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Use of Near-Infrared Spectroscopy for Estimating Fatty Acid Composition in Intact Seeds of Rapeseed

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Abstract

Near-infrared spectroscopy (NIRS) was used as a rapid and nondestructive method to determine the fatty acid composition in intact seed samples of rapeseed (*Brassica napus* L.). A total of 349 samples (about 2 g of intact seeds) were scanned in the reflectance mode of a scanning monochromator, and the reference values for fatty acid composition were measured by gas-liquid chromatography. Calibration equations for individual fatty acids were developed using the regression method of modified partial least-squares with internal cross validation (n = 249). The equations had low SECV (standard errors of cross-validation), and high R^2 (coefficient of determination in calibration) values (> 0.8) except for palmitic and eicosenoic acid. Prediction of an external validation set (n = 100) showed significant correlation between reference values and NIRS estimated values based on the SEP (standard error of prediction), r^2 (coefficient of determination in prediction), and the ratio of standard deviation (SD) of reference data to SEP. The models developed in this study had relatively higher values (> 3.0 and 0.9, respectively) of SD/SEP(C) and r^2 for oleic, linoleic, and erucic acid, characterizing those equations as having good quantitative information. The results indicated that NIRS could be used to rapidly determine the fatty acid composition in rapeseed seeds in the breeding programs for high quality rapeseed oil.

Key words: near-infrared spectroscopy, oleic acid, linoleic acid, erucic acid, rapeseed

Introduction

Rapeseed (*Brassica napus* L.) is one of the most important oilseed crops used as a source of vegetable oil and a substitute for fossil diesel fuel (Cardone et al., 2003; Jang, 2002). Selection for the characters for seed quality such as fatty acid profile and protein content is carried out according to end-use such as edible oil for humans, meal for animal nutrition, and industrial application of biodiesel. Rapeseed oil, contributing approximately 40% of seed weight, is mainly composed of fatty acids such as palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), eicosenoic (20:1), and erucic (22:1) acid (Bang & Lee, 1991; Lee et al., 1994). The major components in rapeseed oil are unsaturated fatty acids (mainly oleic, erucic, and linoleic acid).

The modification of the fatty acid composition of seed oil to develop new genotypes having alternative oil characteristics has been an important objective in quality breeding in rapeseed, and it is required to determine the fatty acid composition of the oil in a large number of breeding lines. Fatty acid composition was usually determined by gas-liquid chromatography equipped with a flame ionization detector (GLC-FID) and a capillary coumn. However, these methods require multi-step sample preparation. For example, fatty acids must be converted to fatty acid methyl esters (FAMEs) for GLC analysis. These analysis methods are time-consuming, expensive, labor-intensive, and also destructive; therefore, they are not adequate for selecting superior lines from a number of rapeseed germplasm lines. Thus, a rapid and nondestructive method is in high demand to evaluate oil quality for rapeseed breeding programs.

Near-infrared spectroscopy (NIRS) has been known as a powerful tool for analysis of chemical and physical properties without sample preparation, and it has been applied for the analysis of quality characteristics in food and agricultural commodities (Batten, 1998; Williams & Norris, 2001). The NIRS has been successfully used to determine diverse compounds in numerous foods and industrial crops such as sesame (Sato et al., 2003; Kim et al., 2006), soybean (Choung et al., 2005), perilla and peanut (Oh et al., 2000), sunflower (Fassio & Cozzonlino, 2004), rice (Wu & Shi, 2004; Kim et al., 2004), maize (Brenna & Berardo, 2004; Baye et al., 2006), and sweet potato (Lu et al., 2006). Though the application of NIRS method to estimate fatty acid composition of intact seeds of rapeseed has already been reported (Velasco & Becker, 1998; Velasco et al., 1999; Font et al., 2006; Wu et al., 2006), it is needed to develop new prediction models to apply to our breeding program for domestic rapeseed germplasm.

The objectives of this study were to improve NIRS application for

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estimating fatty acid composition and to develop high-throughput screening techniques with intact seed samples for rapeseed breeding programs.

Materials and Methods

Seed samples of rapeseed

A total of 349 seed samples of rapeseed germplasm were obtained from the National Genebank, Rural Development Administration, Korea and were used to develop a NIRS prediction model for the determination of individual fatty acid composition. The seeds were stored in desiccators until NIRS and GC analysis.

