Czech J. Genet. Plant Breed., 42, 2006 (4): 132–136

Estimation of Fatty Acid Content in Intact Seeds of Oilseed Rape (*Brassica napus* L.) Lines Using Near-Infrared Spectroscopy

Radoslav KOPRNA[1], Pavel NERUŠIL[3], Oldřich KOLOVRAT[1], Vratislav KUČERA[2] and Alois KOHOUTEK[3]

[1]OSEVA PRO Ltd., dep. Research Institute of Oilseed Crops at Opava, Opava, Czech Republic; [2]Research Institute of Crop Production, Prague-Ruzyně, Czech Republic; [3]Research Station of Grass Ecosystems at Jevíčko, Research Institute of Crop Production, Prague-Ruzyně, Czech Republic

Abstract: Based on NIRS (near infrared reflectance spectroscopy) measurements carried out in a collection of 262 samples of winter oilseed rape with a different content of fatty acids (FA) in oil, calibration equations for the laboratory instrument Foss-NIRSystem 6500 were developed. Calibration was focused on the possibility of screening seed samples of different composition of oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) using NIRS analysis. The reference method was gas chromatography (GC). The content of FA in segregating F_2 generations after crossing between lines with different contents of FA and lines with standard content of FA in oil ranged from 32.3 to 82.0% for C18:1, 10.2–26.8% for C18:2 and 3.3–11.8% for C18:3. The verification of a validation equation in 50 randomly selected samples of F_2 generation in the year 2006 proved high correlation coefficients (r) between NIRS analysis and GC values, r = 0.86 for C18:1, r = 0.82 for C18:2 and r = 0.85 for C18:3. Non-destructive NIRS analysis enables rapid and reliable selection of materials with different composition of FA in the seed of oilseed rape (lines with desirable high content of C18:1 and low content of C18:3).

Keywords: winter oilseed rape; fatty acids; oleic acid; linoleic acid; linolenic acid; NIRS calibration

The main fatty acids (FA) in oil of currently grown cultivars of oilseed rape are oleic acid – C18:1 (59–68%), linoleic acid – C18:2 (17–21%) and linolenic acid – C18:3 (7.8–10%). Oil quality may be improved by developing cultivars with a reduced content of polyunsaturated FA and an increased content of oleic acid (Scarth & McVetty 1999).

The reduction of linolenic acid to 2–3% in rapeseed and the increase of oleic and linoleic acid to 80% and 35%, respectively, has been an important breeding objective. Winter oilseed rape, with high content of C18:1 and low content of C18:3 acid is of interest for nutritional as well as for industrial purposes due to better frying stability of oil (Schierholt & Becker 2001; Carré *et al.* 2003). A lower content of linolenic acid has a positive effect on the oxidation stability of oil. The same criteria are demanded for bio-diesel usage. Linolenic acid is a component of rapeseed oil that is readily oxidized, which results in reduced frying stability and shelf life of the oil (Somers *et al.* 1998).

In winter oilseed rape and other *Brassica* crops there is a natural variability in the content of par-

Supported by the Ministry of Agriculture of the Czech Republic, Projects No. 1G46061 and No. 27006001.

ticular fatty acids. The qualitative characteristics of 253 genotypes of *Brassica napus*, 99 genotypes of *B. campestris* and 49 genotypes of *B. juncea* collected from various parts of China in 1987–1989 were evaluated (Liu & Tang 1992). The content of erucic acid, oleic acid, linoleic acid and palmitic acid in the rapeseed (*B. napus)* was in the range of 0.18–57.33%, 6.7–66.2%, 11.2–33.4% and 1.5–8.9%, respectively.

The inheritance of linolenic acid content in oil is polygenic and is also strongly influenced by the environment (Somers *et al*. 1998). The coefficients of heritability (h^2) for the content of C16:0 (palmitic acid) and C18:0 (stearic acid) were fairly high in phytotron tests (h^2 = 0.89–0.93) (Pleines & Friedt1988). Analysis of variance (in high oleic mutant group) revealed a high heritability for oleic acid content, h^2 = 0.99. Subdividing the doubled haploid population into a high (> 64% C18:1) and a low (< 64% C18:1) oleic acid class showed high heritability (h^2 = 0.94) for C18:1 content within both the high and the low oleic acid types. The oleic acid content in high oleic types of winter oilseed rape was environmentally stable at the three locations tested (Schierholt & Becker 2001). The content of linoleic and linolenic acids is controlled by two major genes with additive effects. However, minor genes also appeared to be expressed (Jourdren *et al.* 1996).

