QUANTITATIVE ANALYSIS OF FATTY ACIDS IN INTACT RAPESEED BY NEAR INFRARED REFLECTANCE SPECTROSCOPY

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INTRODUCTION

Fast, non-destructive, cheap and nevertheless precise, these are the characteristics of analytical methods requested by plant breeders. Near infrared reflectance spectroscopy (NIRS) can satisfy these expectations especially because it allows to determine more than one or two constituents in one measurement.

For rapeseed, some results for the determination of oil and protein by NIRS are known (e.g. Bengtsson 1985; McGregor 1988). It is furthermore possible to check for glucosinolate (GSL) content in the seed within the range from 4 to 35 µmol/g (e.g. Michalski et al. 1987; Wathelet et al. 1989). We have developed equivalent NIRS methods and use these since 1990 for oil, protein and GSL content routine analysis in Göttingen.

The present study describes two new applications of NIRS: the determination of oleic acid in 0 rapeseed and of erucic acid in + rapeseed.

MATERIALS AND METHODS

More than 700 samples of rapeseed were harvested from open- or self-pollinated plants and bulks during the years 1987-1990 at different locations.

The samples were scanned with a NIRS-Monochromator (1100-2500 nm, Pacific Scientific Model 6250) using a sample quantity of 3-5 g seed. The data obtained from measurement of three (calibration) and two (validation) repacks (replication) were averaged. For all samples results of gas chromatography (GC) were available from three different laboratories (Table 1).

Table 1. Analytical data (gas chromatograph) of the calibration and validation sets

Fatty acid	Use	n	Total fatty acids (%) mean	SD	min	max	Labor
C22:1	Calibration	73	46.0	6.69	35.0	58.6	a
	Val 1 1989	44	46.9	6.99	35.5	59.2	a
	Val 2 1990	45	46.9	7.66	35.1	61.1	b
C18:1	Calibration	101	58.2	6.65	39.8	73.2	c
	Val 1 1990	29	58.1	4.16	49.9	64.9	c
	Val 2 1990	34	61.6	4.91	49.8	68.9	b,c

Calibration samples were selected according to spectral variation and content of the interesting fatty acid. The validation sample sets (Val) are independent from the calibration sets.

For development of the NIRS-methods, ISI-Software (Pacific Scientific) was used with multiple linear regression as statistical method for optimal wavelength selection.

RESULTS

Based on two sets of 73 and 101 samples, respectively, NIRS-methods were developed for the determination of C22:1 and C18:1 content (% of total fatty acids; see Table 2). Four or five wavelengths were selected, of which 1750 nm and 1696 nm are the most important. A data transformation to the second derivative gave the best results.

Table 2. Calibration

Fatty acid	SEC(1)	r^2
C22:1	2.585	0.851
C18:1	2.488	0.860

(1) standard error of calibration

Validation of both methods with two different sample sets each confirms the reliability of the methods (Table 3, Fig. 1, Fig. 2). The mean difference (absolute) between GC value and NIRS value was 4 % (C22:1) and 2 % (C18:1), respectively; but there were a few samples, which were considerably over- or underestimated (Table 4). No systematic error could be detected.

Table 3. Validation

Fatty acid	Set	SEP(1)	Bias(2)	SEP(C)(3)	r^2
C22:1	Val 1	4.182	0.54	4.195	0.648
	Val 2	3.358	0.75	3.310	0.814
C18:1	Val 1	2.276	-0.24	2.304	0.694
	Val 2	1.784	0.26	1.792	0.902

(1) standard error of performance
(2) mean difference between GC and NIRS values
(3) standard error of performance, bias-corrected

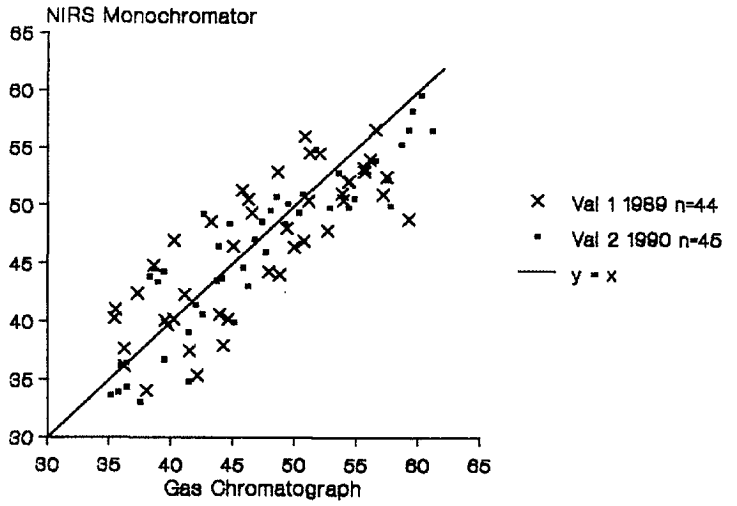

Figure 1. Determination of erucic acid (C22:1 in % of total fatty acids) by NIRS and GC

