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The Analysis of Rapeseed (*Brassica napus* L.) in a Network of NIRS-Instruments

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NIRS-Analysis of Rapeseed

Whole kernel analysis of rapeseed by NIRS was first published by TKACHUK (1981) for the constituents protein and oil. Extensive research into the application of NIRS-analysis for glucosinolate (GSL) determination in rapeseed was done by BISTON and coworkers (BISTON *et al.*, 1987, 1988). First results for the fatty acid analysis in rapeseed were published by REINHARDT *et al.* (1992).

At the Institute of Agronomy and Plant Breeding of the University of Göttingen NIRS-analysis of rapeseed was established by REINHARDT (1992) (Table 1). In the following years many German rapeseed breeders started to use NIRS-analysis in their breeding programs (FRAUEN AND REINHARDT, 1993). All instruments were calibrated separately by means of the same calibration set.

Table 1: Validation parameters for NIRS-analysis of rapeseed

Constituent	Unit	Range	SEP	r
Oil	%	38–58	0.7–1.1	0.95
Protein	%	13–31	0.6–1.0	0.97
GSL	µmol/g	5–36	1.8–2.6	0.92
C18:1	%	39–73	2.5–3.0	0.90
C22:1	%	35–59	2.6–4.0	0.90
Moisture	%	3–36	1.0–1.2	0.99

Source: REINHARDT (1992)

In 1993 a project was started to focus on the reliable management of NIRS-analysis at different laboratories. Instead of calibrating each instrument individually a networking approach was followed. This project, funded by the UNION FÜR OEL- UND PROTEINPFLANZEN (UFOP), Bonn, had the purpose to evaluate different strategies of create a network of NIRS-instruments for rapeseed analysis and to determine its precision.

Networking of NIRS-Instruments

A network of NIRS-instruments is defined as a group of instruments using a single calibration for the analysis of a product. In this terminology, a master instrument is the instrument on which this calibration is developed and the satellites are the instruments on which the calibration is used in routine work (Figure 1). The aim of a network of NIRS-instruments is a precise and accurate analysis by all connected instruments.

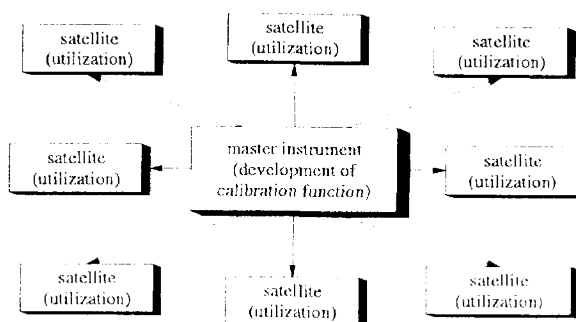

Figure 1: Network of NIRS-instruments using a single calibration function

In the literature several methods to create a network were described (DE NOORD, 1994). The direct transfer of the calibration from the master to the satellites is often associated with a bias. A straightforward approach, therefore, is the direct calibration transfer with bias correction (OSBORNE AND FEARN, 1983).

Another approach is the standardization of instruments first reported by SHENK *et al.* (1985). Spectra of the satelites are modified to look like those of the master instrument. First results on networking of NIRS-instruments for rapeseed using standardized instruments were published by DARDENNE AND BISTON (1990).

A third approach was named "robust calibrations". Calibrations developed on the master instrument are developed in that way that they can be directly transferred to the satellites. Two strategies are reported: calibrating with spectra from several instruments (SHENK, 1992) and using a repeatability file (WESTERHAUS, 1990).

Robust calibrations can be used together with either bias correction or the standardization of instruments. In a first comparison only a calibration developed on the master instrument and its use either with bias correction or standardized instruments was evaluated in different variants. Secondly robust calibrations were evaluated. Combinations of both are reported by TILLMANN (1997).

All instruments used in this study were NIRSYSTEMS instruments from PERSTORP ANALYTICAL. The common wavelength range used for calibration was 1300–2400 nm. The calibration and validation set as well as the samples used for standardization or calculation of the bias correction were independent of each other (see TILLMANN, 1997).

