Determination of the Fatty Acid Composition of Canola, Flax, and Solin by Near-Infrared Spectroscopy

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ABSTRACT: The ability to rapidly measure key FA in oilseed crops would assist in the administration of identity-preserved systems in the grain-handling system or in selection systems in plant-breeding programs. This study shows near-infrared reflectance (NIR) spectroscopy to be a reliable method of determining FA composition in canola, flax, and solin (low-linolenic flax), for oleic acid, linoleic acid, linolenic acid, and iodine value, and to a limited extent for saturated fat. Samples from cultivar trials, harvest surveys, and export shipments were scanned on a NIRSystems 6500 spectrometer (Silver Spring, MD), and calibrations were developed and optimized using modified partial least squares. SE of prediction results for prediction sets of canola, flax, and solin, were, respectively: oleic acid (0.77, 1.03, 0.62%); linoleic acid (0.71, 1.20, 0.37%); linolenic acid (0.42, 0.62, 0.08%); saturated FA (0.23, 0.39, 0.31%); and iodine value (0.63, 0.95, 0.43 units).

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KEY WORDS: Canola, fatty acid composition, flax, NIR, solin.

Over the last 10 years, the production of oilseeds with specialty FA profiles has increased. Canola (low erucic acid rapeseed) cultivars have been developed with high oleic acid and/or low linolenic acid (Table 1). There is pressure to select flax lines with high linolenic acid for human consumption or to meet industrial specifications. This has been highlighted by the recent introduction of high α-linolenic acid (HALA) flax to meet a growing human nutritional demand (Table 1). In solin, linoleic and linolenic acids must meet certain specifications, usually a linoleic acid content of 70% or over and a linolenic acid content below 5% (Table 1). There has also been an interest in lowering the level of saturated FA in oilseed crops, especially canola (1).

Concomitant with the development of these specialty oilseeds is a need to segregate them in the grain-handling system from commodity oilseeds. Within species, canola varieties appear virtually identical, and there is no way to visually distinguish between varieties with high oleic acid and low linolenic acid and those with normal FA composition. However, these specialty oilseeds must be segregated to maintain their market value. Admixture of a small proportion of oilseeds with a specialty FA composition may not do significant harm to the normal commodity oilseeds unless the specialty FA is antinutritional in nature, such as with erucic acid. Contamination with seed having normal FA with a specialty oilseed will cause significant harm to the market value of the specialty seed if the normal oilseed is mixed in to the extent that the contracted FA limits are not met. Although the specialty oilseeds are handled in identity-preserved systems, it is still necessary to have some means of verifying the FA composition of the delivered seed to maintain control of the system. Ideally, the need would be satisfied with a rapid testing method capable of testing multiple constituents along with minimal sample preparation at the delivery point.

A number of publications have indicated that it is possible to determine the content of FA in oilseeds by NIR spectroscopy, and many of these have dealt specifically with *Brassica* oilseeds. Although work was done on the influence of FA on oil content determination in oilseeds (2), the first publication dealing with calibrations for FA on intact rapeseed dealt with oleic and erucic acids (3). Other publications for the *Brassica* species (4–8), sunflower seed (9), soybeans (10), and sesame (11) followed. The methodology is used routinely in our laboratory for determination of iodine value (IV, degree of unsaturation of the oil) in the Canadian Grain Commission's annual crop bulletin for flax (12), and the IV and linoleic and linolenic acid contents in solin (13) are based on NIR calibrations developed over the last several years.

The objective of this study was to evaluate the use of NIR to rapidly determine the FA composition of canola, flax, and solin seed, particularly to determine the oleic acid, linoleic acid, linolenic acid, and saturated FA contents and IV. The study also investigated the variation in NIR results of the absolute (mg FA/g of seed) value of FA due to the variation in oil content.

