

Measurement of glucosinolate content in intact seeds of rapeseed with NIR reflectance spectrometry

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Abstract

Chemical analyses of rapeseed are expensive and time consuming, thus for quick analyses of breeding materials it is necessary to use physical methods. NIR analysis was applied for analysis of glucosinolates. Calibration equations were calculated for total content of glucosinolates and for individual glucosinolates. The calibration database covers 300 samples collected in IHAR during last 3 years. The GC of desulfo glucosinolate silyl derivatives were used as a reference method. Obtained equations show good correlation with reference data and could be used for screening of seed samples before chemical analyses.

Introduction

Glucosinolates are one of basic factors responsible for economical value of rapeseed. Wet chemical analyses of glucosinolate content in rapeseed need expensive chemicals and trained staff. It is necessary to apply physical method – such as NMR or NIR to speed up analyses of breeding materials.

NIR analysis was introduced for analyses of plant materials by Carl Norris (1965). Filters were used in first machines to cut necessary data from spectra. This solution is still used only in simple analyser. More sophisticated are using whole spectra for measurement. The real, wide scale application of NIR technology was started in late seventies, with introduction of personal computers. Calibration of NIR Analyser needs a lot of computations.

Glucosinolates absorb the energy in the near infrared range. Frequency of absorption depends on the matrix, (like rapeseed seeds, rapeseed meal) but mainly depends of chemical structure of glucosinolate especially of alkenyl or indol group characteristic for given glucosinolate. Differences in absorption frequency among glukosinolates make possible calculation of equations for individual glucosinolates.

Early works (Michalski 1987, Renard 1987) show that the glucosinolate measurement using NIR Analyser was possible, but with limited precision. Recent works, published during the International Rapeseed Congress in Canberra, show constant progress in applying NIR technology for rapeseed analysis. Font (1999), Pruvot (1999) and Velasco (1999) applied NIR analysis to measure total glucosinolates as well as single ones. Mollers (1999) investigated the measurement of sinapic acid ester. Patel (1999), Marcroft, Allen (1999) and Potter (1999) measured fat and protein content. Michalski (2000) used NIT technique to measure protein, neutral and acid detergent fibre.

On the market are available commercial calibrations for glucosinolates, but results obtained by applying them for seeds grown in Poland, are far from optimal. The necessary research works were started to make possible more precise measurement of glucosinolates in Polish varieties and lines of winter oilseed rape (both black seeds and yellow seeds) by using NIR spectrometry.

Typical glucosinolate composition in low glucosinolate winter rapeseed is as follows:

- gluconapin — 15%,
- glucobrassicinapin — 5.6%,
- progoitrin — 53%,
- napoleiferin — 2%,
- glucobrassicin — 1%,
- 4-hydroksyglukobrassicin — 20%

Genetical analysis of the chemical results (Krzymanski et al. 1999) show that indol glucosinolate (glucobrassicin and 4-hydroksyglukobrassicin) variability depends practically only on environment and is independent of genotype. Ultimate goal of breeding works is to minimise of alkenyl glucosinolate level because alkenyl glucosinolates are known to be toxic for animals. In low glucosinolate varieties only main alkenyl glucosinolates are important (progoitrin, gluconapin, glucobrassicinapin), others are only small fraction of total content. In this research only the total of all glucosinolates or total of alkenyl glucosinolates, and progoitrin, gluconapin, brassicagluconapin were measured.

Materials and methods

Research materials covered above 300 seed samples of winter oilseed rape collected in 1998-2000 years. The amount of glucosinolates varied from 2 to 40 $\mu\text{M/g}$ seeds, with lion share of samples from lower part of range. Samples were scanned and appropriate chemical data were added (glucosinolates were estimated as desulfo silyl derivatives by GC method). Spectra were measured by NIRSystems 6500 spectrometer and WINISI 1.50 chemometrics software was used for computing of calibration of data.

