Fast prediction of quality parameters in whole seeds of oilseed rape (*Brassica napus* L.)

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ABSTRACT

A calibration equation for NIRSystems 6500 instrument was derived at VSTE Jevíèko using the measurement of broad collection of Czech samples of winter rape, allowing sufficiently accurate prediction of content of dry matter (DM), crude protein (XP), crude fat (XL), glucosinolates (GSL), oleic and linoleic acids in an extremely short time. The prediction accuracy was verified on a validation file (n = 60). The coefficients of determinance (R^2) were 0.83 for XP, 0.71 for XL, and 0.84 for GSL. The prediction accuracy according to the VSTE equation was compared to the prediction accuracy according to the VDLUFA calibration equation (Kassel, FRG) used in EU near infrared spectroscopy network. It was stated that the former was not distinctly worse. Non-destructive NIR-analysis of the whole seed also allows sowing selected seeds in the year of harvest and thus accelerates the breeding cycle.

Keywords: winter oilseed rape; technological quality; NIRS; screening methods; rapeseed analysis

The improvement of chemical composition of rapeseed (*Brassica napus* L.) is one of breeding programme priorities. Glucosinolates (GSL), which make up a constituent of extracted meal, have antinutritive qualities. On the contrary, some GSLs have an important role in plant protection against pests and diseases (Zukalová and Vašák 2002). When bred for quality, genotypes with higher content of crude fat (XL) and crude protein (XP) and with lower content of GSL are of value. When the oil would be used for technical purposes, high proportion of erucid acid in triglycerid fraction is required. However, when the oil is used for human nutrition, presence of erucid acid must be minimised and proportion of oleic and linoleic acids should be increased. The proportion of linolenic acid should be simultaneously decreased because it oxidizes easily.

Since standard methods of an assessment are generally slow and expensive, some screening methods as near infrared reflectance spectroscopy (NIRS) are examined for evaluation of breeding materials, varieties, and also rapeseed sold for technical processing (Velasco and Becker 1998a, b). The main advantage of NIRS for the breeding industry is the prediction of composition of intact seeds without loss of germination capacity. The previous results suggested encouraging possibility of using this method for GSL evaluation (Míka et al. 1997, Koprna and Kolovrat 2001).

The paper aims to present recent improvements in non-destructive rapeseed assessment in such important quality parameters as dry matter (DM), crude protein (XP), crude fat (XL), and total glucosinolate content (GSL). The comparison of prediction accuracy of locally developed calibration equations and those used in the network of German Association of Agricultural Research and Experimental Stations (VDLUFA) over EU-countries with standard laboratory analyses have been done.

MATERIAL AND METHODS

VSTE calibration. The VSTE calibration equation was developed from spectra of 516 samples of rape of the Czech origin. This equation was extended by spectra of 60 samples from variety trials (ÚKZÚZ Brno) and 230 samples of breeding material (VÚO Opava) from harvest years 2000 and 2001. All samples used were predried at < 40°C and possessed residual water content < 100 g/kg. This calibration allows the determination of DM, XL and GSL simultaneously (Table 1). The reference methods used were:

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Table 1. VSTE calibration

Constituent	Unit	*n*	Mean	min–max[1]	SEC[2]	SECV[3]	Reference
DM	%	355	94.7	93.1–96.3	0.25	0.33	drying oven
XL	% in DM	516	46.7	40.2–53.1	0.53	0.59	Soxhlet
GSL	µmol/g DM	227	19.2	0–43.1	4.61	5.50	HPLC and GC

[1]estimated minimum and maximum, [2]standard error of calibration, [3]standard error of cross validation

DM – whole seed drying at 105°C, 4 h (according to ČSN ISO 665)
XP – Nessler flow colorimetric method
XL – extraction of ground samples with ethylether in Soxhlet apparatus (according to ČSN ISO 659)
GSL – gas chromatography – GC (according to ČSN 461039)

The HPLC and GC methods differ with respect to the indol GSL. During the GC procedure these indol GSL are destroyed and therefore are not detected. The indol GSL may contribute between 0.5 and 10 µmol/g DM to the total GSL content.

VDLUFA calibration. The VDLUFA calibration was developed on German rapeseed breeding material mainly. Depending on previous experiences it should have a precision of 0.5% DM for crude oil and crude protein prediction and 2 mmol/g DM for the prediction of the GSL content (Tillmann 1997, VDLUFA 2002, Table 2). This calibration was transferred to the NIRS instrument in Jevíčko by the algorithm of Shenk (1990). The calibration may also be used on samples with less than 100 g/kg residual moisture only.

Validation sample set. The reference methods were the same as above, with the exception of GSL where the HPLC procedure (ISO 9167-1) was used (VÚO Opava).

