Inserts for FOSS NIRS 6500 spinning ring cups

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Abstract

Near infrared reflectance (NIR) analysis has proven be a very efficient tool for elevating seed quality for rapeseed and mustard breeders. Calibrations for oil, protein, fatty acids, glucosinolate, fibre, chlorophyll, etc. are routinely utilized by many laboratories supporting rapeseed breeding efforts around the world. The FOSS NIRS 6500 instrument is a popular model. With the use of an autoloader and the spinning ring cup attachment the analysis is simple and rapid. Intact seed can be utilized which makes it a non-destructive method. The spinning ring cup holds about 4-5 grams of seed and is therefore useful for examining samples harvested from large or small plots and individual 3 meter rows. However the minimum sample size of about 4 grams limits its usefulness for samples from individual plants harvested from field plots or from the greenhouse. We found the inserts sold by FOSS for these spinning ring cups impractical and too expensive for routine use, so we designed our own. A local machine shop made three sizes for us with the following nominal sizes: two gram, one gram and 0.5 gram (Cost: $6.00 each). The inserts allow intact seed samples from individual plants to be analyzed by the FOSS NIR instrument. The insert size choice depends on the size of the smallest samples.

Key words: NIR, sample cup inserts, single plant analysis, oilseeds

Introduction

NIR is widely accepted by oilseed breeders and chemists, replacing several traditional methods of analysis. NIR is used to predict oil, protein, glucosinolate (GSL), fatty acids, fiber and chlorophyll on intact seed samples of rapeseed and mustard (Biston et al. 1987, Daun et al. 1994, Font et al. 2003, Renard et al. 1987, Sato et al. 1998, Velasco et al. 1998, Williams and Sobering 1993, and others). The FOSS NIRS 6500 instrument is popular and its spinning cup attachment allows for simple, rapid analysis. Individual plants harvested from the field or greenhouse often yield an amount of seed which is insufficient to be analyzed in the full size cups. In this paper we describe inserts which can used to allow analysis of these plants.

Material and Methods

Cup Inserts

Inserts were designed for standard sample cups utilized by the autoloader attachment of our NIR (NIRS system model 6500, FOSS NIRSystems, Silver Springs, MD, USA). Aluminum inserts rings were designed to exactly fit the inside of the sample cups. They were 38 mm in diameter by 9 mm thick with an inside diameter of 13 mm, 20 mm and 25 mm, to accommodate approximately 0.5 gram, 1 gram or 2 grams of seed respectively. A machine shop (Nutana Machine Ltd., 2615 1st Ave. N, Saskatoon, SK, Canada, Fax: 1-306-242-2671) manufactured 100 of each size (see Fig. 1). The inserts are held in place using a strip (25 mm by 6 mm) of black foam window insulation attached to the side of the insert. The backings for the inserts are 13, 20, and 25 mm Tegrabond septa (Chromatographic Specialties Ltd.). The inserts are easily removed from the cups when not needed.

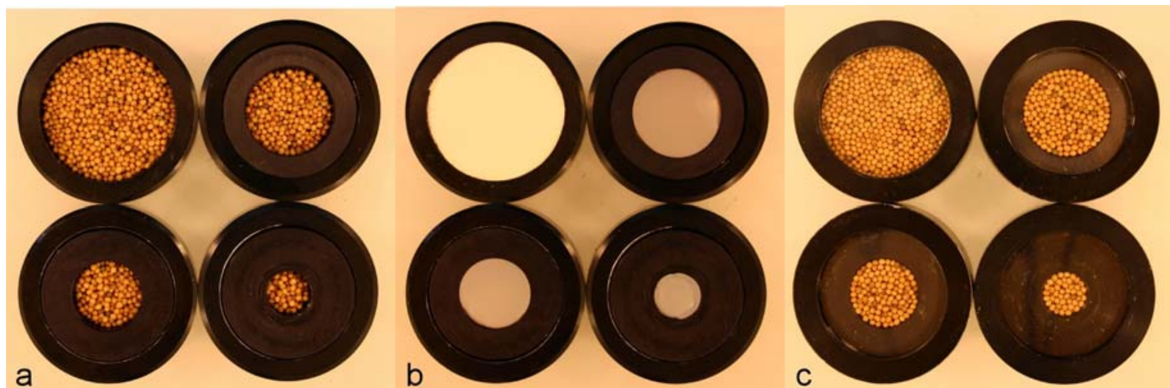

Figure 1: a) inserts filled with seed. b) inserts with backing on. c) inserts front view. Upper left: original cup, Upper right: 2 gram insert. Lower left: 1 gram insert. Lower right: 0.5 gram insert.

