Technical

Fast Analysis of Rapeseed Glucosinolates by Near Infrared Reflectance Spectroscopy

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Industry and plant breeders require fast methods to analyze glucosinolates in rapeseed. We tested the potential of near infrared reflectance spectroscopy (NIRS) for this analysis and developed calibration equations on a large population of whole seeds. Reference methods used are high performance liquid chromatography (HPLC) and gas liquid chromatography (GLC) of desulphoglucosinolates, a glucose-release method after purification on an anion exchange column and a palladium test. In the range from 2 to 107 μM/g and after transformation of the data in first derivative, a correlation coefficient of 0.99 was observed, as well as standard errors of estimated values of 2.15, 2.52, 2.67 and 4.07 for samples analyzed by HPLC, GLC, glucose and the palladium test, respectively. With different wavelengths, a limited calibration test on seeds containing from 4 to 40 μM/g gives a standard error of 1.91 μM/g (HPLC).

Different chemical methods can be used to determine glucosinolate content (HPLC, GLC, glucose, palladium and thymol tests). Unfortunately, all these methods are time consuming. Near infrared, a fast and nondestructive method, has been shown to provide a satisfactory alternative to more traditional methods when dealing with a large number of samples in plant breeding programs, in storage and processing plants and for feed manufacturers. Near infrared reflectance is widely used for the analysis of some components of a variety of plant materials, especially cereal grains and oilseeds. It has been investigated as a method of accurate and rapid quality prediction (1) and has been used increasingly for the determination of protein, oil and moisture content in oilseeds (2,3).

Tkachuk (2) investigated the measurement of glucosinolates by NIRS. Using whole seeds, he found a coefficient of multiple correlation of 0.71. Starr (3) has obtained similar results on ground seeds. In 1987, Renard (4) observed better results (r:0.94, SE:14.3) using six wavelengths, 1445, 1620, 1632, 1640, 2139 and 2208. Preliminary results found in our laboratory (5) showed good results (r:0.99; SE:2.96) when calibrated by HPLC of desulphoglucosinolates. In this paper, we compare correlations among four chemical reference methods and NIRS.

MATERIALS AND METHODS

Materials. Seeds were collected in several areas of Belgium, France, Denmark, Germany and England. After reference analyses were carried out, sets were selected for calibration and prediction purposes (Table 1).

REFERENCE METHODS

HPLC. Glucosinolates were extracted with methanol-water (70:30, v/v), purified on DEAE sephadex A25, desulphated with an enzyme (*Helix pomatia* sulphatase) and injected on a Sperisorb ODS2 HPLC column (25 cm × 4.6, 5 μ) (6).

GLC. After drying the purified glucosinolate extract, desulphoglucosinolates were silylated with a mixture of TMCS, MSHFBA, 1-methylimidazole and acetone. Chromatographic separation was done on a 2% OV7 column on diatomite CLQ (7).

Glucose test. The extracted glucosinolates were absorbed on an ion exchange column (DEAE Sephadex A25) and washed free of interfering nonanionic material. Addition of myrosinase to the ion exchange column released glucose. The glucose was eluted with water and estimated by a standard enzymatic assay (Sigma Chemical Co., St. Louis, Missouri) (7).

Palladium test. Glucosinolates were purified on Ecteolla cellulose columns. A complex formation between Pd and glucosinolates colors the solution. The color response of the Pd-glucosinolate complexes was measured at 425 nm (8).

Near infrared method. Reflectance data were collected with a Mark II PSC1 single beam spectrophotometer controlled by a North Star computer. The data were recorded for 12 g whole rapeseed contained in a normal cylindrical, rotating sample-holder covered by a quartz disk. For each rapeseed sample, an average of two readings were recorded at each 2.0-nm interval from 1100 to 2400 nm for a total of 700 values. The reflectance values were transformed in first derivative

TABLE 1

Characteristics of the Sets Used for Calibration and Prediction

	Calibration				Prediction			
Reference method	n	Range	Mean	SD	n	Range	Mean	SD
Palladium	74	6–107	30.75	24.52	20	12–100	27.97	24.60
Glucose	74	2–89	24.06	22.68	20	7–87	21.19	22.31
GLC	74	2–87	21.61	21.19	20	2–70	18.32	20.83
HPLC	74	4–100	24.75	22.53	20	4–87	20.61	23.19

SD, standard deviation; n, number of samples.