NIRS scanning. Spectra of 60 samples of whole seeds were scanned in Jevíčko on a NIRSystems 6500 instrument equipped with sample transport module, in reflectance range 400–2500 nm, band width 2 nm, measured in small ring cups, in 2 × 2 replications and using ISI 3.01 software. Laboratory analyses were conducted as described in Table 3. For the validation with the VDLUFA calibration, these data were computed to DM basis. The validation is described in terms of standard error of prediction (SEP), bias and standard error of prediction corrected for bias. The bias describes the systematic difference between the analyses done by laboratory method and the NIRS predictions.

RESULTS AND DISCUSSION

The validations for XL, XP and GSL (Tables 4 and 5) demonstrated fair relationships of NIRS analyses to laboratory analyses (coefficient of determination $R^2 = 0.83$ for XP, 0.71 for XL, and 0.84 for GSL). The coefficients of determination are rather low due to the limited range of constituents in the validation set (Tables 2 and 3).

The SEP for oil determination is 1.08% for the VSTE calibration and 0.86% DM for the VDLUFA calibration. When the slight bias for the VSTE calibration is neglected both calibrations show an almost identical SEP(C) of 0.69% and 0.80% DM (Figures 1a and 2a). The SEP is close to what has been observed for the VDLUFA calibration on other datasets.

Table 2. VDLUFA network calibration

Constituent	Unit	*n*	min–max	SEP[1]	From	Reference
XL	% in DM	795	38–58	0.5	1984–1998	NMR
XP	% in DM	307	13–31	0.5	1984–1998	Dumas
GSL	µmol/g DM	434	3–35	2	1985–1998	HPLC
Moisture	%	49	4–14	0.3	1989	drying oven

[1]standard error of prediction from previous experience

Table 3. Validations sample set 2001 from Jevíčko

Constituent	Unit	*n*	min–max	Reference
XL	% in DM	30	46.0–51.5	Soxhlet
XP	% in DM	60	17.3–22.7	Nessler
GSL	µmol/g DM	30	5.1–50.8	GC

Table 4. Validation of the locally developed calibration for the prediction of crude protein, crude fat and glucosinolates in DM in rapeseed

Name	Unit	SEP	Bias	SEP(C)	Slope	R^2	n
XL	% in DM	1.08	0.86*	0.67	0.96	0.79	30
GSL	µmol/g DM	7.04	–1.91	6.89*	1.58	0.74	30

Table 5. Validation of the VDLUFA network calibration for the prediction of crude protein, crude fat and glucosinolates in DM in rapeseed

Name	Unit	SEP	Bias	SEP(C)	Slope	R^2	n	GH
XL	% in DM	0.86	0.35	0.80	0.87	0.71	30	0.99
XP	% in DM	0.90	–0.63*	0.65	0.89	0.83	60	0.88
GSL	µmol/g DM	7.96	5.25*	6.08*	1.60	0.84	30	0.77

Due to sample specific interactions between DM and XL, to reach acceptable accuracy of NIRS prediction, samples must have at least 900 g/kg DM, when scanned with NIRS.

The protein calibration could only be validated for the VDLUFA calibration. Here a slight bias (–0.63% DM) is observed (Figure 2b). This might be due to differences in reference methods (Nessler vs. Dumas). The SEP(C) is 0.65% DM and exactly in the range of the expected value.

The GSL calibrations gave SEPs for the VSTE and VDLUFA calibrations of 7 µmol/g and 7.96 µmol/g DM, respectively (Figures 1b and 2c). The VDLUFA calibrations exhibit a strong bias of 5.25 µmol/g DM. Both calibrations can differentiate between the samples with high (50 µmol/g), medium (30 µmol/g) and low GSL content (20 µmol/g) but fail to differentiate between 5 and 15 µmol/g.

The result is most probably due to the reference method used (GC for validation samples vs. HPLC for calibration development). From Tables 4 and 5 is clear that for both validations the slope is 1.58 and 1.60, which is a clear deviation from 1.0, as is to be expected. Based on experience an SEP of 2 µmol/g would be expected.