Methods to Create a Network By Direct Calibration Transfer with or without Standardization

The variants are described in Table 2. The objective was to compare a direct calibration transfer with or without bias correction (dir, bias) and several variants of standardization. Standardizations using ground material (ISI) and 1 or 10 whole kernel rapeseed samples were investigated. Three single sample standardizations (1R) were evaluated where the samples differed in their H value. The H value measures how representative a sample is for the calibration set: the smaler the H value, the more representative is the sample.

The calibrations for oil, protein, GSL and moisture were transferred to two satellite instruments. The precision of the analysis in this network measured using 36–42 independent validation samples is reported in Table 2. The SED is calculated as standard deviation of the analytical results of the three instruments in the network. The limit for acceptable SEDs is 0.38 times the SECV of the transferred calibration function.

Results In case of the direct calibration transfer (dir) for all constituents except protein the SEDs are exceeding the limits. Precise analyses are only possible if they include either a bias correction (bias) or the instruments are standardized using rapeseed samples (1R, 10R). No differences were found between different single sample standardizations. Standardizing instruments using ground material (ISI) for rapeseed analysis was even harmful for the determination of protein and not acceptable for GSL determination.

Table 2: Variants of networking procedures using bias correction or standardization; Standard Deviation of Analysis (SED) on three instruments

		Protein	Oil	GSL	Moisture
Abbr.	Description	%	%	μmol/g	%
dir	Direct calibration transfer	0.19	0.62*	2.18*	0.21*
bias	• with *bias* correction	0.17	0.21	0.59	0.06
	Standardization				
ISI	• with ISI-Set	0.50*	0.27	0.75*	0.07
10R	• with 10 rapeseed samples	0.14	0.23	0.90*	0.04
1R-0,6	• with 1 rapeseed sample (H 0,6)	0.23	0.26	0.57	0.07
1R-1,1	• with 1 rapeseed sample (H 1,1)	0.19	0.24	0.66	0.08
1R-1,6	• with 1 rapeseed sample (H 1,6)	0.21	0.21	0.60	0.07
	n	42	42	36	42

Asterisks mark unacceptable results.

Table 3: Variants of networking procedures using robust calibrations; Standard Deviation of Analysis (SED) on three instruments

		Protein	Oil	GSL
Abbr.	Description	%	%	μmol/g
1I	Spectra of one instrument	0.19	0.62*	2.18*
3I	Spectra of three instruments	0.26	0.94*	3.65*
	Repeatability file			
1I-R1	• with file 1	0.18	0.21	0.66
1I-R2	• with file 2	0.19	1.44*	1.95*
	n	42	42	36

Asterisks mark unacceptable results.

Methods to Create a Network by Robust Calibrations

Three procedures to develope robust calibrations were evaluated (Table 3). First calibrations were developed with spectra from three instruments (3I). These were the master and two other instruments differing from the satellites. Furthermore two sets of calibrations were developed with spectra from the master instrument including a repeatability file (WESTERHAUS, 1990). Repeatability file 1 (1I-R1) was built with the spectra of 10 fixed samples measured on the master and the two satellites under controlled conditions (temperature and humidity). Repeatability file 2 (1I-R2) was built with the spectra of 1 fixed sample measured on 15 NIRS-instruments under varying conditions.

Calibrations were developed for the constituents protein, oil and GSL. These calibrations were directly transferred to the two satellite instruments, next to a control variant (1I). The calibrations of the control variant were developed solely with spectra from the master instrument using no repeatability file.

Results The control variant (1I) gave acceptable results only for protein determination (Table 3). The calibrations developed using spectra from three instruments (3I) produced worse results[1]. Very precise analyses were reached when a robust calibration was developed using repeatability file 1. The calibrations were directly transferable to the satellites. This was not true for repeatability file 2.

Combinations of robust calibrations and standardization of instruments were also studied, but no additional information was gathered. The interested reader is referred to TILLMANN (1997).