MATERIALS AND METHODS

Samples. Canola samples were from locations across western Canada, with the majority of the 1,995 samples coming from the 1999 Western Canada Canola/Rapeseed Recommending Committee Co-operative trials. The study included FA variant samples from the 1998–1999 western Canada harvest survey and 1998 cargoes. The 275 flax samples, including 36 HALA flax and 134 solin samples, were obtained from the 1997 to 2001 harvest survey, from the Agriculture and Agri-Food Canada Morden Research Station, and from Pizzey's Milling

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TABLE 1
Approximate FA Composition of *Brassica* and *Linum* Seed Variants Used in This Study[a]

Seed type	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Saturated FA[b] (%)	Iodine value
Brassica rapa L.					
Conventional	59	22	12	6	120
Brassica napus L.					
Conventional	62	21	9	7	115
Low linolenic acid	64	25	3	7	108
High oleic/low linolenic acid	72	18	3	6	98
Linum usitatissimum L.					
Flax	17	15	59	9	194
HALA flax[c]	13	11	69	7	210
Solin	16	72	2	10	142

[a] Canadian Grain Commission data; the total FA may not add up to 100% because minor FA were excluded.
[b] Sum saturated FA (14:0 + 16:0 + 18:0 + 20:0 + 22:0 + 24:0).
[c] HALA, high α-linolenic acid flax.

(Angusville, Manitoba, Canada). All seeds were free of foreign material, and the moisture content varied from 4 to 8%.

NIR measurements. Reflectance spectra for canola (log 1/*R*) were recorded at 2-nm intervals from 400 to 2500 nm with an NIRSystems 6500 scanning monochromator (FOSS NIRSystems Inc., Silver Spring, MD) using NSAS v3.53. Flax and solin samples were scanned using WinISI v1.05. The samples were scanned using the coarse sample cell holding approximately 120 g of seed when full. The sample cell was adjusted to 3/4, 1/2, or 1/4 full as required. The canola spectra were converted to WinISI v1.05, sorted by linolenic acid content, and then divided into calibration and prediction sets. The flax and solin spectra were sorted by linolenic and linoleic acid content, respectively, before splitting them into calibration and prediction sets. The WinISI software removed spectral outliers and nearest neighbors from the calibration set. Calibrations were developed using modified partial least squares, first or second derivative math treatments, and standard normal variant and detrend scatter correction. The calibrations were tested on an independent prediction set (Table 2) that included spectral outliers. The SE of prediction (SEP), coefficient of determination (r^2), and ratio of SEP to SD of the prediction set (RPD) were used to appraise the analytical efficiency of the NIR calibrations (14). SEP is the SD of differences between the reference and NIR values for an independent data set, so the smaller the SEP, the better. RPD values of 5 are ideally required for quality control, but values as low as 3 can be used for screening purposes.

Reference method. FA composition was determined by GC according to AOCS Official Methods Ch 1-91 and Ce 1-62 (15). Methyl esters were prepared from hexane extracts of seed using base-catalyzed hydrolysis and were separated and quantified by GC on a 15 m by 0.32 mm column with a 0.25 µm SUPELCOWAX 10 coating (Supelco, Bellefonte, PA). FA were expressed as percentages of total FA and as milligrams of FA per gram of seed (4). IV, a measure of the overall unsaturation of the oils, was calculated from the FA composition as determined by AOCS Recommended Practice Cd 1c-85 (15). FA content per gram of seed was derived by multiplying the relative FA by the predicted NIR oil content and factoring in the M.W. of the particular FA and the TG molecule.

RESULTS AND DISCUSSION

Canola. The NIR prediction for oleic acid (18:1) in canola (Table 2, Fig. 1) had a SEP of less than 0.8% and an RPD of 3.5. This suggested that NIR could be used as a tool for screening samples for oleic acid. Similarly, the results for linolenic acid (SEP = 0.42% and RPD = 5.8) indicated that NIR would be an excellent tool for selecting samples with different levels of linolenic acid. However, the current main interest in the canola industry is at the lower end of the linolenic acid (18:3) range (1–4%), and the calibration results had a high degree of dispersion at the low end of the range (Fig. 1). More work is required to improve the linolenic calibration in order to have confidence in the results.

The best NIR calibration result in canola was for IV with an RPD of 7.6, although this parameter is becoming less meaningful since it is so easy to obtain information on individual FA (16). It is possible, however, to discriminate a low linolenic/high oleic acid canola from a conventional canola based on the IV parameters shown in Table 1. NIR was moderately successful in determining total saturated FA with an RPD of 2.8. The total saturated FA NIR calibration equations gave better results than the individual saturated FA such as palmitic acid (16:0) or stearic acid (18:0). Part of the success of this calibration is due to the inclusion of *B. rapa* samples with their lower saturated fats, which therefore increase variability within the data set. With diminishing production of *B. rapa* varieties in western Canada, the "success" of the saturated fat calibration becomes questionable. The problem in developing a good saturated FA calibration might be overcome by including in the data set a wider range of saturated fat from *B. napus* varieties modified to give high or low saturated fats.