The prediction of GLS content shows that NIRS provides more accurate results in the whole range of values of natural variability (Figure 2b, c) than glucotest (Koprna and Kolovrat 2001). It can also be used for identification of genotypes with GSL values above set limit, which

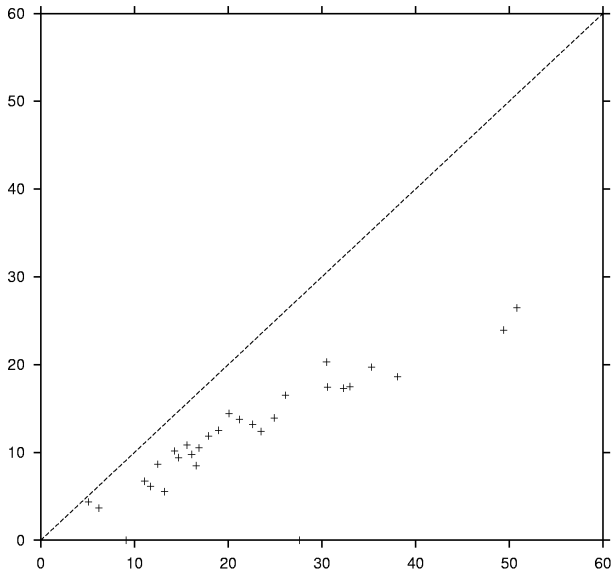

Figure 1a. Validation of VSTE calibration for crude oil content in whole rapeseeds

Linear regression equation: $y = 0.96x + 2.70$
Label x-axis: crude oil content (%, Soxhlet)
Label y-axis: crude oil content (%, NIRS)

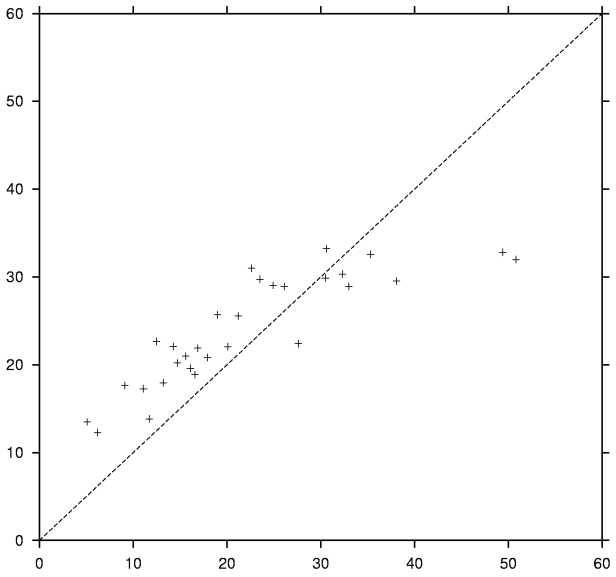

Figure 1b. Validation of VSTE calibration for total GSL content in whole rapeseeds

Linear regression equation: $y = 1.57x - 15.76$
Label x-axis: GSL content (µmol/g DM, GC)
Label y-axis: GSL content (µmol/g DM, NIRS)

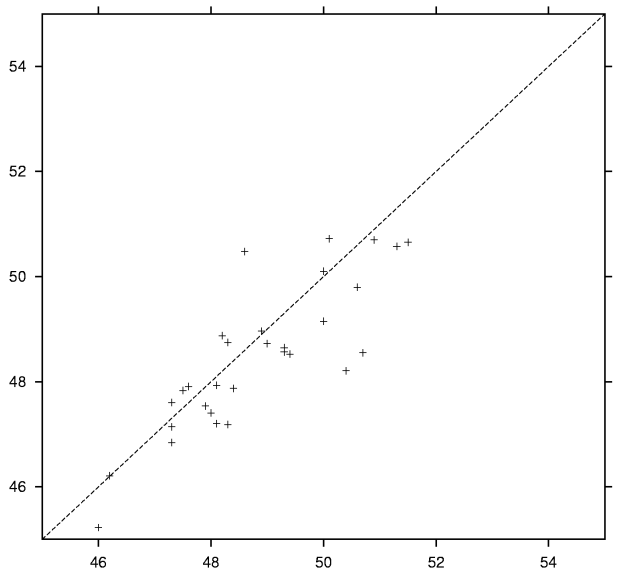

Figure 2a. Validation of VDLUFA calibration for crude crude oil content in whole rapeseeds

Linear regression equation: $y = 0.87x + 6.45$
Label x-axis: crude oil content (%, Soxhlet)
Label y-axis: crude oil content (%, NIRS)

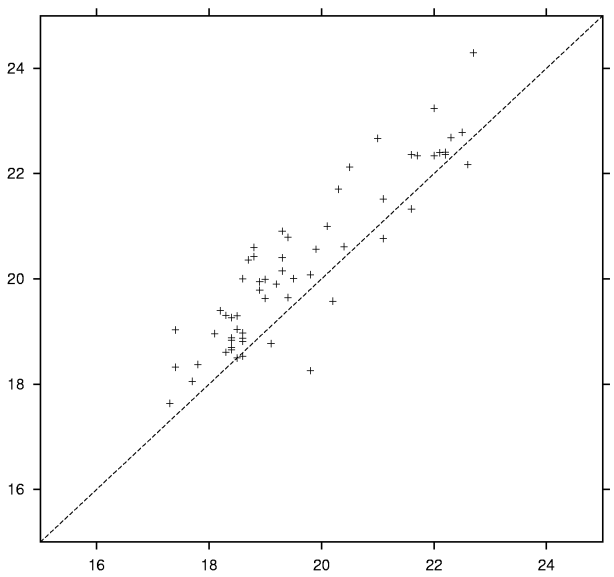

Figure 2b. Validation of VDLUFA calibration for crude protein content in whole rapeseeds