Seed Material and NIRS analysis

Seed samples used for calibration of the NIRS instrument were selected from seven oilseed species that normally enter

our laboratory for analysis including: *Brassica napus, Brassica juncea, Brassica rapa, Brassica carinata, Sinapis alba, Camelina sativa and Linum usitatissimum.* Two sets of samples were created, one for calibration (1365 samples) and a separate set for validation (272). Intact seed samples, dried at 40°, were in placed in the cups, scanned and the entire spectral range of the instrument collected. The same seed samples were scanned again using the insert rings and spectra collected. Equation generation and data transformation were done using WINISI III (version 1.50e) software. The modified partial least squares (MPLS) regression method was chosen as well as scatter and detrend correction. All wavelengths were utilized and a second derivative mathematical treatment (2,4,4,1) was applied. Calibrations were created for oil, protein and insoluble fibre content, seed color, individual fatty acids and GSL contents, both total and individual.

Reference Methods

Oil content, protein content, fibre content, seed color, fatty acid composition and GSL content were determined by reference methods available in the laboratory. Oil content was estimated on 20-25 g intact seed samples with a pulsed NMR instrument (Bruker Minispec, 10 MHz magnet, 40 mm probe assembly, Bruker Optics Ltd, Milton, Ontario, Canada), calibrated according to manufacturer's instruction and corrected using standards for each species. Protein content was determined with an LECO FP-428 on 0.5 gram intact seed samples. Acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (NDF) contents were measured with an ANKOM^{200} Fiber analyzer (ANKOM Technology, Macedon, NY, USA). The manufacturer's standard procedures were followed. Seed color (whiteness index, WI) was determined with a HunterLab Miniscan colorimeter. Oil, protein and fibre contents are reported as percent of dry seed.

Seed fatty acid composition was determined by gas chromatography (GC). Three gram seed samples were ground with 14 mm stainless steel balls in 4 ml hexane in 20 ml PET scintillation vials (Wheaton) placed in an Eberbach reciprocating shaker for 1 hour, after which a 10 µl aliquot was placed in a GC autosampler vial containing 50 µl hexane and treated with 100 µl 0.8% metallic sodium in methanol (Thies, 1971). After 15 minutes 50 µl 0.2 M $NaPO_4$ pH 7 was added, methanol and hexane evaporated under a stream of air (1 minute), and then 0.5 ml of heptane was added. The samples were injected into an FID equipped GC (model 6890 Agilent Technologies, Santa Clara, CA, USA; column: HP-Innowax, 7.5 m × 0.25 mm × 0.5 µm, hydrogen, constant flow, 1.3 ml/min; injector: 280°, 1 µl, split 1:40; oven: 190 - 240°, 20°/min., final time 0.6 min.; detector 300°). Oleic (18:1), linoleic (18:2), linolenic (18:3) and erucic (22:1) are expressed as percentages of all fatty acids detected. Two additional parameters are calculated, oil hydrogen density (HD) and iodine value (IV).

The GSL content of the seed was determined by GC of trimethylsilylated derivatives of desulphated GSLs by a modification of the method of Thies (1976). Samples (1 g) were ground with steel balls in a mixture of 5 ml methanol, 2 ml 1 mM benzyl GSL, 0.2 ml barium/lead acetate in 20 ml PET vials, then placed in a reciprocating shaker for 1 hour. After centrifugation 1 ml was pipetted onto 0.125 ml DEAE-Sephadex A-25 in Bio-Rad microcolumns. The columns were washed with 70% methanol, 6% acetic acid, water and 0.02 M pyridine actetate pH 5.8. Then 0.05 ml of arylsulfatase (type H-1, *Helix pomatia*, Sigma Aldrich) was added. After overnight incubation the desulpho-GSLs were eluted with water, evaporated to dryness at 60° under a stream of air, derivatized with 0.3 ml silylation reagent (pyridine: N,O-bis(trimethylsilyl)-acetamide: chlorotrimethylsilane, 18:10:1) and injected into an FID equipped GC (column: DB-1, 15 m × 0.25 mm × 1.0 µm, hydrogen, constant flow, 1.3 ml/min.; injector: 270°, 1 µl, split 1:30; oven: 260 - 320°, 20°/min, final time 0.8 min.; detector 340°). Individual and total GSLs are expressed in µmoles/gm seed.