NIR calibrations based on milligrams FA/gram of seed or "absolute" FA content of seed gave better statistical results than calibrations based on the percentage or "relative" amounts of FA, particularly for oleic acid (Table 3). NIR calibrations based on

TABLE 2
Calibration and Prediction Data for FA Components for Seed Types Used in the Study

	Calibration set							Prediction set								
Component	*n*	Mean	SD	Min.	Max.	Range	SECV[a]	*n*	Mean	SD	Min.	Max.	Range	r^2[b]	SEP[c]	RPD[d]
Canola																
16:0	610	3.8	0.3	2.9	4.7	1.9	0.13	997	3.8	0.3	2.9	4.9	2.1	0.819	0.13	2.3
18:0	605	1.9	0.2	1.4	2.8	1.4	0.11	997	1.9	0.2	1.3	2.7	1.4	0.729	0.13	1.9
18:1	704	61.9	2.5	55.1	76.5	21.4	0.62	997	61.9	2.7	54.1	75.5	21.4	0.919	0.77	3.5
18:2	707	19.3	2.1	10.7	29.0	18.3	0.64	997	19.1	2.2	10.4	28.2	17.9	0.893	0.71	3.0
18:3	704	9.2	2.6	1.4	14.4	13.1	0.36	997	9.3	2.4	1.5	14.5	13.0	0.971	0.42	5.8
Saturated FA[e]	707	7.0	0.6	5.2	8.8	3.6	0.19	997	7.0	0.6	5.1	9.3	4.1	0.874	0.23	2.8
Iodine value	698	112.4	4.7	94.9	126.1	31.2	0.47	997	112.5	4.8	95.5	126.5	31.0	0.983	0.63	7.6
mg 18:1/g seed	702	234.8	20.2	177.8	295.4	117.5	2.41	997	235.3	19.2	173.8	308.6	134.8	0.976	3.00	6.4
mg 18:2/g seed	702	73.0	9.0	42.1	118.0	75.9	2.40	997	72.5	9.1	37.9	116.2	78.3	0.914	2.69	3.4
mg 18:3/g seed	707	34.8	10.1	5.0	54.3	49.3	1.41	997	35.3	9.5	5.4	54.3	48.9	0.972	1.62	5.9
mg Sats/g seed	707	26.4	2.3	18.8	31.9	13.1	0.73	997	26.4	2.4	18.7	33.0	14.2	0.880	0.83	2.9
Flax																
16:0	56	5.2	0.5	4.3	6.2	1.9	0.28	137	5.2	0.5	4.2	6.4	2.2	0.600	0.31	1.6
18:0	57	3.4	0.7	2.3	5.0	2.7	0.28	137	3.3	0.6	2.3	4.9	2.6	0.699	0.33	1.8
18:1	57	17.3	3.2	10.4	25.3	14.9	0.82	137	17.4	3.1	10.0	25.8	15.8	0.893	1.03	3.1
18:2	59	14.6	2.0	10.8	18.7	7.9	0.98	137	14.4	1.9	10.7	21.2	10.5	0.603	1.20	1.6
18:3	59	59.0	5.1	47.7	71.7	24.0	0.65	137	59.2	4.6	47.5	71.8	24.3	0.982	0.62	7.5
Saturated FA[e]	56	8.6	1.0	6.7	10.6	3.9	0.27	137	8.5	0.8	6.7	11.3	4.5	0.780	0.39	2.1
Iodine value	58	194.5	8.2	177.7	214.4	36.7	0.97	137	194.7	7.6	177.7	215.0	37.3	0.984	0.95	8.0
Solin																
16:0	55	5.9	0.4	5.2	6.6	1.4	0.19	55	6.0	0.4	5.2	6.6	1.4	0.688	0.23	1.8
18:0	55	3.8	0.3	3.0	5.7	2.6	0.12	55	4.0	0.4	3.1	4.6	1.5	0.933	0.10	3.8
18:1	55	15.8	1.1	13.2	19.1	5.9	0.54	55	16.1	1.4	13.9	19.8	5.9	0.802	0.62	2.2
18:2	67	70.9	1.8	66.1	75.2	9.1	0.33	67	70.8	1.7	66.9	74.1	7.3	0.958	0.37	4.6
18:3	67	2.0	0.2	1.5	2.7	1.2	0.09	67	2.0	0.2	1.6	2.4	0.8	0.804	0.08	2.2
Saturated FA[e]	55	10.3	0.7	8.6	11.8	3.2	0.35	55	10.5	0.8	9.0	11.8	2.8	0.839	0.31	2.4
Iodine value	67	141.8	2.2	135.6	147.0	11.4	0.47	67	141.6	2.1	137.5	145.8	8.3	0.960	0.43	5.0