Linear regression equation: $y = 0.89x + 1.56$
Label x-axis: crude protein content (%, Nessler)
Label y-axis: crude protein content (%, NIRS)

is significant in breeding programmes. Slightly higher SEP value (Figure 2b) is definitely influenced by disunity of used reference laboratory method, i.e. the content of GLS was determined with GC for about half of samples, the rest with HPLC. HPLC method is generally preferred because it is more accurate, but GC method is still used in this country for capacity reasons.

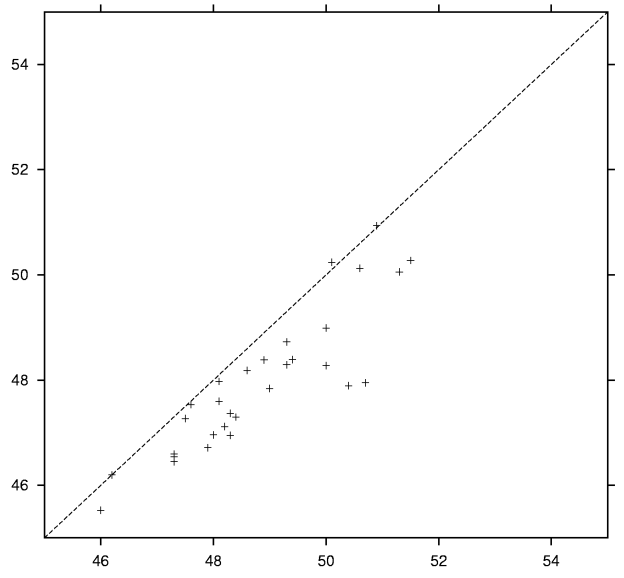

Figure 2c. Validation of VDLUFA calibration for total GSL content in whole rapeseeds

Linear regression equation: $y = 1.87x - 14.78$
Label x-axis: GSL content (µmol/g DM, GC)
Label y-axis: GSL content (µmol/g DM, NIRS)

The global H (GH) is the Mahalanobis distance. It describes the distance of an individual samples from the centre of population of the calibration samples. It serves for determination whether a sample is an outlier (critical value is usually > 3). The samples from Jevíčko are not different from the VDLUFA calibration samples to cause unexpected problems.

The study demonstrated that NIRS technology can analyse whole rapeseeds in extremely short time period on all significant parameters of quality. This allows to process selected samples immediately after harvest and to sow materials selected according to quality criteria in the same year when they were harvested. Both calibrations (VSTE and VDLUFA) are able to predict fatty acid composition (C18:1 and C22:1) for unknown samples as well as the named constituents, but no validations were performed in this study.

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ABSTRAKT

Rychlá predikce parametrů kvality celých semen řepky (*Brassica napus* L.)

Na základě NIR-měření (spektroskopie v blízké infračervené oblasti) obsáhlé kolekce českých vzorků ozimé řepky byla ve VÚRV Praha, VSTE Jevíčko vypracována kalibrační rovnice pro přístroj NIRSystems 6500. Rovnice umožňuje extrémně rychlou a přitom přijatelně přesnou predikci obsahu sušiny (DM), dusíkatých látek (XP), tuku (XL), glukosinolátů (GSL), kyseliny olejové a linolové. Přesnost predikce byla ověřena na validačním souboru (n = 60) tuzemských vzorků. Koeficienty determinace (R^2) činily pro XP 0,83, resp. 0,71 pro XL a 0,84 pro GSL. Přesnost predikce obsahu XP, XL a GSL podle kalibrační rovnice VSTE byla srovnávána s přesností predikce podle kalibrační rovnice VDLUFA (Kassel, BRD), využívané v síti NIRS ve státech EU. Ukázalo se, že není zřetelně horší a danému účelu plně vyhovuje. Nedestrukční analýza celých semen řepky umožňuje, aby ještě v roce sklizně byly vybrané materiály vysety, a významně se tak urychlil šlechtitelský cyklus při vývoji nových odrůd.

Klíčová slova: řepka olejka; technologická kvalita; NIRS; metody screeningu; laboratorní analýzy semen řepky

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