A number of authors (van de Voort *et al.* 1992; Williams & Sobering 1992; Sato 2002; Míka *et al.* 2003) confirmed the suitability of NIRS (Near Infrared Reflectance Spectroscopy) as a non-destructive, rapid, cheap and relatively accurate method for determining the seed quality (content of glucosinolates, oil, proteins, dry matter and fatty acids) of oilseed crops. In all measured components including FA high significance of the correlation between NIRS and the reference method (GC) was found (Velasco *et al.* 1998, 1999a, b). The aim of this study was calibration of NIRS instrument for the effective screening selection of oilseed rape lines with different fatty acid contents for utilization in Czech breeding programme.

MATERIAL AND METHODS

Materials

For calibration of NIRS intact seed samples of winter oilseed rape breeding materials were used (Table 1). All plants were isolated with pollen-proof non-woven fabric at the blooming time. For gas chromatography (GC) analysis and NIRS calibration the same seed lot of isolated plants was used.

To derive the calibration equation for the prediction of the FA content – oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3), three groups including totally 262 samples (conditioned seeds of 92–94% DM) from three harvest years (2001, 2004 and 2005) were used. Seed samples from the

Table 1. Characteristics of seed samples used for NIRS calibration from the harvest years 2001, 2004 and 2005 (reference method GC)

Harvest year of calibration samples	Constituent (fatty acid)	*n*	Mean (% in oil)	Range (% in oil)
	C18:1	95	66.6	32.3–82.0
2001 (F_2 generation)	C18:2	95	18.7	10.2–26.8
	C18:3	95	7.4	4.2–11.8
	C18:1	91	53.0	23.7–72.8
2004 (hybrid generation)	C18:2	91	17.9	10.2–30.6
	C18:3	91	8.7	6.1–12.3
	C18:1	76	70.2	60.5–75.3
2005 (F_2 generation)	C18:2	76	16.5	11.4–24.2
	C18:3	76	6.0	3.3–8.5
	C18:1	262	62.9	23.7–82.0
Total 2001–2005	C18:2	262	17.8	10.2–30.6
	C18:3	262	7.5	3.3–12.3

Table 2. Validation sample set 2006 from RIOC in Opava (reference method GLC)

Constituent (fatty acid)	*n*	Mean (% in oil)	Range (% in oil)
C18:1	50	71.8	63.6–80.0
C18:2	50	15.3	7.6–20.6
C18:3	50	4.8	2.2–7.1

year 2001 came from the plants of the segregating F_2 generation of crosses between lines with different composition of FA (OPMK-2107 × OPMK-2166). Seed samples from the year 2004 came from crosses between original self-incompatible lines and domestic breeding lines. Samples from the year 2005 came from the plants of the F_2 generation of crosses between lines with different composition of FA (OP-1016 × OP-BN-08).

For calibration verification, 50 randomly selected seed samples from the segregating F_2 generation were used. They came from the cross between the line with a modified content of FA (OP-1016) and cultivars (Liprima, Madrigal, Ontario, Action, Viking, Olpop, Jesper, OP-BN-07, Oponent, Oksana and Lisek) with the standard content of FA in oil (Table 2).

Determination of the content of FA in oilseed rape seeds by the reference method of gas chromatography (GC)

Preparation of methyl esters of fatty acid. Sodium methoxide (5 ml) was added to approximately 0.5 g of extracted oil. The mixture was boiled under nitrogen bubbling for 15 min in an esterification unit consisting also of a reflux. Then 2 ml of 3.1N solution of dry hydrogen chloride in methanol was added, and the solution was boiled for another 10 min (Kolovrat 1985). Approximately 40 ml of water was added to this sample, and the mixture was extracted twice using 5 ml of petroleum ether. Joint petroleum ether extracts were washed with water into the neutral reaction and at a temperature of 50°C they were reduced to a 1/5 of the volume. The prepared sample was used for coating on the gas chromatograph.

Chromatographic determination

The analysis was carried out on the gas chromatograph CHROM 5 (Laboratorní přístroje Praha, ČSSR) with a FID detector on the glass column 2.5 m long and an internal diameter of 3 mm, filled with stationary phase GP 3% SP-2310/2% SP-2300 on the carrier Chromosorb WAW 100/120 mesh using this thermal program: 190°C (2 min), 2°C per min to 220°C. For the chromatographic record, the TZ 4200 recorder was used.

