

## **A new NIRS method for high throughput analysis of oleic, linoleic and linolenic acid content of single seeds in oilseed rape**

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### **Abstract**

A high-throughput Near-Infrared Reflectance Spectroscopy (NIRS) method using an automated sample presentation unit for single seeds of oilseed rape and a new kind of spectrometer equipped with a photodiode array detector was developed. First analyses have been accomplished with a throughput of about 500 seeds per hour. Seeds from segregating F<sub>2</sub> populations of different origin were analysed by NIRS and gas liquid chromatography (GLC). Calibration equations were developed and validated. The coefficients of determination in cross validation were 0.81 for oleic acid, 0.76 for linoleic acid and 0.57 for linolenic acid. The ratio standard deviation/standard error in cross validation (SD/SECV) ranged from 2.4 for oleic acid over 2.1 for linoleic acid to 1.7 for linolenic acid. Using four different external validation sets, the coefficients of determination in external validation varied between 0.44 and 0.88 for oleic acid, between 0.31 and 0.72 for linoleic acid and between 0.11 to 0.33 for linolenic acid. The results show that the new high-throughput method can be applied to predict the oleic and linoleic acid content of single rapeseeds. However, the calibration seed sample set need to be extended by more seeds derived from different crosses and environments before robust results can be expected in routine analysis.

**Key words:** fatty acids, quality, single-seed NIRS, Near-Infrared Reflectance Spectroscopy, HOLL, high oleic, low linolenic, photodiode array detector

### **Introduction**

Rapeseed with a high content of oleic acid (18:1) and with a low content of linoleic (18:2) and linolenic acid (18:3) has a large potential for applications in the food and oleochemical industry (Möllers 2004). The aim of the present project is to develop a Near-Infrared Reflectance Spectroscopy (NIRS) method for high throughput analysis of oleic and linolenic acid content of single seeds in oilseed rape. Since the fatty acid composition of the seed oil is mainly determined by the genotype of the embryo, such a method could be useful for the non-destructive analysis of segregating F<sub>2</sub>-seed populations. Fatty acid composition of single rapeseeds has been successfully determined earlier by applying single seed adapters to standard NIRS equipment (Sato et al. 1998, Velasco et al. 1999).

### **Material and Methods**

**Technology:** Two innovations allow for a non-destructive high throughput determination of fatty acid composition in single seeds of oilseed rape: an automated sample presentation unit (PPM unum, constructed by Ingenieurbüro Steps, Jena, and VDLUFA, Kassel) and a new kind of spectrometer (ZEISS MCS 611). A schematic drawing of the automated sample presentation unit is shown in Figure 1. The tubes of a spindle move through a holding tank containing seeds of oilseed rape. As the tubes are connected to a vacuum pump, a single seed is aspirated by the tube (1) and transported to a set of three optic fibres (2). The outer two fibres transmit white light of a halogen lamp to the sample seed, while the inner fibre transmits the reflected light to a spectrometer. The spectrometer is equipped with a novel photodiode array detector that is able to record the full spectrum (1340–2000 nm) at once within milliseconds. Finally, the single seed is stripped off into a downspout (3) and arrives at a free position on a microtiter tray, which then moves automatically to the next free position.

**Calibration development:** Four breeding companies involved in this project provided seeds for NIRS analysis: In total 1051 single seeds from different crosses segregating for oleic, linoleic and linolenic acid were scanned by NIRS (1340–2000 nm) and subsequently analysed by gas liquid chromatography (GLC) for their fatty acid content. NIRS calibrations were developed using WinISI 1.61 software. Between 1014 and 1018 sample spectra were used for development of calibration equations.

**External validation:** In order to evaluate the performance of the calibration equations in routine analysis, cross validations and external validations have been accomplished. For external validations, spectra from seeds obtained from the four different breeding companies were separately removed from the primary calibration set and used as four different validation sets (V1-V4). New calibrations (C1-C4) were developed in each case from the remaining spectra. These calibrations were used to predict the fatty acid contents of the validation sets not represented in the calibration.

