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# Determination of Seed Lipid and Protein Contents in Perilla and Peanut by Near-Infrared Reflectance Spectroscopy

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**ABSTRACT: Near-infrared reflectance spectroscopy (NIRS) was used to estimate the lipid and protein contents in ground seed samples of perilla (*Perilla frutescens* Brit.) and peanut (*Arachis hypogaea* L.). A total of 46 perilla and 80 peanut calibration samples and 23 perilla and 46 peanut NIRS validation samples were used for NIRS equation development and validation, respectively. Validation of these NIRS equations showed a range of very low bias (-0.05 to 0.13%) and standard error of prediction corrected for bias (0.224 to 0.803%) and very high coefficient of determination ($R^2$) (0.962 to 0.985). It was concluded that NIRS could be adapted as a mass screening method for lipid and protein contents in perilla and peanut seed.**

***Keywords*** **:** perilla, peanut, NIRS, lipid, protein.

Soxhlet and kjeldahl method are widely used to analyse the lipid and protein contents of oil seed crops. These techniques are reliable and accurate, but also expensive, and time consuming as well as requiring the use of toxic solvents or chemicals. For many applications, e.g., screening for lipid and protein content in plant breeding system, a more rapid and simple method is needed.

Near-infrared reflectance spectroscopy (NIRS) is a multitrait technique that fulfills most of the requirements for rapid, reliable, and cost-effective screening for several seed quality traits in intact-seed samples of many crops, i.e., *Brassica* oil seed species, soybean, sunflower, and corn (Velasco *et al.*, 1997; Pazdernik *et al.*, 1997; Perez-Vich *et al.*, 1998).

However, NIRS applications and studies were insufficient in Korea (Jung *et al.*, 1998). In some crop, such as rice, soybean, pea and barley, NIRS was used to analyse amylose, protein, starch, lipid, β-glucan, and ash contents (Hwang *et al.*, 1994; Kim *et al.*, 1996; Jung *et al.*, 1998; Kim *et al.*,1995). But analysis using NIRS in perilla and peanut was not reported in Korea.

Therefore in this study, we report the potential use of NIRS to estimate the oil and protein contents in ground seed of perilla and peanut.

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## MATERIALS AND METHODS

### Sample preparation

Seed samples (20 g) of 69 germplasms of perilla (*Perilla frutescens* Brit.) and 126 germplasms of peanut (*Arachis hypogaea* L.) were ground in a homogenizer with 10,000 rpm and sieved with a 1.0 mm screen. The ground samples were well-mixed and used for the analysis of lipid and protein contents by NIRS and standard methods.

### Chemical measurements

The oil content was determined by soxhlet method with Buchi B-811 extraction system. 2 g of ground seed sample was extracted for two hours and dried for ten minutes. This condition was confirmed in preconditioning experiment (data not shown). 0.2 g of ground sample was digested by Buchi B-435 digestion system and Buchi B-412 scrubber with 20 ml of sulfuric acid and 3 g of catalyst ($CuSO_4 : K_2SO_4 = 1 : 9$). Percent nitrogen was calculated by Buch B-339 auto kjeldahl system and was converted to percent protein with the factor 6.25 in both perilla and peanut.

### NIRS scanning

Samples were scanned on a monochrometer NIR systems model 6500 by using a standard cell cup. The reflectance spectra (log 1/R) from 400 to 2500 nm were recorded at 2-nm intervals.

### NIRS calibration

NIRS calibration equations were developed for lipid and protein content. Table 1 shows the lipid and protein content of calibration and prediction sets for perilla and peanut seed. Both sample sets represented a uniform distribution within the range of lipid and protein content, analysed by standard methods. The procedure of Barnes *et al.* (1989) was modified for the log (1/R) spectra analysis. First-derivative transformation, De-trand, and standard normal variate (SNV)

**Table 1.** Laboratory reference value statistics for lipid and protein content based on perilla and peanut samples.

| | | Perilla | | | | Peanut | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | n | Mean | Range | SD | n | Mean | Range | SD |
| | | | % | | | | % | | |
| Calibration | Lipid | 46 | 42.49 | 25.19~49.73 | 6.40 | 80 | 51.07 | 45.19~55.30 | 2.21 |
| | Protein | 46 | 21.76 | 17.68~29.31 | 2.51 | 80 | 28.20 | 24.72~31.57 | 1.39 |
| Prediction | Lipid | 23 | 42.98 | 28.28~49.51 | 5.59 | 46 | 51.43 | 46.43~56.70 | 2.40 |
| | Protein | 23 | 22.05 | 18.30~26.79 | 2.51 | 46 | 27.76 | 24.50~31.65 | 1.29 |

scatter corrections were applied to the log (1/R) spectra, and calibration equations were developed by using modified partial least squares (MPLS) regression (WinISI v.1.02a).