NIRS calibration

Seed samples were measured using the instrument Foss-NIRSystems 6500 (Company NIRSystems, Inc., Silver Spring, USA), in small circular cups in two parallel replications using the software WinISI

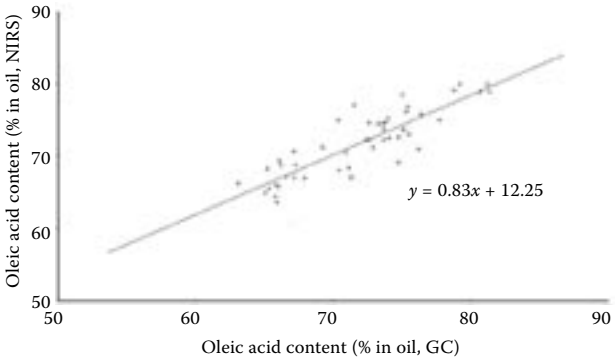

Figure 1. Validation of calibration equation re06mk.eqa for oleic acid content in the intact oilseed rape seeds

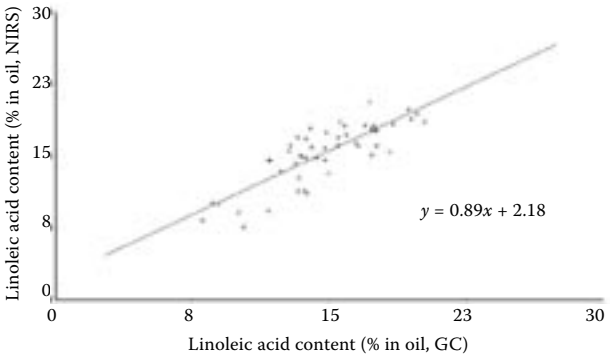

Figure 2. Validation of calibration equation re06mk.eqa for linoleic acid content in the intact oilseed rape seeds

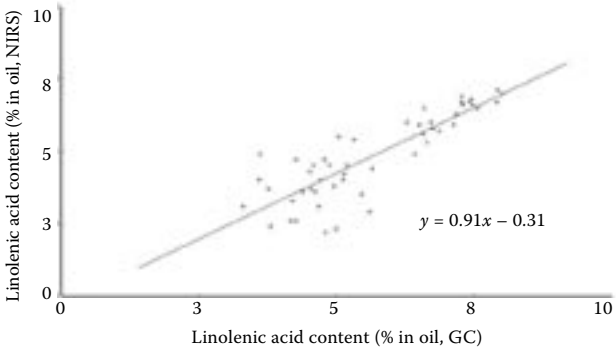

Figure 3. Validation of calibration equation re06mk.eqa for linolenic acid content in the intact oilseed rape seeds

II, vers. 1.50. Sample scanning was performed in a reflectance mode with the range of wavelengths from 400 to 2500 nm and a step of 2 nm. For mathematical processing of the calibration equation a regression method Modified PLS, scatter SNV and Detrend, math treatment 2.5.5.1 (Shenk 1990) were used. The accuracy of prediction of this calibration equation re06mk.eqa was verified on an independent validation set of 50 samples of breeding materials from the harvest year 2006 (Table 2).

RESULTS AND DISCUSSION

The assessed content of the FA (C18:1 = 32.3 to 82.0%, C18:2 = 10.2–26.8%, C18:3 = 3.3–11.8%) in the oil of plants of the segregating F_2 generation (Table 1) according to GC suggests sufficient genotype variability. These results correspond with the statement of Liu and Tang (1992) about the existence of natural variability in the content of FA in *Brassica* crops.

In validation of the calibration equation to assess C18:1 content, favourably low standard error of prediction (SEP) of 2.39% was found (Figure 1). This value of SEP enables to use NIRS prediction based on this equation for reliable detection of genotypes with a high content of C18:1 (above 70% in oil). The low value of SEP (1.89%) for C18:2 offers an opportunity for effective selection of genotypes with the content of this FA below 15% in oil (Figure 2). In validation to assess the C18:3 content SEP value 1.13% enables effective selection of lines with the content of this FA below 5% (Figure 3). The values of obtained statistic data for the FA are presented in Table 3.

The validation for C18:1, C18:2 and C18:3 content demonstrated fair relationships of NIRS analyses to the reference method GC (Table 3). Correlation coefficient was $r = 0.86$ for C18:1, $r = 0.82$ for C18:2 and $r = 0.85$ for C18:3 (all significant at $P \leq 0.001$). The obtained high values of correlation coefficients are in accordance with the results presented by Velasco *et al.* (1998, 1999a). A comparison of cross validation of calibration re06mk.eqa with the reference method GC revealed even a higher value of the correlation coefficient for the content of C18:2 and C18:3, compared with the value reported by Velasco *et al.* (1999a).

These results confirm the conclusions drawn by Williams and Sobering (1992), Velasco and Becker (1998), Velasco *et al.* (1999a) that NIRS analysis may facilitate effective selection of oilseed rape genotypes with the desired content of FA in oil similar to other significant parameters of quality (Míka *et al.* 2003). NIRS method enables the analysis of intact rape seeds in an extremely short time without loss of their germination capacity. This allows the processing of selected samples immediately after harvest and sowing of materials selected according to quality criteria in the same year when they are harvested. The advantages of NIRS analysis become more apparent with large sample collections of the same character.