[a] SECV, SE of cross-validation: describes the SE between reference and NIR values in the calibration set by sequentially removing samples from the calibration process.
[b] r^2, coefficient of determination.
[c] SEP, SE of prediction.
[d] RPD, ratio of the SEP to the SD of the prediction data set.
[e] Sum saturated FA (14:0 + 16:0 + 18:0 + 20:0 + 22:0 + 24:0). Sats, saturated FA.

the relative amount of a particular FA do not take into account the oil content of the seed. For example, a canola seed with the exact same relative oleic acid content but with different total oil contents will have different levels of absolute or actual oleic acid contents. Since NIR calibration development is based on the Beer–Lambert's Law, i.e., that the absorbance varies linearly with the analyte concentration, it would make sense that better statistical results should be achieved with a calibration based on absolute FA content. These results are similar to those achieved with the oleic acid content in ground corn (17).

The limiting factor for calibrating the NIR with absolute vs. relative FA content is that the absolute FA content was calculated with the NIR oil content (4). Since NIR calibrations generally have a higher degree of error than the reference method, this may be why we did not see dramatic improvements in all the FA. Moreover, when we converted the absolute amounts (absolute FA content/NIR oil content) back to relative amounts of a FA (Table 3), we lost any improvements we had with the absolute FA results. The recalculated results gave coefficients of determination, SEP, and RPD identical to those from our original relative FA calibration.

Flax. The prediction set (Table 2) gave the best NIR calibration equations for linolenic acid (Fig. 1) and IV, with SEP of 0.62% and 0.95 units, respectively. RPD of over 7.0 indicate that the method would be good for quality-control purposes. Oleic acid gave reasonable results, with a coefficient of determination of 0.893, SEP of 1.03%, and RPD of 3.1. Saturated FA and linoleic acid showed little potential for NIR screening at the present time, mainly because the low variability of these constituents in the samples made development of calibrations difficult.

Solin. For solin (Table 2), NIR calibrations predicted linoleic acid (Fig. 1) and IV for selection purposes based on the low SEP, coefficient of determinations of over 0.95, and RPD of over 4. Linolenic acid calibrations, although having low coefficient of determination values of 0.804, had low SEP values of less than 0.08%. This indicates that one would have confidence in meeting the low linolenic acid specifications for solin. Saturated FA, with the exception of stearic acid, did not give useful calibrations.

In conclusion, our study suggested that NIR technology could be used to measure linolenic acid in canola and flax, linoleic acid in solin, and IV in canola, flax, and solin accurately.

