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Analysis of Protein and Moisture Contents in Pea(*Pisum sativum* L.) Using Near-Infrared Reflectance Spectroscopy

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ABSTRACT

This study was conducted to establish a rapid analysis method for determining protein and moisture contents of pea. Ninety and eighty pea (*Pisum sativum* L.) lines were analyzed to determine protein and moisture contents, respectively using near-infrared reflectance spectroscopy. Simple correlations (γ) of protein content in a ground sample and an intact grain sample by an automatic regression method were 0.978 and 0.910, respectively. Simple correlations by partial least square regression/principal component analysis (PLS/PCA) methods were 0.982 and 0. 925, respectively. Standard error of performance (SEP) in protein content was the lowest value, 0.446 in ground sample by PLS/PCA methods. Simple correlation of moisture content was the highest at 0.871 in ground samples when using a standard regression method. Accuracy for the moisture content was slightly lower than for protein content. It was concluded that the NIRS method would be applicable only for rapid determination of protein content in pea.

Key words : pea, near-infrared reflectance spectroscopy (NIRS), protein, moisture.

Near-infrared reflectance spectroscopy (NIRS) has been proved itself to be rapid, accurate, and economical in a wide range of analytical applications (AACC, 1986). William Herschel discovered the infrared region in 1800. In the 19th century, scientists proposed that matter was composed of molecules with groups of atoms. When light of specific wavelengths collided with certain molecules, bonds vibrated and part of the light energy was absorbed by the molecules and changed to heat energy (Allen, 1983). Karl Norris established a quantity analysis system using NIRS in the 1960s (AACC, 1986; Allen, 1983). In the early 1970s, the advantages of NIRS were recognized and it became a practical tool for determining protein and moisture content of wheat in America and Canada (Allen, 1983; Kim et al., 1996).

Chemical components of many crops have been determined using NIRS in the field of food industry (Czuchajowska & Pomoranz, 1991; Krishnan et al., 1994). Recently, NIRS have been applied in the area of quality analysis (AACC, 1986). However, studies on NIRS were insufficient in Korea. Cho et al. (1990) reported that the NIRS method is suitable for determining total sugar and capsicine content in dried red pepper. Hwang et al. (1994) reported that amylose and protein contents of rice were analyzed using NIRS. Kim et al. (1995) reported that protein, starch, β-glucan, and ash contents of covered barley were analyzed using NIRS with filter type. Analysis using NIRS in pea was carried out for methionine, starch, and protein contents among chemical components (Davies & Wright, 1984; Davies et al., 1985; Williams et al., 1985).

This study was conducted to get information on the analysis potential and basis data to increase selection efficiency and to develop high quality lines in early generation using rapid, accurate NIRS methods.

MATERIALS AND METHODS

Peas (*Pisum sativum* L.) were obtained from the germplasm collection of the National Yeongnam Agricultural Experiment Station. Ninety lines were used to evaluate the NIRS method for determining protein content. Eighty lines were used for determining moisture content. Two kinds of samples, intact and ground ones were used for NIRS analysis. A Heico sample mill was used to grind samples using a 1.0 mm screen. Standard and coarse cups were filled with ground and intact grain samples, respectively. Protein content was compared with the Leco 40 (nitrogen autoanalyzer) method and moisture content was compared with the AOAC (1984) method.

A 'NIRS 6500' instrument was used with monochrometer types. Wavelength ranged from 400 nm to 2500 nm. Two sample lots for protein content analysis were prepared; the first lot with 61 lines for calibration, and the second lot with 29 lines for prediction. The second sample lot was not used for the calibration. Two sample lots for moisture content were 55 and 25 lines, respectively. Both sample lots represented a uniform distribution within the range of protein and moisture content, analyzed by standard methods. The NSAS program was used for the calibration and prediction. Optimum wavelengths for prediction of protein and moisture contents were determined by log 1/R and transformed to the 2nd derivative and 4th derivative of log 1/R using the calibration samples as described by Stark et al. (1983) Accuracy was determined as SEP and simple correlation (γ) using the prediction samples.

RESULTS AND DISCUSSION

Means, standard deviation, and ranges of the cali-

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Table 1. Composition of pea sample sets.

Content	Calibration (%)				Prediction (%)			
	High	Low	Mean	SD†	High	Low	Mean	SD
Protein	34.4	23.1	27.7	2.5	31.1	23.4	27.6	2.3
Moisture	13.1	10.7	11.6	0.5	12.7	10.9	11.6	0.5

† Standard deviation.