## RESULTS AND DISCUSSION

Fig. 1. Shows the average spectra from 69 perilla and 126 peanut samples scanned by NIRS as meal. The average spectrum of the perilla samples showed maximum absorption values at 1204, 1494, 1714, 1758, 1934, 2144, 2308 and 2346 nm, similar with those of sunflower oil (Perez-Vich *et al*, 1998; Sato *et al.*, 1995; Sato *et al.*, 1991), except for the peak at 1494, 1934 nm. The average spectrum of the peanut samples had similar pattern of wave length in maximum absorption values with that of perilla.

The NIRS equations for ground seed lipid and protein showed low standard error of calibration (SEC) and standard error of cross-validation (SECV) values in both perilla and peanut. And high $R^2$ and 1-VR values in both perilla and peanut were obtained (Table 2). Additionally, the predicted means for lipid and protein contents of perilla and peanut were similar to the means based on the chemical analysis. During the calculation of calibration equation, samples, which have high critical T, H, or X value, were eliminated.

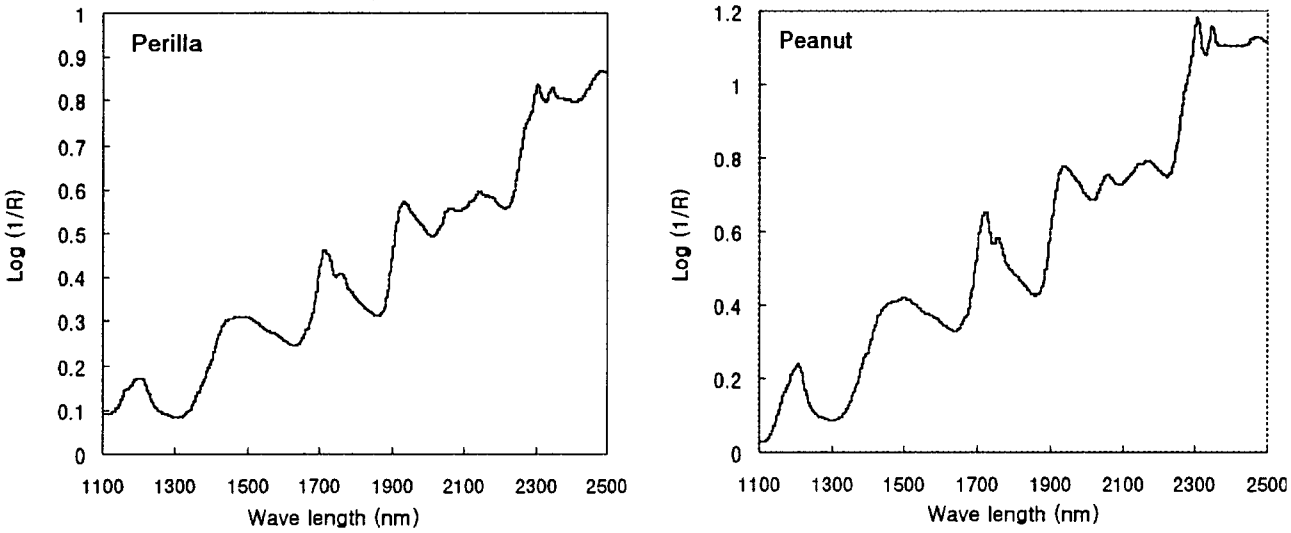


**Fig. 1.** Average near-infrared reflectance spectroscopy spectra of perilla and peanut samples scanned as meal.

**Table 2.** Near-infrared spectroscopy calibration equation statistics for lipid and protein content of perilla and peanut seed on a dry matter basis.