A Network of NIRS-Instruments in a Collaborative Study

A network of NIRS-instruments was set up using conclusions from the above studies. A single sample standardization procedure with a fixed rapeseed sample was used to standardize each of the 13 satellite instrument to the master. Furthermore under calibration development a repeatability file was used based on a single sample measured on each instrument in the network.

The precision of rapeseed analysis in such a network was measured for the constituents protein, oil and GSL. 10 samples were distributed and scanned on each instrument four times. The same 10 samples were also analyzed by conventional procedures for oil (SOXHLET, NMR) and GSL (HPLC, XRF).

Participation was voluntary and no attempt was made to homogenize analyses across laboratories. The distribution of the participants to the different methods was rather unequal (Table 4). Especially the reproducibility standard deviation of the XRF methods differed markedly from those in the corresponding ISO norm (ISO 9167-2, 1994), whereas the other here reported repeatability and reproducibility standard deviations compared favourable to the corresponding norms (ISO 659, 1996; ISO 5511, 1992; ISO 9167-1, 1992). The results of the collaborative study were determined according to ISO 5725 (1994).

Results The repeatability and reproducibility standard deviations for NIRS-analysis of oil determination was 0.36% and 0.66%, respectively. The repeatability standard deviation was in the range of that of the SOXHLET method for oil determination but twice that of the NMR method.

[1] These can be explained by what is known in statistics under "errors in the variable modell".

Table 4: Collaborative trial of NIRS-analysis in a network

Constituent	Unit	Range	Method	N	p	σ_r	σ_R
Oil	%	45.36 – 50.44	SOXHLET	15–20	4–5	0.28	0.71
			NMR	35–40	9–10	0.19	0.91
			NIRS	51–52	13	0.36	0.66
GSL	μmol/g	7.89 – 16.70	HPLC	15–20	4–5	0.86	1.09
			XRF	11–12	3	0.77	3.37
			NIRS	50–52	13	0.83	1.96
Protein	%	18.33 – 22.86	NIRS	51–52	13	0.28	0.40

N = total number of determinations on each sample, p = number of labs
σ_r = repeatability standard deviation, arithmetic mean for 10 samples
σ_R = reproducibility standard deviation, arithmetic mean for 10 samples

This means that in a single laboratory two single determinations of the oil content by NMR are on average half as far apart as oil determinations by the NIRS method.

The reproducibility standard deviation was slightly lower for the NIRS method than for especially the NMR method of oil determination. This means that by using a network of NIRS-instruments the differences in analyses from laboratory to laboratory can be minimized.

The repeatability standard deviation for GSL determination was for all methods about 0.8 μmol/g. The reporducibility standard deviation was lowest for the HPLC method with 1.09 μmol/g, followed by the NIRS method with 1.96 μmol/g. These figures are well within the range of the ISO norm for the HPLC method (ISO 9167-1, 1992).

The figures for protein determination showed the homogeneity of analytical results in a network. These findings in general support the results of many other ring tests, where NIRS analysis seldomly reached the repeatability of the official analytical methods but most often surpassed these in reproducibility.

Conclusion

NIRS analysis has been described in several reports as fast and inexpensive. Most authors suggested an application in breeding programs. In this study a network of NIRS instruments was evaluated.

First methods for setting up a network were investigated. Each of the following procedures gave homogeneous analytical results in a network: a) direct calibration transfer with bias correction, b) standardization with either 10 or 1 rapeseed sample and c) a robust calibration using a repeatability file covering spectra from all satellites. Networking of NIRS-instruments is a superior method of know-how transfer to practical application.

In a second step a network of 13 NIRS-instruments was set up using the above findings. The precision of oil and GSL analyses in this network was comparable to those of the official analytical methods (SOXHLET, NMR, HPLC, XFR) with exception of the repeatability of the oil determination.

The results of this collaborative study clearly showed that the NIRS method of rapeseed analysis is as precise as the official methods. There-

fore, the introduction of NIRS should be supported in areas where precise analytical results need to be available quickly and inexpensive.