Chemical analysis for fatty acid composition

About 2 g of each sample was powdered using an Ultra-Turax T8 homogenizer (IKA-Werke GmbH & Co. KG, 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., Korea), and filtered with filter paper (Whatman No. 1, Whatman plc., UK). The residues were extracted twice again, and the final volume of each extract solution was exactly adjusted to 100 mL. For fatty acid analysis, a 1 mL aliquot of *n*-hexane extract was transferred into the 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 and allowed to react at 100 °C for 10 min and then cooled. After 0.5 mL of 14% BF3 (boron trifluoride) solution was added, it was allowed to react at 100 °C for 10 min for the esterification of fatty acids. After cooling, 1.5 mL of *n*-hexane and 1.0 mL of distilled water were mixed, 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 before injection for fatty acid analysis. The GLC system 6890N GC (Agilent Technologies Co., USA) equipped with a flame ionization detector (FID) and a HP-Innowax capillary column (30 m length × 0.25 mm i.d., film 0.25 μm, J&W Scientific, Agilent Technologies Co., USA) 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 then held for 10 min. The injector and detector port temperatures were kept 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 for each sample. Individual fatty acids were expressed as percentage of the total fatty acids.

Spectra collection and pretreatment

The NIR spectroscopic analysis was performed using a near-infrared scanning monochromator (NIRSystem model 6500, Foss NIRSystems Inc., MD, USA) in the reflectance mode. Intact seed samples (about 2 g) were placed in a standard ring cup and then scanned. Reflectance energy readings were references to corresponding readings from an internal ceramic disc. Each spectrum was recorded once from each sample, and was obtained as average of 32 successive scans over the sample, plus 16 scans over the standard ceramic before and after scanning the samples. All spectral data were recorded as the logarithm of reciprocal of reflectance (log 1/*R*) in the wavelength range from 400 to 2500 nm, at 2 nm intervals. The scanning procedure could be finished within about 1.5 min per sample.

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, USA). The distance between a sample and its neighbor was measured as Mahalanobis distance (*H* distance) called the neighborhood *H*. The Score algorithm ranks spectra according to *H* distance from the average spectrum, and provides spectral boundaries to eliminate outliers with $H > 3.0$ and similar samples with $H < 0.6$ (Shenk & Westerhaus, 1991b). Therefore, the final number of samples for calibration and validation was variable 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 = 349) were randomly split into two sets for calibration and validation using WinISI program. The calibration set (249 samples) was used to calibrate and cross-validate the equation derived, and internal cross validation was used to avoid overfitting of the equations (Shenk & Westerhaus, 1996). The other 100 samples as an external validation set were used to test the goodness of fit of the developed equations using random samples not included in calibration sample set (Windham et al., 1989).

Data processing

The equations for NIRS prediction were developed using the Global program in WinISI software with the regression method of modified partial least-squares (MPLS) using wavelengths of entire visible (400-1100 nm) and near-infrared (1100-2500 nm) region at every 8 nm. Various mathematical treatments using the raw optical spectrum (log 1/*R*), or first or second derivatives of the 1/*R* data, were used to maximize the calibration results, with several combinations of smoothing and gap size. For example, in 2,4,4,1, the first number indicates the order of derivative function (two is the second derivative of log 1/*R*), the second number is the gap (the length in nm) in data points over which the derivative is calculated, the third number represents the number of data points (segment length) used in first smoothing, and the fourth number is the number of data points in the second smoothing which is normally set at 1 for no 2nd smoothing (Shenk & Westerhaus, 1993). To reduce the noise, the smoothing way of Savitzky-Golay (Savitzky & Golay, 1964) was used, with a gap of four data points. This gap was chosen after trials of several different gap sizes and was found to be adequate. In addition to derivatives, scatter correction using standard normal variate and detrending (SNVD) were applied for the calibration to reduce the differences in spectra related to physical characteristics such as particle size and

Table 1. Descriptive statistics for fatty acid composition of intact seed samples of rapeseed used in both calibration and validation.