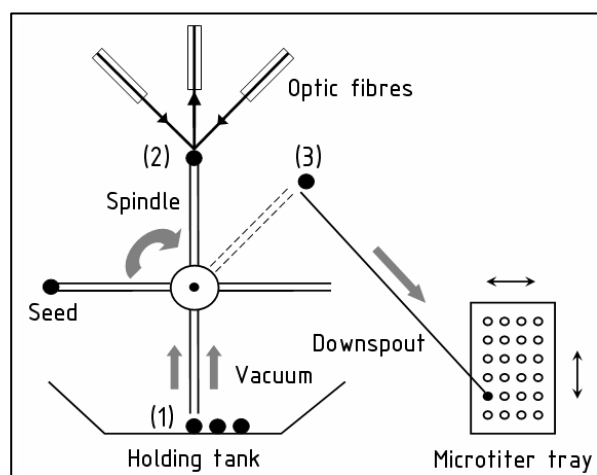


Figure 1. Principle of the automated sample presentation unit (PPM unum) for single rapeseeds constructed by Ingenieurbüro Steps (Jena) and VDLUFA (Kassel)

## Results

In the complete calibration seed sample set the oleic acid content ranged from 43% to 89% with a standard deviation of 5.3% (Tab. 1). The standard error of cross validation (SECV) was 2.22 and the coefficient of determination in cross validation ( $r(cv)^2$ ) was 0.81 (see also Fig. 2). The ratio SD/SECV was 2.4. Linoleic acid content ranged from 2.5% to 29% with a standard deviation of 4.8%. The SECV was 2.31 and the coefficient of determination in cross validation was 0.76. Linolenic acid content ranged from 1% to 13% with a standard deviation of 1.6. The SECV was 0.96 and the coefficient of determination in cross validation was 0.57. The analyses have been accomplished with a throughput of about 500 seeds per hour.

Table 1. NIRS calibration and cross validation statistics for the percentage of oleic (18:1), linoleic (18:2) and linolenic acid (18:3) in the oil of intact single seeds of oilseed rape. Values as percentage of total fatty acids. Mean, standard deviation (SD) and range are GLC data. SEC standard error of calibration, SECV standard error of cross validation,  $r(c)^2$  coefficient of determination in calibration,  $r(cv)^2$  coefficient of determination in cross validation

Fatty acid	Mean	SD	Range	SEC	$r(c)^2$	SECV	$r(cv)^2$	SD/SECV
18:1	76.3	5.3	55.3 - 88.9	2.11	0.83	2.22	0.81	2.4
18:2	13.5	4.8	2.5 - 28.9	2.16	0.79	2.31	0.76	2.1
18:3	3.8	1.6	1.1 - 12.9	0.93	0.59	0.96	0.57	1.7

For external validation between 147 and 309 seed spectra were removed from the primary calibration set (Tab. 2). The calibrations developed with the remaining seed spectra showed for all three different fatty acids similar values for the SECV and for the coefficients of determination in cross validation; for comparison see Table 1 and Table 2. For all three fatty acids, the standard deviations of the four validations sets were lower compared to the primary calibration sets. For oleic and linoleic acid the standard errors of prediction corrected for the systematic error (SEP(C)) were in most cases much higher than the standard errors of calibration (SEC) of the primary calibration set. The SEP(C) was also large in comparison to the standard deviations of the

validation sets. For oleic acid, the coefficients of determination in external validation ( $r(e)^2$ ) ranged between 0.44 and 0.88 for the four different validation sets. For linoleic and linolenic acid the coefficients of determination in external validation ranged from 0.31 to 0.72 and from 0.11 to 0.33, respectively.