Discussion

The calibrations are presented in Table 1 and respectively on the plots in Figures 2 to 6. The robustness of calibration data was analyzed by plotting them at 3D plot in score space. As it is visible at plot (Fig. 1), the set is divided into three subsets, which correspond with subsets of yellow-seed samples, black-seed samples and samples collected from other than IHAR's sources. Optimal situation is, when the plot show globe evenly filled by samples. Pattern in Figure 1 is of course far from optimal, and need more samples covering wide spectral variation and filling gaps, present on the plot. The statistical data and results of calibration are shown in Table 1 and graphically, in Figures 2-6. Calibration error for this range was about 1,4 $\mu\text{M/}$ of glucosinolates per g seeds (total of alkenyl glucosinolates) and 1.8 $\mu\text{M /g}$ seeds (total of all glucosinolates), where prediction error was respectively 1.7 $\mu\text{M/g}$ seeds and 2.5 $\mu\text{M/g}$ seeds what is comparable to wet chemistry analysis error.

Conclusions

The above results show the opportunity of applying NIRS method as method for screening glucosinolate level for breeding purposes in Poland.

In the future it is planned to add another constituents (protein, fat content, moisture). This should make possible for breeders more full preliminary estimation of quality of rapeseed and white mustard genotypes.

Although the calibration set of 320 seed samples was fairly big, it is still too narrow in spectral terms, and it is necessary to enhance the calibration set with samples which fill the gaps in score space and make it more robust and reliable.

In the further work there will be added new samples on the basis of glucosinolate content (above 20 μM of total) and the spectral uniqueness, (global and local “H” parameters) to enhance the calibration robustness.

It was shown that measurements of individual main alkenyl glucosinolates were also possible, and that it can be a serious advantage in breeding work.

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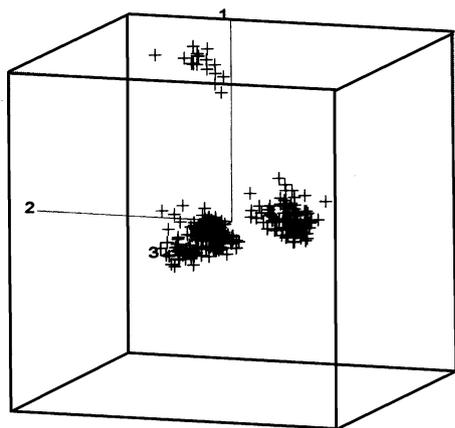


Fig. 1. Sample pattern in the space scores (1-2-3)