Fatty acids	Calibration (n = 249)			Validation (n = 100)		
	Mean[a]	Range	SD	Mean[a]	Range	SD
Palmitic	4.01	2.0-7.4	0.90	4.09	2.1-7.9	0.84
Stearic	1.61	0.8-3.7	0.55	1.59	0.2-2.9	0.51
Oleic	32.9	6.2-70.4	19.3	34.2	8.3-66.6	19.2
Linoleic	15.9	5.7-26.3	3.47	16.1	8.4-25.1	3.80
Linolenic	7.70	1.8-13.6	1.72	7.56	2.7-14.0	1.62
Eicosenoic	9.00	0.6-29.6	5.43	8.68	1.0-18.4	5.15
Erucic	28.8	0.0-62.9	20.1	27.8	0.0-63.4	20.8

[a]Individual fatty acid composition are expressed as percentage of the total fatty acids in the seed oil, respectively; SD, standard deviation of mean.

path length of samples (Barnes et al., 1989; Shenk & Westerhaus, 1991a).

The best predicted equations for each chemical component were selected on the basis of minimizing the standard error of cross validation (SECV) and increasing the coefficient of determination (R^2) (Windham et al., 1989). The ratios of standard deviation (SD) of reference data to SECV and the corrected standard error of prediction (SEP(C)) were used as criteria to evaluate the performance of calibrations and the accuracy of equations, respectively (Williams & Sobering, 1996). The developed equations were monitored with the Monitor program in WinISI software, using the validation set (n = 100).

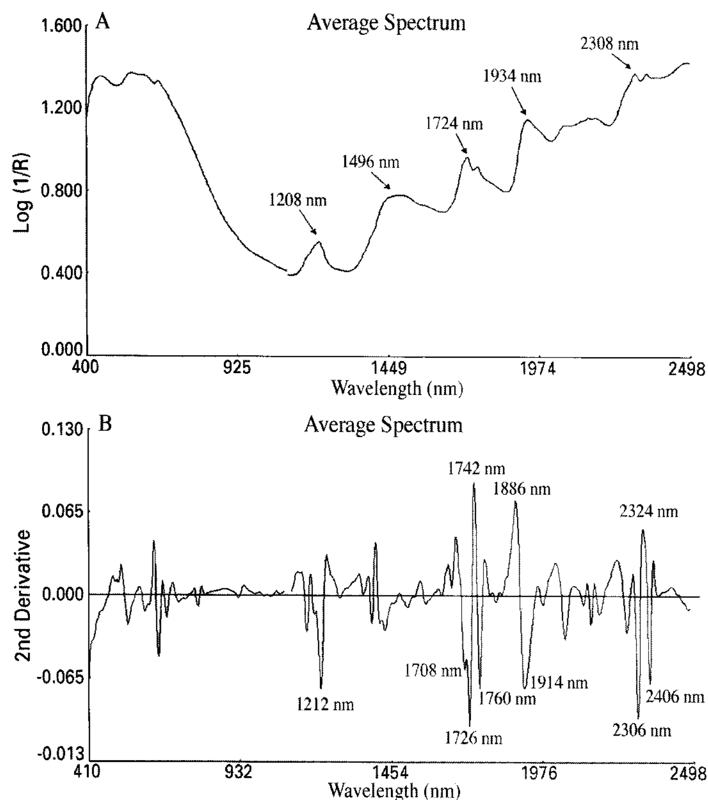

Fig. 1. Raw spectrum (log 1/R; A) and second derivative (B) of NIRS average spectrum of intact seeds of rapeseed.

Results and Discussion

Spectral analysis

The raw NIR reflectance and second derivative spectra of intact seed samples are shown in Fig. 1. The main absorption bands are observed at 1208 nm related to C-H stretching 2nd overtone ($-CH_2$), 1496 nm related to C-H stretching 1st overtone, 1724 nm related to C-O (oil) and C-H stretching 1st overtone ($-CH_2$), 1936 nm related to O-H bending 2nd overtone (water), and 2308 nm related to C-H bending 2nd overtone (oil). The information of functional group in spectrum was searched from WinISI software. The overall spectrum shows strong absorption bands related with oil and water, and is similar to those of other oil crops such as perilla, peanut, soybean, and sesame, especially, in near-infrared region (Oh et al., 2000; Choung et al., 2005; Kim et al., 2006).

The second derivative spectra had a trough corresponding to each peak in the original spectra, removing the overlapping peaks and baseline effects (Osborne et al., 1993). The average spectrum of the second derivative (Fig. 1) showed absorption bands at 1212, 1726, 1760, and 2306 nm related to hydrocarbon (-CH) in the NIR region.