Table 1: Summary of reference method data for calibration and validation sample sets

Constituent	Calibration Set						Validation Set					
	N	Mean	Min.	Max.	StD	Rge	N	Mean	Min.	Max.	StD	Rge
Oil	1057	42.1	24.8	52.9	5.3	28.1	212	41.7	25.9	52.5	5.4	26.6
Protein	1350	28.5	14.9	42.7	4.0	27.8	271	28.8	18.9	40.8	3.9	21.9
NDF	460	16.7	9.5	25.0	2.6	15.4	91	16.3	10.4	23.6	2.9	13.2
ADF	456	11.2	5.1	20.8	2.7	15.7	91	10.7	5.6	18.6	2.6	13.0
ADL	460	2.7	0.3	9.4	1.8	9.2	91	2.4	0.4	7.7	1.7	7.3
18:1	707	44.2	6.2	76.5	22.1	70.3	139	44.1	6.1	73.9	21.7	67.8
18:2	707	19.9	7.9	75.6	5.3	67.7	139	21.2	8.9	74.6	8.8	65.7
18:3	707	12.9	1.3	69.4	11.1	68.0	139	12.5	1.8	62.6	10.9	60.8
22:1	707	9.4	0.0	55.3	16.1	55.3	139	9.6	0.0	55.9	16.6	55.9
IV	707	119.5	92.2	210.5	19.3	118.3	139	120.0	96.7	201.1	19.2	104.4
HD	707	11.64	10.97	11.97	0.16	1.00	139	11.64	11.03	11.95	0.16	0.92
Sinigrin	598	56.1	0.0	169.3	59.0	169.3	121	54.2	0.0	167.2	58.9	167.2
Sinalbin	598	6.0	0.0	194.7	30.1	194.7	121	8.2	0.0	204.9	36.2	204.9
aGSL	598	67.2	0.1	170.2	56.0	170.1	121	65.6	0.0	168.1	56.1	168.1
tGSL	598	79.7	1.7	200.5	56.0	198.8	121	78.8	0.8	212.0	57.6	211.3
WI	943	-17.6	-42.7	2.8	12.9	45.4	184	-19.0	-41.2	2.2	12.8	43.4

* N – number of samples with reference method data; StD – standard deviation; Rge – range of values found; aGSL – total aliphatic glucosinolates; tGSL – total glucosinolate content

Results

Reference data is shown in Table 1. For all constituents a broad range of values is observed, covering the expected ranges to be seen at our laboratory. The NIRS statistics of the second derivative equations for the full cup and 1 gram size are shown in Table 2. A good correlation between the NIRS prediction and reference data is found for most of the constituents for both

the standard cup and the reduced sample size using the 1 gram inserts. Generally there is little or no decline in NIR prediction efficiency between the full size cup and the inserts as estimated by RSQ and SEP values. The bias and slope of the equations are also mostly acceptable. The RPD and RER values (Williams and Sobering, 1993) for oil, protein, 18:1, 18:3, IV, HD, GSLs and seed colour indicate that the NIR predictions either with full size cup or the 1 gram insert may be used as a selection tool for breeders. Of the fatty predictions, HD and IV are the strongest. Calibrations for 18:2 and the insoluble fibre constituents are less impressive, but ADL is certainly useful for breeding purposes.

Table 2: NIR validation set statistics for the second derivative equations for the full size cups and 1 gram inserts

Constituent	Full size cups						1 gram inserts					
	SEP	Bias	Slope	RSQ	RER	RPD	SEP	Bias	Slope	RSQ	RER	RPD
Oil	0.8	0.06	1.01	0.980	34.6	7.0	0.7	-0.03	1.00	0.982	36.8	7.5
Protein	0.6	0.02	1.00	0.980	39.6	7.1	0.6	0.08	1.00	0.976	36.2	6.4
NDF	1.2	0.19	1.05	0.820	10.8	2.3	1.4	0.16	1.11	0.784	9.8	2.1
ADF	1.1	0.06	0.99	0.817	11.8	2.3	1.1	0.05	0.97	0.819	11.8	2.4
ADL	0.4	0.02	1.02	0.934	16.3	3.9	0.4	0.02	0.99	0.942	17.4	4.2
18:1	2.8	-0.04	1.02	0.983	24.1	7.7	3.6	-0.16	1.00	0.973	18.9	6.1
18:2	2.9	0.55	1.24	0.929	22.7	3.0	2.5	0.33	1.13	0.934	26.6	3.6
18:3	1.1	-0.07	1.03	0.990	55.0	9.9	1.5	-0.04	1.02	0.982	41.2	7.4
22:1	2.6	0.01	1.03	0.976	21.3	6.3	2.6	0.13	1.00	0.976	21.9	6.5
IV	1.4	0.10	1.01	0.995	73.7	13.5	1.9	0.16	1.01	0.991	56.2	10.3
HD	0.01	0.00	1.03	0.993	65.9	11.5	0.02	0.00	1.01	0.989	54.3	9.5
Sinigrin	7.9	-1.07	0.98	0.982	21.1	7.4	8.4	-0.25	0.98	0.980	20.0	7.0
Sinalbin	9.3	-0.53	1.00	0.934	22.1	3.9	7.1	-0.73	0.98	0.963	29.1	5.1
aGSL	4.8	-0.36	0.99	0.993	35.2	11.7	4.0	-0.24	0.99	0.995	41.6	13.9
tGSL	10.4	-0.94	0.98	0.968	20.4	5.6	7.6	-1.42	0.99	0.983	27.6	7.5
WI	1.9	-0.13	1.01	0.977	22.3	6.6	1.8	0.01	1.01	0.981	24.4	7.2