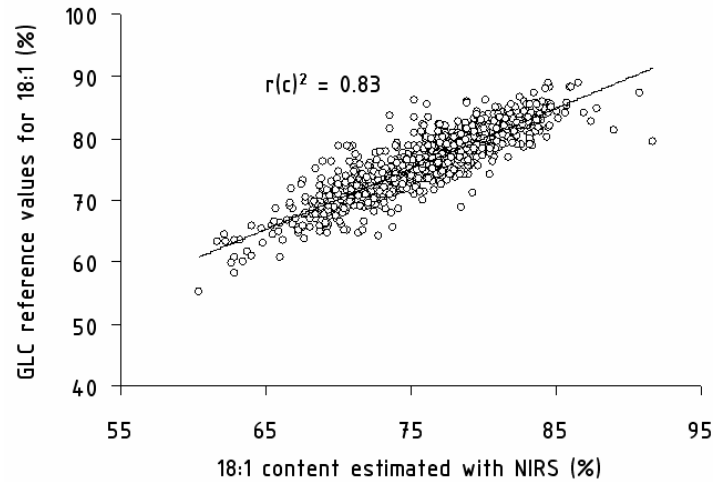


Figure 2. Relationship between oleic acid (18:1) content as determined by GLC and by NIRS

Table 2. NIRS external validation statistics for the percentage of oleic (18:1), linoleic (18:2) and linolenic acid (18:3) in the oil of intact single seeds of oilseed rape. Values as percentage of total fatty acids. *Mean* and standard deviation (*SD*) are GLC data. *SEP(C)* standard error of prediction corrected by systematic error,  $r(e)^2$  coefficient of determination in external validation

Fatty acid	Calibration			External Validation					
	Name	SECV	$r(cv)^2$	Name	N	Mean	SD	SEP(C)	$r(e)^2$
18:1	C1	2.2	0.82	V1	295	75.5	4.9	2.7	0.71
	C2	2.3	0.81	V2	309	80.4	4.1	3.5	0.44
	C3	1.9	0.86	V3	300	74.2	4.2	3.7	0.47
	C4	2.1	0.80	V4	147	76.0	4.6	2.9	0.88
18:2	C1	2.3	0.79	V1	295	14.6	4.3	2.9	0.60
	C2	2.6	0.73	V2	309	10.6	3.7	3.2	0.35
	C3	2.1	0.76	V3	300	14.0	3.4	3.9	0.31
	C4	2.1	0.76	V4	147	13.8	4.1	3.8	0.72
18:3	C1	1.0	0.51	V1	295	4.0	0.9	1.1	0.23
	C2	1.1	0.36	V2	309	2.6	0.6	0.9	0.14
	C3	0.9	0.60	V3	300	4.4	0.7	1.2	0.33
	C4	0.9	0.65	V4	147	3.9	0.8	2.0	0.11

## Discussion

The preliminary results from this study show that the new high-throughput NIRS method can in principle be used to predict the oleic and linoleic acid content of single rapeseeds. However, the standard error in cross validation still appears high in comparison to the standard deviation ( $SD/SECV=2.4$  and  $2.1$ ), indicating that an efficient selection will be possible only in  $F_2$  seed populations showing a large variation (Fontaine et al. 2001). The comparison of the coefficient of determination in calibration,  $r(c)^2$ , with the coefficient of determination in cross validation,  $r(cv)^2$ , in Table 2 shows similar values, indicating a spectral homogenous seed population. Nevertheless, in external validation using different subsets of the primary calibration set, mostly inferior results were obtained, although calibration statistics ( $SECV$ ,  $r(cv)^2$ ) were similar. This indicates that the calibration set need to be extended by including more sample spectra from seeds derived from different crosses and different environments to become more robust in routine applications.

The development of a reliable calibration equation for linolenic acid showed to be more difficult. The  $SECV$  was high in comparison to the standard deviation ( $SD/SECV=1.7$ ) and the results obtained in external validation

show that a selection in routine applications will not be possible. Velasco et al. (1999) also reported inferior calibration statistics for linolenic acid in comparison to oleic acid, applying standard NIRS and single seed adapter ring cups. It needs to be shown whether the calibration can be improved and be made more robust by including more seed material from different crosses and environments.

### Conclusions

The results obtained so far show that the new high-throughput NIRS technology is a promising method for the fast and non-destructive estimation of the fatty acid content of single seeds of oilseed rape. The analyses have been accomplished with a throughput of about 500 seeds per hour. However, it should be kept in mind that a considerable amount of time is also needed for handling, storage and checking the spectra for correct sorting, outliers, etc. More spectra from individual seed samples derived from different crosses and environments need to be included in the calibrations to make them more robust in validations and practical applications.

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