TABLE 2

Calibrations and Predictions for Glucosinolate Content by NIRS

Reference method	Calibration			Prediction			
	Math[a]	SEC[b]	MC[c]	RMS[d]	r[e]	p[f]	bias
Palladium	D10D 10/0	3.15	0.992	4.07	0.988	0.945	-0.419
Glucose	D10D 10/0	3.15	0.990	2.67	0.993	0.973	-0.257
GLC	D10D 10/0	3.71	0.985	2.52	0.993	0.969	0.436
HPLC	D10D 10/0	2.84	0.993	2.15	0.996	0.978	0.261

[a]Mathematical algorithm.
[b]Coefficient of multiple correlation.
[c]Correlation.
[d]Standard error calibration.
[e]Root mean square.
[f]Slope.

and were compared with the laboratory values by stepwise regression.

The highest correlation coefficient (r) and the lowest standard errors of estimate (SE) were used as criteria for evaluating the best predicting calibration equation. The equation was tested by using it to predict the same constituent in another set. Results obtained were then compared with those determined by standard laboratory methods.

RESULTS AND DISCUSSION

The optimal wavelengths selected by the stepwise regression program for the prediction of glucosinolate content in the range from 2 to 107 μM are the following: 1624-1636-1640 nm. The wavelengths are similar to those published in the literature. This spectral area seems to be very specific and highly sensitive.

Table 2 gives results of calibration and prediction of glucosinolate content by NIRS according to the reference methods used (HPLC, GLC, glucose and Pd tests). Better results have been observed after transformation of the data in first derivative (smoothing, 10 nm; cap, 10 nm). A correlation coefficient of 0.99 was found, as well as a standard error of estimated values of 2.15 μM/g of whole seeds, for samples analyzed by HPLC. Calibration made by GLC is not so good because indolylglucosinolates and glucosinolates containing sulfur cannot be measured satisfactorily by GLC. The glucose test can also be used to calibrate NIR, especially if the laboratory has no sophisticated equipment such as HPLC or GLC. Palladium calibration gives a high standard error.

The precision of all those methods is very similar. The samples have been analyzed chemically twice to ensure that the error of the mean was negligible. Complementary calibration tests have been realized on samples containing from 4 to 40 μM/g by HPLC. With the same wavelengths (1624-1636-1640), precision was similar; however, a new optimization of the wavelengths (1620-1638-1644 and 1666), in first derivative of the smoothing (4 nm) and the cap (4 nm), gives a standard error of estimated values of 1.91 μM/g.

These results indicate that NIRS is a suitable method for the prediction of glucosinolate content in whole rapeseed. The speed for analyzing glucosinolates and possibly moisture, protein and oil (1 min for 4 constituents), and the nondestruction of the seed make this technique well adapted for breeding purposes as well as for quality control in oil factories and in feed manufacturing.

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REFERENCES

1. Clark, D.H., *U.S. Dept. Agric. Handbook. 643*:7 (1985).
2. Tkachuk, R., *J. Am. Oil Chem. Soc. 58*:819 (1981).
3. Starr, C., J. Suttle, A.G. Morgan and D.B. Smith, *J. Agric. Sc. Camb. 104*:317 (1985).
4. Renard, M., C. Bernard, M. Deschamps, V. Furtoss, M. Lila, A. Quinsac, J.M. Regnier and D. Ribailler, in *Glucosinolates in Rapeseeds: Analytical Aspects*, edited by J-P. Wathelet, Martinus Nijhoff Publishers, Dordrecht, The Netherlands, 1987, pp. 173-176.
5. Wathelet, J-P., in *Glucosinolates in Rapeseeds: Analytical Aspects*, Martinus Nijhoff Publishers, Dordrecht, The Netherlands, 1987, pp. 163-172.
6. Spinks, E., K. Sones and G.R. Fenwick, *Fette, Seifen, Anstrichm. 86*:228 (1984).
7. Heaney, R.K., E. Spinks, A. Hanley and G.R. Fenwick, *Technical Bulletin: Analysis of Glucosinolates in Rapeseed*, Food Research Institute, Norwich, 1986.
8. Möller, P., A. Plöger and H. Sorensen, in *Advances in the Production and Utilisation of Cruciferous Crops*, edited by H. Sorensen, Martinus Nijhoff Publishers, Dordrecht, The Netherlands, 1985, pp. 97-110.

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