Table 2. Near-infrared reflectance spectroscopy analysis of protein content in pea by standard and automatic regression methods.

Samples	Format	Wavelength(nm)	MR†	SEP‡	γ§
Ground	D2OD¶	1976/1508/1606/898 S#	0.994	0.488	0.976
	D2OD	1390/1990/2470/1900/1540/2020 A††	0.962	0.467	0.978
Intact grain	D2OD	1282/1266/1684 S	0.941	0.962	0.908
	D2OD	910/460/850/790/1240/1810/1270 A	0.914	0.952	0.910

† Multiple correlation. ‡ Standard error of performance. § Simple correlation.
¶ D2OD = Second derivative of log 1/R signal. # Standard regression method. †† Automatic regression method.

bration and prediction samples, for protein and moisture contents by standard methods were shown in Table 1. The protein content of 61 lines for calibration ranged from 23.1 to 34.4%, and moisture content of 55 lines for calibration ranged from 10.7 to 13.1%. Protein and moisture content of lines for prediction ranged from 23.4 to 31.1% and from 10.9 to 12.7%, respectively. These were within the content ranges of calibration samples. Calibration preparation was obtained by standard and automatic regression methods, and PCA/PLS method as described by Williams et al. (1991).

Table 2 gives values of multiple correlation, SEP and simple correlation (γ) of protein content between NIRS and Leco 40 results using standard and automatic regression methods in NIRS analysis.

As shown in Table 2, multiple correlation (MR) and SEP of protein content in ground samples by standard regression method were a little higher than those by the automatic regression method. However, simple correlation which was represented as accuracy of calibration showed little difference among the methods. Six ranges of wavelength such as 1390, 1990, 2470, 1900, 1540, and 2020 nm were selected. Simple correlation by standard regression method was also a little higher than the automatic regression method in intact grain samples. In comparison between samples, simple correlation were higher in ground samples than intact grain samples. Calibrations were transformed to the 2nd derivative from log 1/R in standard and automatic regression methods. Low simple correlation in intact grain samples was considered due to particle size. Norris & Williams (1984) reported that particle size was one of the most important factors in NIRS analysis, and particle size in NIRS analysis causes a change in the amount of radiation scattered by samples (AACC, 1986).

SEP and simple correlation by PCA/PLS methods were a little higher than standard and automatic regression methods in ground and intact grain samples (Table 3). As shown in Table 3, simple correlations in ground and intact grain samples were 0.980 and 0.925, respectively. Davies & Wright (1984) reported that simple correlation for protein content in pea flour using NIRS (filter type instrument) was 0.95. Therefore, this study corresponded with Davies'. Kim et al. (1995) reported that SEP by a standard regression method for protein content in barley was 0.257, and Kim et al. (1995) reported that SEP for protein content in intact soybean seeds was 0.75.

Table 4 gives the values of multiple correlation, standard error of performance (SEP), and simple correlation (γ) of the moisture content for the calibration samples and 25 prediction samples between NIRS and AOAC analysis results using standard and automatic regression methods. The best calibration was obtained from the standard regression method. SEP and simple correlation

Table 3. Near-infrared reflectance spectroscopy analysis of protein content in pea by principal component analysis/partial least square regression(PCA/PLS) methods.

Samples	Format	Factors	MR	SEC†	SEP	γ
Ground	D2OD	11	0.998	0.183	0.446	0.980
Intact grain	Log 1/R	13	0.970	0.733	0.869	0.925

† Standard error of calibration.

Table 4. Near-infrared reflectance spectroscopy analysis of moisture content in pea by conventional methods.

Samples	Format	Wavelength(nm)	MR†	SEP‡	γ§
Ground	D4OD¶	1860/970/2384 S#	0.923	0.227	0.871
	Log 1/R	2410/1930/1900/1870/1810/2380 A††	0.852	0.251	0.839
Intact grain	Log 1/R	1336 S	0.322	0.379	0.568
	Log 1/R	1330 A	0.086	0.379	0.569

† Multiple correlation. ‡ Standard error of performance. § Simple correlation.
¶ Fourth derivative of log 1/R signal. # Standard regression method. †† Automatic regression method.

Table 5. Near-infrared reflectance spectroscopy analysis of moisture ontent in pea by principal component analysis/partial least square regressin(PCA/PLS) methods.