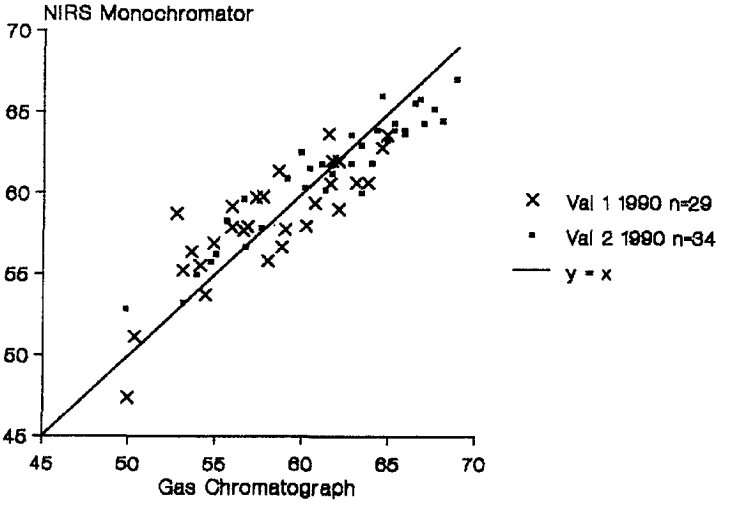

Figure 2. Determination of oleic acid (C18:1 in % of total fatty acids) by NIRS and GC

Table 4. Relative frequency of differences between results obtained from GC and NIRS (sample set=100)

Set	Difference regarding fatty acid %								
	< 3	< 4	< 5	< 6	< 7	< 8	< 9	<10	<11
C22:1									
Cal	76.8	8.2	9.6	5.5					
Val 1	40.9	15.9	18.2	11.4	11.4				2.3
Val 2	62.3	8.9	13.3	8.9	4.4	2.2			
C18:1									
Cal	78.2	14.9	3.0	4.0					
Val 1	86.2	10.3			3.4				
Val 2	91.1	8.8							

DISCUSSION

Absorption of energy in the NIR-range depends on molecule groups, but is not very specific. Since the different fatty acids have the same absorbing molecule groups ($-CH_2-$), it is difficult to determine single fatty acids quantitatively.

Experiences with different fatty acids revealed that differences in chain length may cause shifts of bands in the NIR region between 1740 and 1770 nm (Holman and Edmondson, 1956). Spectra of oleic acid and methyl-oleate have strong bands in the range from 1650 to 1780 nm (Panford and DeMan, 1990). Good agreement could be found between these results and the most important wavelengths used in the demonstrated methods.

It has been not possible to determine two fatty acids in one sample. Only the dominating fatty acid can be estimated by NIRS with the demonstrated accuracy. For erucic acid a functionality between selected wavelengths and content could be noticed - samples without erucic acid have contents from -5 to 15 % C22:1 estimated by NIRS. But high erucic acid samples with oleic acid content of about 14 % (GC) exhibit a content of > 70 % C18:1 by NIRS! So as a conclusion C18:1 determination in + rape is not practicable. Probably at the wavelengths, selected for the determination of oleic acid both fatty acids exhibit strong absorption bands. It might be supposed that the estimation of oleic acid is influenced directly by the oil content. But there is no difference between the correlation coefficients of C18:1 (NIRS) vs oil and C18:1 (GC) vs oil content (Table 5). It can be concluded that the selected wavelengths are obviously specific for oleic acid.

Table 5. Correlation between oleic acid content and oil content (C18:1 content between 39.8 % - 73.2 %)

	C18:1 (NIRS) - oil content	C18:1 (GC) - oil content	oil content min	oil content max
Cal	0.649***	0.604***	30.8	52.8
Val 1	0.582***	0.424*	33.8	51.3
Val 2	-0.190 ns	-0.240 ns	37.2	48.9

With the help of the presented methods developed for a NIRS monochromator it is now possible to determine oil, protein, glucosinolate, dry matter and oleic acid content in 0 rapeseed and same materials, and the erucic acid content instead of oleic acid in + rapeseed in less than one minute.

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REFERENCES

BENGTSSON, L. 1985. Some experiences of using different analytical methods in screening for oil and protein content in rapeseed. Fette Seifen Anstrichmittel 87: 262-265.

HOLMAN, R.T. and EDMONDSON, P.R. 1956. Near-infrared spectra of fatty acids and some related substances. Anal. Chem. 28: 1533-1538.

McGREGOR, D.I. 1988. New trends in the analytical chemistry of Brassica seed. 3rd Chemical Congress of North America, Toronto, Canada, 5.-10. Juni 1988.

MICHALSKI, K., KRZYMANSKI, J., BYCZYNSKA, B. 1987. Determination of glucosinolate in intact seeds of winter rape (B. napus) by near infrared reflectance method. Proc. 7th Int. Rapeseed Congress, Poznan, Poland, Vol. 6: 1541-1546.

PANFORD, J.A. and DeMAN, J.M. 1990. Determination of oil content of seed by NIR - Influence of fatty acid composition on wavelength selection. J. Am. Oil Chem. Soc. 67: 473-482.

WATHELET, J.-P., DARDENNE, P., MARLIER, M., SEVERIN, M. and BISTON, R. 1989. Fast analysis of quality of rapeseed by NIRS (protein, oil and glucosinolates). Proc. XII. Eucarpia Congress, Göttingen, Germany, Abstr. Vol. II: 31-3.