park, S. H.; Choung, M. G. Nondestructive determination of lignans and lignan glycosides in sesame seeds by near infrared reflectance spectroscopy. *J. Agric. Food Chem.* **2006**, *54*, 4544−4550.

(11) Choung, M. G.; Kang, S. T.; Han, W. Y.; Baek, I. Y.; Kim, H. K.; Kim, K. S.; Park, S. H. Determination of fatty acid composition in soybean seed using near infrared reflectance spectroscopy. *Korean J. Breed.* **2005**, *37*, 197−202.

(12) Oh, K. W.; Choung, M. G.; Pae, S. B.; Jung, C. S.; Kim, B. J.; Kwon, Y. C.; Kim, J. T.; Kwack, Y. H. Determination of seed lipid and protein contents in perilla and peanut by near-infrared reflectance spectroscopy. *Korean J. Crop Sci*. **2000**, *45*, 339−342.

(13) Fassio, A.; Cozzolino, D. Non-destructive prediction of chemical composition in sunflower seeds by near infrared spectroscopy. *Ind. Crop Prod.* **2004**, *20*, 321−329.

(14) Wu, J. G.; Shi, C. H. Prediction of grain weight, brown rice weight and amylase content in single grains using near-infrared reflectance spectroscopy. *Field Crop Res.* **2004**, *87*, 13−21.

(15) Kim, Y. H.; Kang, C. S.; Lee, Y. S. Quantification of tocopherol and tocotrienol content in rice bran by near infrared reflectance spectroscopy. *Korean J. Crop Sci.* **2004**, *49*, 211−215.

(16) Brenna, O. V.; Berardo, N. Application of near-infrared reflectance spectroscopy (NIRS) to the evaluation of carotenoids content in maize. *J. Agric. Food Chem.* **2004**, *52*, 5577−5582.

(17) Baye, M. T.; Pearson, C. T.; Settles, A. M. Development of a calibration to predict maize seed composition using single kernel near infrared spectroscopy. *J. Cereal Sci.* **2006**, *43*, 236−243.

(18) Lu, G.; Huang, H.; Zhang, D. Prediction of sweetpotato starch physiochemical quality and pasting properties using near-infrared reflectance spectroscopy. *Food Chem.* **2006**, *94*, 632−639.

(19) Shenk, J. S.; Westerhaus, M. O. Population definition, sample selection and calibration procedures for near infrared reflectance spectroscopy. *Crop Sci.* **1991**, *31*, 469−474.

(20) Shenk, J. S.; Westerhaus, M. O. New standardization and calibration procedures for NIRS analytical systems. *Crop Sci.* **1991**, *31*, 1694−1696.

(21) Shenk, J. S.; Westerhaus, M. O. Population structuring of near infrared spectra and modified partial least squares regression. *Crop Sci.* **1991**, *31*, 1548−1555.

(22) Barnes, R. J.; Dhanoa, M. S.; Lister, S. J. Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *Appl. Spectrosc.* **1989**, *43*, 772−777.

(23) Shenk, J. S.; Westerhaus, M. O. Calibration the ISI way. In *Near Infrared Spectroscopy: The Future Waves*; Davis, A. M. C., Williams, P. C., Eds.; NIR Publications: Chichester, U.K., 1996; pp 198−202.

(24) Windham, W. R.; Mertens, D. R.; Barton, F. E. Protocol for NIRS calibration: sample selection and equation development and validation. In *Near Infrared Reflectance Spectroscopy (NIRS): Analysis of Forage Quality*; Marten, G. C., Ed.; USDA Agricultural Handbook; Y.S. GPO: Washington, DC, 1989; p 643.

(25) Williams, P. C.; Sobering, D. C. How do we do it: a brief summary of the methods we use in developing near infrared calibration. In *Near Infrared Spectroscopy: The Future Waves*; Davis, A. M. C., Williams, P. C., Eds.; NIR Publications: Chichester, U.K., 1996; pp 185−188.

(26) Murray, I. The NIR spectra of homologous series of organic compounds. In *Proceedings of the International NIR/NIT Conference*; Hollo, J., Kaffka, K. J., Gonczy, J. L., Eds.; Akademiai Kiado: Budapest, Hungary, 1986; pp 13−28.

(27) Osborne, B. G.; Fearn, T.; Hindle, P. H. *Practical NIR Spectroscopy with Applications in Food and Beverage Analysis*; Longman Scientific and Technical: Harlow, U.K., 1993.
(28) Hourant, P.; Baeten, V.; Morales, M. T.; Meurens, M.; Aparicio, R. Oil and fat classification by selected bands of near-infrared spectroscopy. *Appl. Spectrosc.* **2000**, *54*, 1168−1174.
(29) Velasco, L.; Becker, H. C. Estimating the fatty acid composition of the oil in intact-seed rapeseed (*Brassica napus* L.) by near-infrared reflectance spectroscopy. *Euphytica* **1998**, *101*, 221−230.

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