Samples	Format	Factors	MR	SEC†	SEP	γ
Ground	D4OD‡	5	0.915	0.222	0.237	0.828
Intact grain	Log 1/R	2	0.323	0.506	0.387	0.543

† Standard error of calibration. ‡ Fourth derivative of log 1/R signal.

were 0.227 and 0.871, in the calibration transformed to the 4th derivative of log 1/R, respectively. However, SEP and simple correlation values in intact grain samples were low. SEP and simple correlation for moisture content were 0.237 and 0.823 by PCA/PLS methods (Table 5). Based on our results, it seemed to be difficult to analyze moisture content of intact pea grain samples using NIRS. However, Stermer et al. (1976) reported that simple correlation of moisture content in intact sorghum and corn grain was 0.959 and 0.993, respectively. Law & Tkachuk (1977) also reported that simple correlation of moisture content in intact wheat grain was 0.966. In this study, a standard sample cup was used to analyze moisture content in intact pea grain samples by NIRS. NIRS requires amount to quantify intact grain samples. However, the standard sample cup holds only a small quantity. Therefore, I assumed that a gap between pea grain samples have a bad effect in analysis of pea moisture content by NIRS. Accordingly, NIRS analysis for moisture content in intact pea grain samples require further examination to be accepted rapid and accurate analysis.

REFERENCES

AACC. 1986. Near-Infrared technology in the agricultural and food industries. St. Paul, Minnesota, USA.

Allen Butler, L. 1983. The history and background of NIR. Cereal Chemist 4:238-240.

AOAC. 1984. Offical method of analysis. 14th Ed. Arlington, Virginia, USA.

Cho, R. K., J. H. Hong, H. K. Kim, and H. M. Park. 1990. Rapid quality evalution of dried red pepper by near-infrared spectroscopy. Korean J. Food Sci. 22(6):675-680.

Czuchajowska, Z. and Y. Pomoranz. 1991. Evalution of vital dry gluten composition and functionality in breadmaking by near-infrared reflectance spectroscopy. Cereal Foods World 36(5):439-446.

Davies, M. C. and D. J. Wright. 1984. Determination of protein in pea flour by Near Infrared analysis. J. Sci. Food Agric. 35:1034-1039.

_____, D. T. Coxon, G. M. Gavrel, and D. J. Wright. 1985. Determination of starch and lipid in pea flour by Near Infrared Reflectance analysis. The effect of pea genotype on starch and lipid content. J. Sci. Food Agric. 36:49-54.

Hwang, H. G., R. K. Cho, J. K. Sohn, and S. K. Lee. 1994. Rapid evalution of chemical components of rice grain using near infrared spectroscopy. Korean J. Crop Sci. 39(1):7-14.

Kim, B. J., E. H. Park, and C. S. Jung. 1996. Effect of chemical contents variation in covered barley seed on near infrared reflectance spectroscopy. Korean J. Crop Sci. 41(3):354-361.

_____, _____, and H. S. Suh. 1995a. Use of Near Infrared Reflectance Spectroscopy for determination of grain components in barley. Korean J. Crop Sci. 40(6):716-722.

Kim, Y. H., H. T. Yun, W. K. Chung, and S. D. Kim. 1995b. Determination of seed component contents in whole soybean seeds by near infrared spectroscopic analysis. RDA. J. Agri. Sci. 37(2):91-94.

Krishnan, P. G., W. J. Park, K. D. Kephart, D. L. Reeves, and G. L. Yarrow. 1994. Measurement of protein and oil content of oat cultivars using near-infrared reflectance spectroscopy. Cereal Foods World 39(2):105-108.

Law, D. P. and R. Tkachuk. 1977. Determination of moisture content in wheat by near-infrared diffuse reflectance spectrophotometry. Cereal Chem. 54(4):874-881.

Norris, K. H. and P. C. Williams. 1984. Optimization of mathematical treatments of raw near-infrared signal in the measurement of protein in hard red spring wheat. I. Influence of particle size. Cereal Chem. 61(2):158-165.

Stark, E., D. Honigs, and H. Mark. 1983. Optimized wave-length search and calibration techniques for NIRA. Cereal Food World 28:571.

Stermer, R. A., Y. Pomeranz, and R. J. McGinty. 1976. Infrared reflectance spectroscopy for estimation of moisture of whole grain. Cereal Chem. 54(2):345-351.

Williams, P. C., H. M. Cordeiro, and M. F. T. Harnden. 1991. Analysis of oat bran products by Near-Infrared Reflectance Spectroscopy. Cereal Foods World 36:107-110

_____, S. L. Mackenzie, and P. M. Starkey. 1985. Determination of methionine in peas by Near-Infrared Reflectance Spectroscopy(NIRS). J. Agric. Food Chem. 33:811-815.