Reference analysis of fatty acid composition

The descriptive statistics including mean, standard deviation (SD), and range, for individual fatty acid composition of rapeseed samples used in the calibration and validation sets, are shown in Table 1. Each reference value of fatty acid composition in a validation sample set was similar to those in the calibration sample set. Mean values of individual fatty acid composition were 4.0% of palmitic acid (PA), 1.6% of stearic acid (ST), 32.9% of oleic acid (OL), 15.9% of linoleic acid (LN), 7.7% of linolenic acid (LNL), 9.0% of eicosenoic acid (EIC), and 28.8% of erucic acid (ERU) in the calibration set, showing similar values to those in the validation set. Among 349 rapeseed germplasms used in this study, a total of 70 samples had zero-content of erucic acid.

Table 2. Equation statistics using regression model (MPLS) and scatter correction for NIRS prediction of fatty acid composition in the calibration set (n = 249) of intact seed samples of rapeseed.

Fatty acids	N	Mean	SD	T	Calibration		Cross-Validation		RSC
					SEC	R^2	1-VR	SECV	
Palmitic	240	3.94	0.78	6	0.355	0.795	0.719	0.418	1.87
Stearic	240	1.58	0.50	8	0.193	0.850	0.798	0.224	2.23
Oleic	241	32.6	19.1	11	2.679	0.980	0.969	3.371	5.67
Linoleic	240	16.0	3.31	10	1.005	0.908	0.847	1.296	2.55
Linolenic	239	7.79	1.61	11	0.626	0.848	0.755	0.795	2.03
Eicosenoic	243	8.90	5.28	3	3.701	0.509	0.465	3.867	1.37
Erucic	242	29.2	19.9	10	2.606	0.983	0.974	3.239	6.14

N, number of samples used to develop the model; SD, standard deviation of mean; T, Number of terms, number of PLS loading factors in the regression model MPLS; SEC, standard error of calibration; R^2, coefficient of determination of calibration; 1-VR, one minus the ratio of unexplained variance divided by variance; SECV, standard error of cross-validation; RSC, SD/SECV: the ratio of SD (standard deviation of reference data) to SECV in the calibration set.

Calibration models for fatty acid composition

In developing NIRS models for each fatty acid composition, the statistics of calibrations and cross-validations are shown in Table 2, including the standard error of calibration (SEC) and the coefficient of determination in calibration (R^2). The one minus the ratio of unexplained variance divided by variance (1-VR), the standard error of cross validation (SECV), and number of terms (T) are also shown in Table 2. The performances of the different equations obtained in the calibration were determined from cross-validation as an internal cross validation method. Internal validation was used to avoid overfitting of the equations by selecting the minimum number of PLS terms in each model (Shenk & Westerhaus, 1996). 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 of each fatty acid composition (except 0.509 of EIC), showing higher values of R^2 (0.795-0.983) and 1-VR, and lower values of SEC and SECV than the different mathematical treatments tested. The equations for each fatty acid composition using mathematical treatment 2,4,4,1 were selected considering RSC

(SD/SECV) values more than 2.0 as the selection criteria of models. The reliable equations for OL (32.6% in oil), LN (16.0% in oil), and ERU (29.2% in oil), major fatty acids in rapeseed oil had high values of R^2 (0.980, 0.908, and 0.983, respectively) and SD/SECV (5.67, 2.55, and 6.14, respectively), indicating close relationship between reference values and NIRS estimated values, but not for PA, ST, LNL, and EIC having relatively lower R^2 (below 0.9) and lower 1-VR values. The best calibration models for OL, LN, and ERU were developed with the mathematical approach over the visible and near-infrared segment (400-2500 nm), and the equations could be used for screening the fatty acid composition in intact seeds of rapeseed.

Table 3. Monitoring statistics for fatty acid composition in the external validation set (n = 100) of intact seeds of rapeseed.