| | Perilla | | | | | | Peanut | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | SEC† | SECV | $R^2$ | 1-VR | n | Mean | SEC | SECV | $R^2$ | 1-VR |
| | | % | | | | | | % | | | | |
| Lipid | 45 | 42.39 | 0.473 | 0.675 | 0.995 | 0.989 | 74 | 51.04 | 0.242 | 0.294 | 0.986 | 0.983 |
| Protein | 46 | 21.76 | 0.251 | 0.414 | 0.990 | 0.973 | 77 | 28.14 | 0.181 | 0.205 | 0.981 | 0.977 |

†SEC; standard error of calibration, SECV; standard error of cross-validation, 1-VR; one minus the ratio of unexplained variance to total variance.

**Table 3.** The performance of the lipid and protein calibration equations based on validation statistics from a new sets of perilla and peanut samples.

| | Perilla | | | | | | Peanut | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | SD | Bias† | SEP(C)‡ | $R^2$ | n | Mean | SD | Bias | SEP(C) | $R^2$ |
| | | | -------- % -------- | | | | | | -------- % -------- | | | |
| Lipid | 22 | 42.81 | 5.53 | 0.13 | 0.803 | 0.981 | 36 | 51.42 | 2.38 | -0.05 | 0.382 | 0.985 |
| Protein | 23 | 22.06 | 2.51 | -0.01 | 0.538 | 0.981 | 43 | 27.61 | 1.14 | 0.03 | 0.224 | 0.962 |

†Bias; difference between reference method and predicted mean, ‡SEP(C); standard error of prediction corrected for bias.