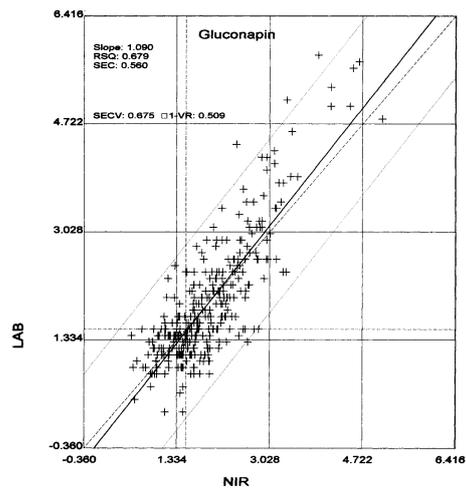


Fig. 2. Gluconapin calibration plot

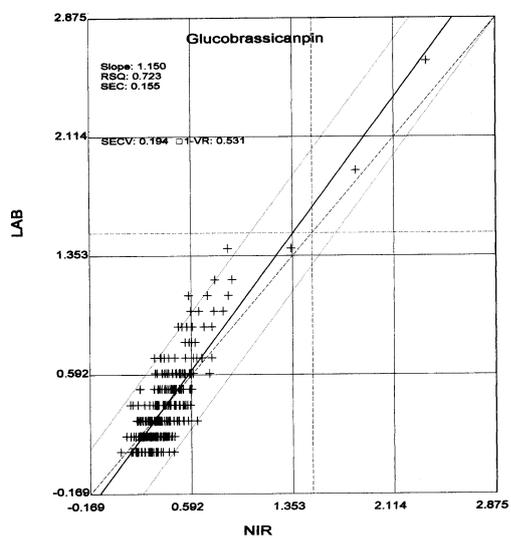


Fig. 3. Glucobrassicinapin calibration plot

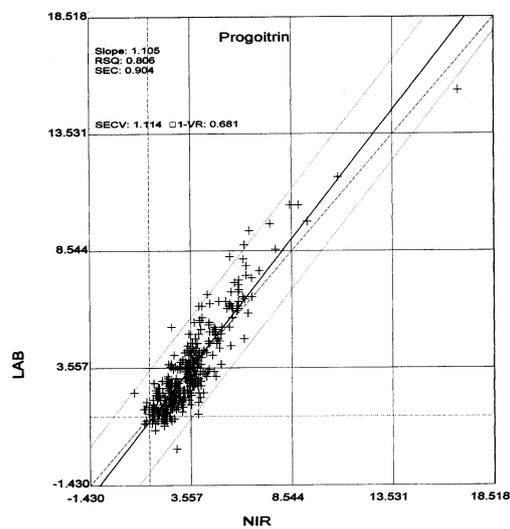


Fig. 4. Progoitrin calibration plot

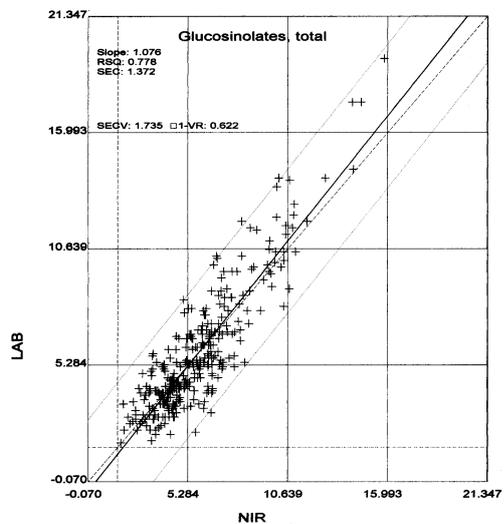


Fig. 5. Sum of glucosinotates calibration plot

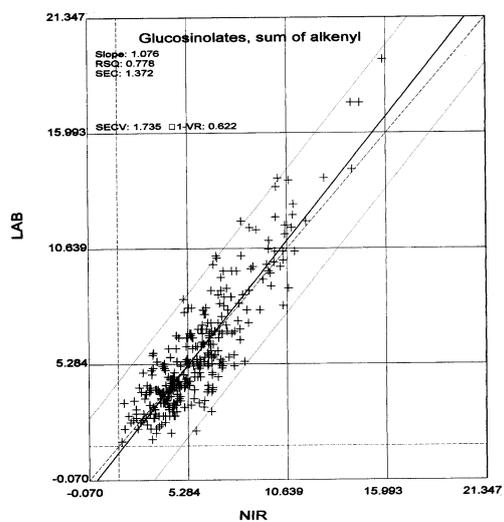


Fig. 6. Total glucosinolate calibration plot

Table 1

Calibration equation statistics

| Glucosinolates | Range [$\mu\text{M/g}$ seeds] | Correlation coefficient | Standard error of calibration [$\mu\text{M/g}$ seeds] | Standard error of validation [$\mu\text{M/g}$ seeds] |
|--------------------|-----------------------------------|----------------------------|--|---|
| Total alkenyl | 1,7–19 | 0,81 | 1,41 | 1,75 |
| Total | 4,4–32 | 0,80 | 1,5 | 2,0 |
| Progoitrin | 0,1–18 | 0,60 | 0,9 | 1,16 |
| Gluconapin | 0,2–4,7 | 0,63 | 0,5 | 0,6 |
| Glucobrassicinapin | 0,1–2,7 | 0,72 | 0,16 | 0,2 |