Fatty acids	N	Mean	SD	Bias	r^2	SEP(C)	Slope	RSP
Palmitic	96	4.03	0.75	0.000	0.719	0.400	0.916	1.88
Stearic	93	1.57	0.49	-0.050	0.812	0.213	0.957	2.30
Oleic	97	34.6	19.2	0.184	0.965	3.610	0.984	5.32
Linoleic	93	16.1	3.64	0.385	0.901	1.147	1.008	3.17
Linolenic	96	7.71	1.45	0.055	0.728	0.758	1.015	1.91
Eicosenoic	99	8.60	5.12	-0.400	0.436	3.970	0.772	1.29
Erucic	98	28.4	20.6	0.121	0.976	3.175	0.993	6.48

N, number of samples used to monitor the model; SD, standard deviation of mean; Bias, average difference between reference and NIRS values; r^2, coefficient of determination of cross-validation; SEP(C), the corrected standard error of prediction; Slope, the steepness of a straight line curve; RSP, SD/SEP(C): the ratio of SD of reference data to SEP(C) in the external validation set.

External validation for fatty acid composition

The predicted statistics for fatty acid composition are shown in Table 3. The prediction statistics included bias, the standard error of prediction (SEP), the coefficient of determination in prediction (r^2), the corrected standard error of prediction (SEP(C)), and SD/ SEP(C) values, which were factors used to evaluate the reliability of the calibration model. The r^2 and SD/SEP(C) values for PA, ST, LNL, and EIC were lower (< 0.9 and 3.0, respectively), indicating a poor correlation between reference values and NIRS estimated values similar to those of calibration. The predictions for OL, LN, and ERU were confirmed by higher values of r^2 (0.965, 0.901, and 0.976, respectively) and SD/SEP(C) (5.32, 3.17, and 6.48, respectively). The SD/SEP(C) value as the cutoff point for evaluating the accuracy of equations was 3.0 in this study, which was recommended for screening purposes (Williams & Sobering, 1996). Figure 2 represented laboratory reference values against NIRS predicted values in the validation set for individual fatty acid composition (OL, LN, and ERU), showing also the relationship between NIRS and reference. These results demonstrated the accurate prediction capacities of the calibration models for OL, LN, and ERU using a nondestructive NIRS method in rapeseed. With larger sample size and broader variation of reference data set, the calibration models for the determination of PA, ST, LNL, and EIC could be sufficiently used for massive screening of breeding lines in spite of less accuracy of models.

There were similar reports (Velasco & Becker, 1998; Font et al., 2006; Wu et al., 2006) to our results, estimating the fatty acid composition in rapeseed oil using NIRS with intact seed samples except for eicosenoic acid (r^2 = 0.69). Velasco et al. (1999) reported a reliable estimation of oleic acid and erucic acid composition in single seeds