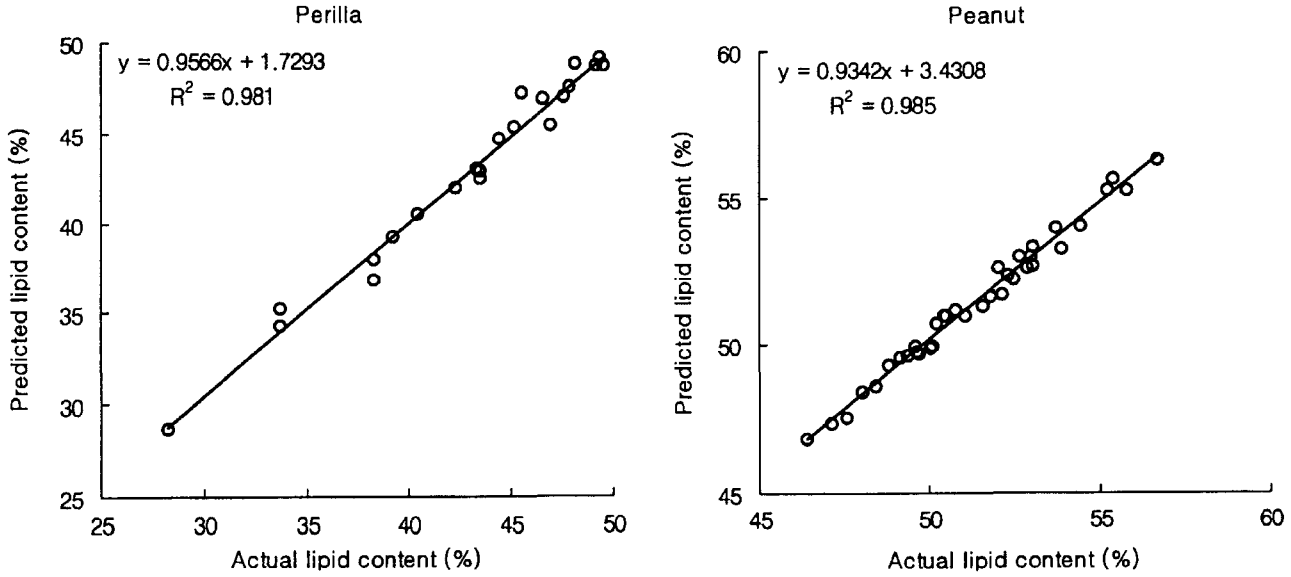


**Fig. 2.** Relationship between the actual and the predicted lipid contents in perilla and peanut samples.

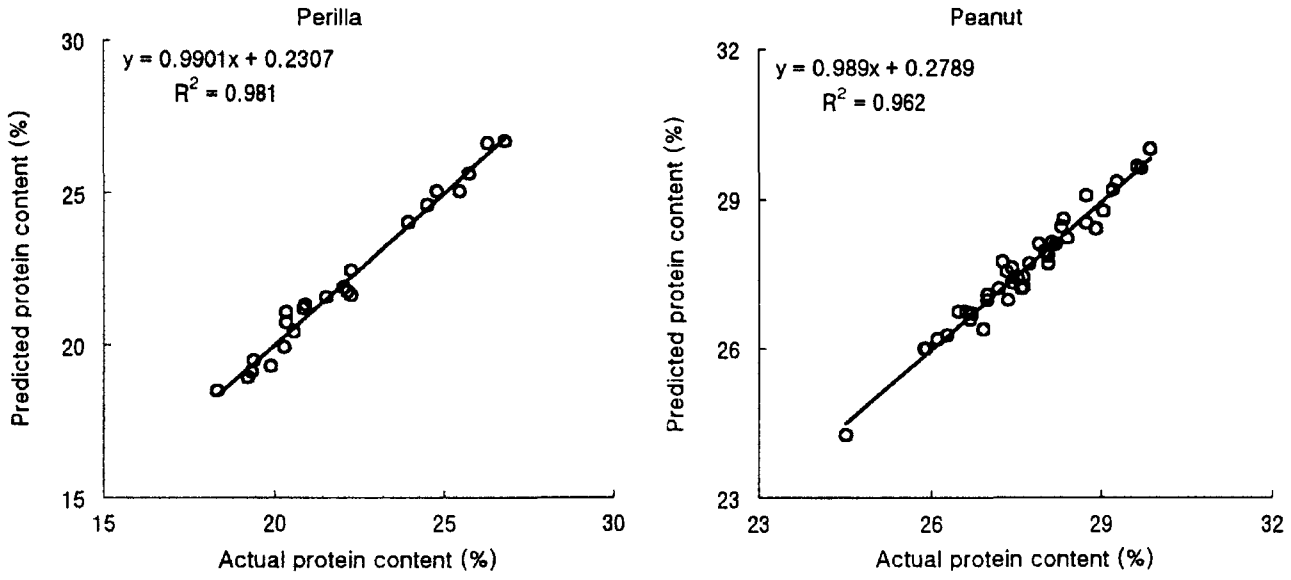


**Fig. 3.** Relationship between the actual and the predicted protein content in perilla and peanut samples.

Therefore the numbers of used sample are not equal with that of original sample sets.

Based on the bias, SEP(C) (standard error of prediction corrected for bias), and $R^2$ the equations from the NIRS for lipid and protein of perilla and peanut were accurately predicting the contents of validation set (Table 3). Fig. 2 dem-

onstrates the accuracy of the ground seed lipid equations for lipid of perilla and peanut on the basis of the relationship between the actual lipid values calculated from soxhlet and the predicted lipid values from the NIRS. Protein content was also accurately predicted by the NIRS equations in perilla and peanut validation sets (Fig. 3). The prediction samples of perilla and peanut had standardized H-distances of 3.0 or less from the mean of the calibration set (Pazdernik *et al.*, 1997). Therefore the numbers of used sample are not equal with that of original sample sets.

These result indicate that NIRS can be used as a gross screening method to evaluate large numbers of perilla and peanut germplasms and breeding populations for lipid and protein quickly. Following the NIRS screening process, soxhlet and kjeldahl with greater precision can be used further to identify the best lines within a smaller and elite group of lines initially selected by NIRS. The main advantage of NIRS is that it eliminates the need to analyze the majority of the samples by standard methods.

Future research should aim at developing equations for the intact seed and improving the accuracy and sample range. The equations developed in this study was obtained from the plants grown in only one year and one place Milyang, Kyungnam, Korea, which limits their overall utility. Pazdernik *et al.* (1997) reported that the addition of appropriate future samples collected from different locations and years needs to expand the equations. The addition of a larger range of samples containing a broader range of lipid and protein contents also should improve the predictive accuracy of these equations.

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