References

BISTON R., DARDENNE P., CWIKOWSKI M., WATHELET J. AND SEVERIN M. (1987): Analysis of quality parameters of whole rapeseed by NIRS. *World Crops Prod Util Descr*, **13**:163–172.

BISTON R., DARDENNE P., CWIKOWSKI M., MARLIER M., SEVERIN M. AND WATHALET J.P. (1988): Fast Analysis of Rapeseed Glucosinolates by Near Infrared Reflectance Spectroscopy. *JAOCS*, **65**:1599f.

DARDENNE P. AND BISTON R. (1990): Standardization procedure and NIR instrument network. In: Biston R. and Bartiaux-Thill N. (Eds.), *Proc Third Int Conf Near Infrared Spectrosc*, Agricultural Research Centre Publishing, Gembloux, B, pp. 655–662.

DE NOORD O. (1994): Multivariate calibration standardization. *Chemom Intel Lab System*, **25**:85–97.

FRAUEN M. AND REINHARDT T. (1993): Einsatz des NIRS-Verfahrens in der Qualitätszüchtung bei Winterraps. In: *Ber. 44. Arb.Tagung Saatzuchtleiter*, Vereinigung Österreichischer Pflanzenzüchter, Gumpenstein, A, pp. 161–164.

ISO 5511 (1992): *Oilseeds — Determination of oil content — Method using continuous-wave low-resolution nuclear magnetic resonance spectroscopy (Rapid method)*. International Standardisation Organisation, Genf, CH.

ISO 5725 (1994): *Accuracy (truness and precision) of measurement methods and results*. International Standardisation Organisation, Genf, CH.

ISO 659 (1996): *Oilseeds — Determination of hexane extract (or light petroleum extract), called "oil content"*. International Standardisation Organisation, Genf, CH.

ISO 9167-1 (1992): *Rapeseed — Determination of glucosinolate content — Part 1: Method using high-performance liquid chromatography*. International Standardisation Organisation, Genf, CH.

ISO 9167-2 (1994): *Rapeseed — Determination of glucosinolate content — Part 2: Method using x-ray fluorescence spectrometry*. International Standardisation Organisation, Genf, CH.

OSBORNE B. AND FEARN T. (1983): Collaborative evaluation of universal calibrations for the measurement of protein and moisture in flour by near infrared reflectance. *J Food Technol*, **18**:453–460.

REINHARDT T. (1992): *Entwicklung und Anwendung von Nah-Infrarot-spektroskopischen Methoden für die Bestimmung von Öl-, Protein-, Glucosinolat-, Feuchte- und Fettsäure-Gehalten in intakter Rapssaat*. Cuvillier Verlag, Göttingen.

REINHARDT T.C., PAUL C. AND RÖBBELEN G. (1992): Quantitative analysis of fatty acids in intact rapeseed by NIRS. In: Murray I. and Cowe I. (Eds.), *Making light work*, VCH, pp. 323–327.

SHENK J. (1992): Networking and calibration transfer. In: Murray I. and Cowe I. (Eds.), *Making light work: Advances in Near Infrared Spectroscopy*, VCH, Weinheim, pp. 223–226.

SHENK J. AND WESTERHAUS M. (1991): New standardization and calibration procedures for NIRS analytical systems. *Crop Sci*, **31**(6):1694–1696.

SHENK J., WESTERHAUS M. AND TEMPLETON JR W. (1985): Calibration transfer between near infrared reflectance spectrophotometers. *Crop Sci*, **25**:159–161.

TILLMANN P. (1997): *Qualitätsuntersuchung von Raps mit der vernetzten Nahinfrarotspektroskopie (NIRS)*. Ph.D. thesis, Universität Göttingen.

TKACHUK R. (1981): Oil and protein analysis of whole rapeseed kernels by near infrared reflectance spectroscopy. *JAOCS*, **58**:819–822.

WESTERHAUS M. (1990): Improving repeatability of NIR calibrations across instruments. In: Biston R. and Bartiaux-Thill N. (Eds.), *Proc Third Int Conf Near Infrared Spectrosc*, Agricultural Research Centre Publishing, Gembloux, B, pp. 671–674.