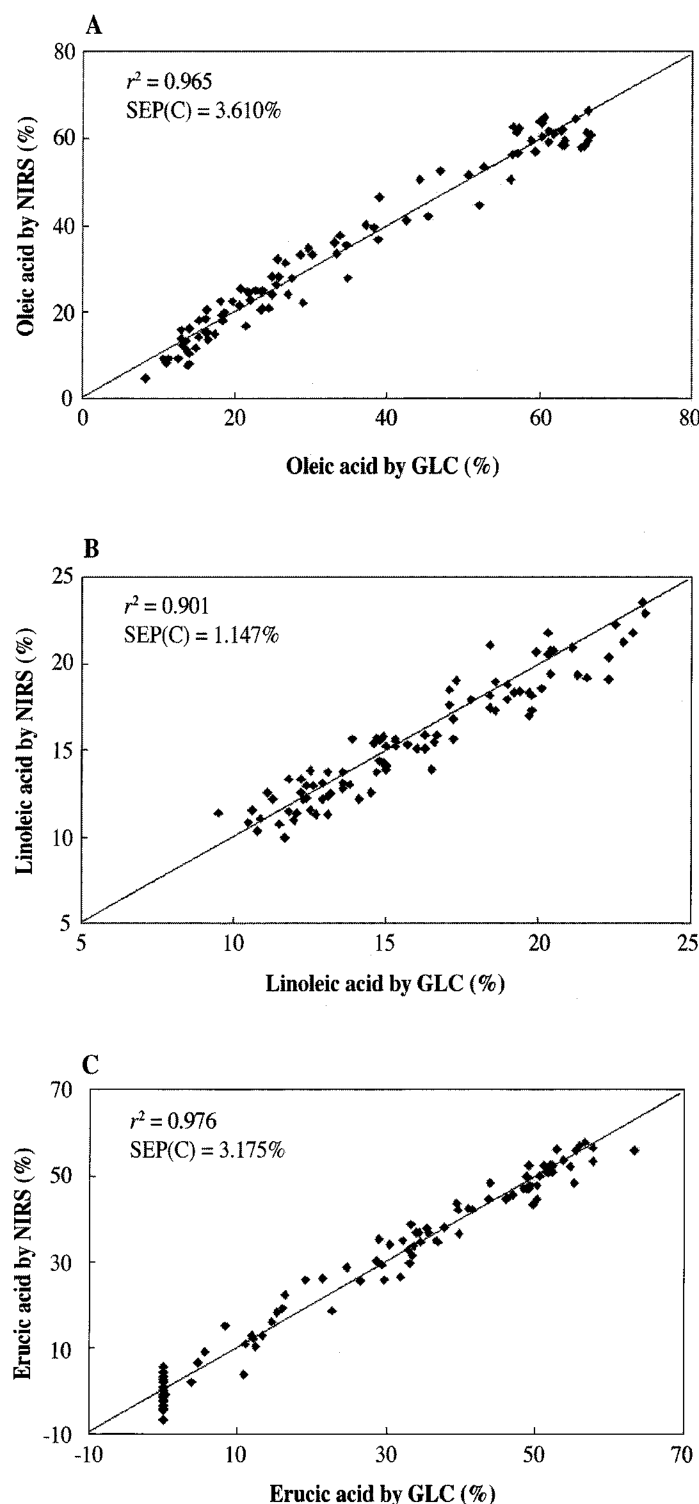

Fig. 2. Scatter plots of NIRS vs. reference values for oleic (A), linoleic (B), and erucic (C) acid in the external validation set (n = 100) of intact seed samples of rapeseed.

was possible by NIRS technique, though not validated for linoleic (r = 0.75) and linolenic acid (r = 0.73). These results showed better models using single seed sample for estimating the fatty acid composition than in this study, but the authors think that our results are meaningful in points of using Korean germplasms storing at National Genebank and developing appropriate models suitable to our breeding materials

because NIR model could be different according to growing condition as well as genotypes. In future study, we need to develop new NIR models with small amount of intact seeds and single seed in estimating contents of chemical components in many other crops as well as rapeseed.

It is concluded that the determinations of OL, LN, and ERU composition in oil can be predicted with reliable accuracy using NIRS analysis of intact seeds of rapeseed, and the presence of larger population of rapeseed covering a wide range of chemical values was required to obtain the accurate prediction of PA, ST, LNL, and EIC.

This nondestructive NIRS method could simplify the analysis of qualitative components, because extraction steps with organic solvents were not required and instrumental analysis was completed in a few minutes. In addition, the NIRS has the additional advantage that it may be carried out simultaneously the estimation of contents of other quality components such as oil, protein, and functional compounds. These analytical characteristics were critical factors for the quality evaluation of nutritional food, the selection of superior breeding lines, and the characterization of new germplasm. For the analysis of numerous samples, the NIRS method can replace chromatographic methods such as GLC. The development of these NIR equations for individual fatty acids is only a first step though the NIRS is a practical method. Therefore, the equations should be updated and improved with samples from different germplasm accessions collected under various environments.

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References

Bang JK, Lee JI. 1991. Oil content and fatty acid composition of rapeseed. Korean J. Crop Sci. 36: 62-78

Barnes RJ, Dhanoa MS, Lister SJ. 1989. Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. Appl. Spectrosc. 43: 772-777

Batten GD. 1998. Plant analysis using near infrared reflectance spectroscopy: the potential and the limitations. Aust. J. Expl. Agric. 38: 697-706

Baye MT, Pearson CT, Settles AM. 2006. Development of a calibration to predict maize seed composition using single kernel near infrared spectroscopy. J. Cereal Sci. 43: 236-243

Brenna OV, Berardo N. 2004. Application of near-infrared reflectance spectroscopy (NIRS) to the evaluation of carotenoids content in Maize. J. Agric. Food Chem. 52: 5577-5582

Cardone M, Mazzoncini M, Menini S, Rocco V, Senatore A, Seggiani M, Vitolo S. 2003. *Brassica carinata* as an alternative oil crop for the production of biodiesel in Italy: agronomic evaluation, fuel production by transesterification and characterization. Biomass Bioenerg. 25: 623-636