The influence of a harvest year may decrease prediction accuracy in some cases, especially when

Table 3. Validation of the calibration re06mk.eqa for FA in rapeseeds

Constituent (fatty acid)	SEP[1]	Bias	SEP(C)[2]	Slope	r^3	n
C18:1 (% in oil)	2.39	–0.13	2.42	0.83	0.86***	50
C18:2 (% in oil)	1.89	0.53	1.84	0.89	0.82***	50
C18:3 (% in oil)	1.13	–0.84*	0.77	0.91	0.85***	50

[1]standard error of prediction; [2]standard error of prediction corrected for bias; [3]correlation coefficient (***$P \leq 0.001$)

the course of weather is significantly different from the year when the samples from which the calibration was calculated were collected. The necessity of recalibration for the given harvest year must be tested and statistically evaluated by the programme WinISI II, version 1.50 before using the existing equation. However, if our calibration equation is already sufficiently robust, the harvest year influence does not show very much and recalibration is not necessary.

References

Carré P., Dartenuc C., Evrard J., Judde A., Labalette F., Raoux E., Renard M. (2003): Frying stability of rapeseed oils with modified fatty acid composition. In: Proc. 11th Int. Rapeseed Congr., Copenhagen, 540–543.

Jourdren C., Simonneaux D., Renard M. (1996): Selection on pollen for linolenic acid content in rapeseed *Brassica napus* L. Plant Breeding, **115**: 11–15.

Kolovrat O. (1985): Esterification of the fatty acids of rapeseed oil. Rostlinná výroba, **31**: 777–782. (in Czech)

Liu R.J., Tang Z.J. (1992): Study on quality characters in rapeseed germplasm resources. Journal of Southwest Agricultural University, **13**: 537–542.

Míka V., Tillmann P., Koprna R., Nerušil P., Kučera V. (2003): Fast prediction of quality parameters in whole seeds of oilseed rape (*Brassica napus* L.). Plant, Soil and Environment, **49**: 141–145.

Pleines S., Friedt W. (1988): Breeding for improved C-18 fatty acid composition in rapeseed (*Brassica napus* L.). Fat Science Technology, **90**: 167–171

Sato T. (2002): New estimation method for fatty acid composition in oil using near infrared spectroscopy. Bioscience, Biotechnology and Biochemistry, **66**: 2543–2548.

Scarth R., McVetty P. (1999): Designer oil canola a review of new food-grade *Brassica* oils with focus on high oleic, low linoleic types. In: Proc. 10th Int. Rapeseed Congr., Canberra-Australia. (CD-ROM)

Schierholt A., Becker H.C. (2001): Environmental variability and heritability of high oleic acid content in winter oilseed rape. Plant Breeding, **120**: 63–66.

Shenk J.S. (1990): Standardizing NIRS instruments. Proc. 3rd Int. Conf. Near Infrared Spectroscopy. Agriculture Research Centre Publication, Brussels, 649–654.

Somers D.J., Friesen K.R.D., Rakow G. (1998): Identification of molecular markers associated with linoleic acid desaturation in *Brassica napus*. Theoretical and Applied Genetics, **96**: 897–903.

van de Voort F.R., Sedman J., Emo G., Ismail A.A. (1992): Rapid and direct iodine value and saponification number determination on fats and oils by attenuated total reflectance. Fourier transform infrared spectroscopy. Journal of the American Oli Chemists' Society, **69**: 1118–1121.

Velasco L., Becker H.C. (1998): Estimating the fatty acid composition of the oil in intact-seed rapeseed (*Brassica napus* L.) by near-infrared spectroscopy. Euphytica, **101**: 221–230.

Velasco L., Goffman F.D., Becker H.C. (1998): Variability for the fatty acid composition of the seed oil in a germplasm collection of the genus *Brassica*. Genetic Resources and Crop Evolution, **45**: 371–382.

Velasco L., Möllers C., Becker H.C. (1999a): Estimation of seed weight, oil content and fatty acid composition in intact single seeds of rapeseed (*Brassica napus* L.) by near-infrared reflectance spectroscopy. Euphytica, **106**: 79–85.

Velasco L., Peréz-Vich B., Fernández-Martínez J.M. (1999b): Crop quality utilization. Crop Science, **39**: 219–222.

Williams P., Sobering D. (1992): Whole-seed grain analysis by near-infrared transmittance and reflectance: a comparison. In: Hildrum, K.I. et al. (eds.): Bridging the Gap between Data Analysis and NIR Application. Near Infra-red Spectroscopy. Ellis Horwood, Chicester, 441–446.

Received for publication September 27, 2006
Accepted after corrections November 26, 2006

Corresponding author:

Ing. Radoslav Koprna, OSEVA PRO s.r.o., o.z. Výzkumný ústav olejnin Opava, Purkyňova 10, 746 01 Opava, Česká republika
tel.: + 420 553 624 160, fax: + 420 553 624 388, e-mail: opava@oseva.cz