* RSQ- coefficient of determination; SECV – standard error of cross validation; SEP – standard error of prediction; RPD –StD of validation / SEP (Williams and Sobering, 1993); RER –range of validation / SEP

Table 3: Use of NIR fatty acid 1 gram prediction in a F_2 plant population segregating for 18:1 and 18:3

Constituent	GC Data					NIR Prediction Statistics						
	N	Mean	Min.	Max.	Rge	StD	SEP	Bias	Slope	RSQ	RER	RPD
18:1	100	72.0	57.0	85.1	28.1	8.0	3.4	-0.68	0.87	0.845	8.3	2.4
18:2	100	14.7	2.8	26.0	23.2	7.7	2.1	-0.46	1.01	0.926	10.9	3.6
18:3	100	4.6	2.1	10.0	7.9	1.7	1.6	0.92	0.66	0.605	5.1	1.1
IV	100	101.6	87.4	119.0	31.6	7.6	2.1	1.23	0.90	0.960	15.1	3.6
HD	100	11.73	11.60	11.83	0.22	0.05	0.02	-0.01	0.90	0.951	14.9	3.5

As a test for the ability of the inserts to be used for fatty acid selection 940 F_2 plants of three breeding populations of *Brassica napus* canola, segregating for 18:1 and 18:3 and seed color, were processed by the NIR with the 1 gram inserts. These populations were unrelated to the samples that were used for calibration and validation sets and the plants were pre-selected for yellow seed color. 100 plants were chosen which represented the entire range of HD and IV values predicted by the NIR and included 48 considered to be candidates for advancement. These plants were analyzed for fatty acid composition by GC. In this case the sample size was 30 seeds to avoid destruction of valuable seed as much as possible. The comparison of NIR prediction values and reference method data is shown in Table 3. Because of the small sample size used for GC analysis it is to be expected that the correlation would be affected due to sampling error, but nevertheless the SEP values of all fatty acids are similar to the original validation set SEP values. There is a strong correlation of the NIR values for IV (RSQ: 0.960) and HD (RSQ: 0.951) and RER and RPD values indicate that these two can be selected for effectively by NIR. Most of the plants selected by NIR using HD and IV for advancement were also selected by GC for advancement using the complete fatty acid information valuable. Using the NIR eliminated the need to do GC analysis on all 940 plants. The correlation between NIR and GC for the individual fatty acids 18:1, 18:2 and 18:3 is less strong, but RER and RPD statistics suggest that NIR predictions based on them might also be useful.

Discussion

Under western Canadian conditions it is a frequent occurrence that individual plants will yield less than five grams of seed. For example, of 1600 plants harvested from a yellow mustard 2006 isolation only approximately 400 yielded 4 grams of seed or more meaning that probably half of the plants could not processed by the FOSS NIR using the full size cups, however with the 1 gram inserts over 90% can be. Others have reported the construction of small size cups (Sato et al. 1998) and inserts (Velasco et al. 1999) which have been used for the single seed analysis, but the inserts described here fit our analytical needs for single plant analysis. The fact that 1 gram inserts are seen to have little negative impact of the NIR predictions is probably a result of duplicating the material (anodized aluminum) used in the original full size sample cups.

Conclusions

The 1 gram inserts created for the FOSS NIRS 6500 sample cups have little negative impact on the efficacy of NIR predictions for oil, protein, fatty acids, glucosinolates, seed color and fibre. They are easy to use, inexpensive and provide breeders access to non-destructive NIR predictions for samples with limited amounts of seed (seed harvested from individual plants). The results for the 0.5 gram and 2 gram inserts are not reported here, but it can be expected that the results with them would be similar.

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