Choung MG, Kang ST, Han WY, Baek IY, Kim HK, Kim KS, Park SH. 2005. Determination of fatty acid composition in soybean seed using near infrared reflectance spectroscopy. Korean J. Breed. 37: 197-202

Fassio A, Cozzolino D. 2004. Non-destructive prediction of chemical composition in sunflower seeds by near infrared spectroscopy. Ind. Crop Prod. 20: 321-329

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

Jang YS. 2002. Prospect and situation of quality improvement in oilseed rape. Korean J. Crop. Sci. 47(S): 175-185

Kim KS, Park SH, Choung MG. 2006. Nondestructive determination of lignans and lignan glycosides in sesame seeds by near infrared reflectance spectroscopy. J. Agric. Food Chem. 54: 4544-4550

Kim YH, Kang CS, Lee YS. 2004. Quantification of tocopherol and tocotrienol content in rice bran by near infrared reflectance spectroscopy. Korean J. Crop. Sci. 49: 211-215

Lee JI, Jung DH, Ryu SN. 1994. Breeding for improvement of fatty acid composition in rapeseed. XXI. Oil quality of fatty acid improved varieties in Cheju area and future production strategy. Korean J. Crop Sci. 39: 165-170

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

Oh KW, Choung MG, Pae SB, Jung CS, Kim BJ, Kwon YC, Kim JT, Kwack YH. 2000. Determination of seed lipid and protein contents in perilla and peanut by near-infrared reflectance spectroscopy. Korean J. Crop Sci. 45: 339-342

Osborne BG, Fearn T, Hindle PH. 1993. Practical NIR spectroscopy with applications in food and beverage analysis, Longman Scientific and Technical, Harlow, U.K.

Savitzky A, Golay MJE. 1964. Smoothing and differentiation of data by simplified least squares procedures. Anal. Chem. 8: 1627-1639

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

Shenk JS, Westerhaus MO. 1991a. Population structuring of near infrared spectra and modified partial least squares regression. Crop Sci. 31: 1548-1555

Shenk JS, Westerhaus MO. 1991b. Population definition, sample selection and calibration procedures for near infrared reflectance spectroscopy. Crop Sci. 31: 469-474

Shenk JS, Westerhaus MO. 1993. Analysis of agriculture and food products by near infrared reflectance spectroscopy, Infrasoft International, MD, USA

Shenk JS, Westerhaus MO. 1996. Calibration the ISI way. In Near Infrared Spectroscopy: The Future Waves, Davis A. M. C. and P. C. Williams, eds., pp. 198-202. NIR Publications, Chichester, U.K.

Velasco L, Becker HC. 1998. Estimating the fatty acid composition of the oil in intact-seed rapeseed (*Brassica napus* L.) by near infrared reflectance spectroscopy. Euphytica 101: 221-230

Velasco L, Perez-Vich B, Fernandez-Martinez JM. 1999. Nondestructive screening for oleic and linoleic acid in single sunflower achenes by near-infrared reflectance spectroscopy. Crop Sci. 39: 219-222

Velasco L, Möllers C, Becker HC. 1999. Estimation of seed weight, oil content and fatty acid composition in intact single seeds of rapeseed (*Brassica napus* L.) by near-infrared reflectance spectroscopy. Euphytica 106: 79-85

Williams P, Norris K. 2001. Near infrared technology in the agricultural and food industries, 2nd ed., American Association of Cereal Chemists Inc., St. Paul, MN, USA

Williams PC, Sobering DC. 1996. 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. and P. C. Williams, eds., pp. 185-188. NIR Publications, Chichester, U.K.

Windham WR, Mertens DR, Barton FE. 1989. 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., p. 643. USDA Agriculture Handbook, Washington DC, USA

Wu JG, Shi CH. 2004. Prediction of grain weight, brown rice weight and amylose content in single rice grains using near-infrared reflectance spectroscopy. Field Crop Res. 87: 13-21

Wu JG, Shi CH, Zhang HZ. 2006. Study on developing calibration models of fat acid composition in intact rapeseed by near infrared reflectance spectroscopy. Spectrosc. Spect. Anal. 26: 259-262