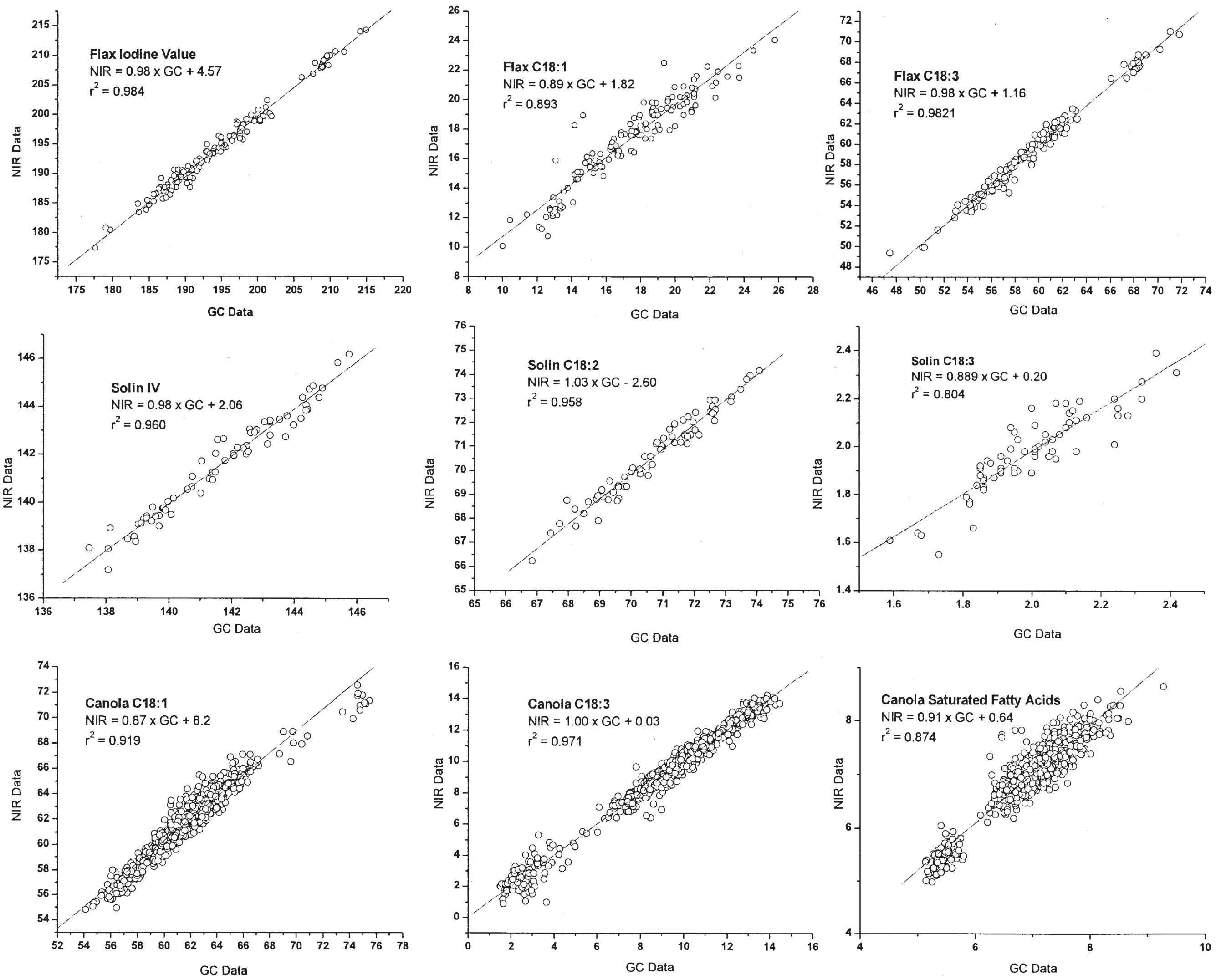

FIG. 1. Regression plots for key FA components of flax, solin, and canola. Statistical analyses showed that none of the slopes were significantly different from 1 and that the intercepts were not significantly different from zero. This confirms the accuracy of the NIR calibration over the region of interest.

The canola linolenic acid calibration still needs improvement in the lower range. NIR showed good potential in screening for oleic acid in canola and flax. Other FA, including total saturated FA, showed potential for estimation by NIR, but more work needs to be done. Other researchers have observed that calibrations will usually be improved when there is large variability in the particular FA (5).

Correction of NIR scans for oil content using NIR-derived oil contents resulted in improvements in calibration for FA in canola, but the results were derived as in milligrams of

TABLE 3
Comparisons of Calibrations Based on Absolute FA Content and Relative Amount of FA

		Absolute FA as mg/g in seed							Relative amount (recalculated) as % FA on the basis of NIR oil content						
FA	*n*	Mean	SD	Min.	Max.	r^{2}[a]	SEP[b]	RPD[c]	Mean	SD	Min.	Max.	r^2	SEP	RPD
18:1	997	235	19	174	309	0.976	3.0	6.4	61.9	2.7	54.1	75.5	0.911	0.8	3.3
18:2	997	73	9	38	116	0.914	2.7	3.4	19.1	2.2	10.4	28.2	0.895	0.7	3.1
18:3	997	35	10	5	54	0.972	1.6	5.9	9.3	2.4	1.5	14.5	0.970	0.4	5.6
Saturated FA[d]	997	26	2	19	33	0.880	0.8	2.9	7.0	0.6	5.1	9.3	0.882	0.2	2.9

[a] r^2, coefficient of determination.
[b] SEP, SE of prediction.
[c] RPD, ratio of the SEP to the SD of the prediction data set.
[d] Sum saturated FA (14:0 + 16:0 + 18:0 + 20:0 + 22:0 + 24:0).

FA/gram of seed. Recalculation of the corrected data back to relative percentages resulted in a loss of improvement.

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