Measurement of Oil in Whole Flaxseed by Near-Infrared Reflectance Spectroscopy

R.S. Bhatty
Crop Development Centre, Department of Crop Science and Plant Ecology, University of Saskatchewan, Saskatoon, SK, Canada S7N 0W0

A near-infrared reflectance (NIR) Infralyzer 500 was calibrated for determination of oil with samples of ground and whole flaxseed grown over three years. Wavelength selection by the computer software interfaced with the Infralyzer, analytical and regression statistic data, such as standard deviation of laboratory analysis (SD_x), correlation coefficient, standard error of estimate (SEE), standard error of prediction (SEP), and the SD_x/SEP ratio showed that calibration of the instrument with whole flaxseed was equal in precision to that obtained with the ground flaxseed. Growth location or seed moisture content had no effect on oil content of whole flaxseed determined by the NIR. The whole seed calibration allowed rapid, nondestructive screening for oil in flaxseed at greatly reduced cost.

KEY WORDS: Flaxseed, near-infrared reflectance, oil determination.

Flax, the sixth largest oilseed crop in the world, is grown for industrial (linseed) oil, primarily because of its high α-linolenic acid (ALA) content. Green and Marshall (1) reported a range of 34.6 to 46.4% oil in a diverse collection of 201 accessions of *Linum usitatissimum* L. species. Cool northern climes of the Prairies (latitude 40° to 57° north), where most of the Canadian flaxseed is grown, delay maturity of the crop and provide a longer period for oil and fatty acid synthesis (2). Canadian flaxseed (No. 1 Canada Western flaxseed) contained, on average of many years' data, 43.8% oil and 56.8% ALA (3).

In an oilseed breeding program, seed oil needs to be determined rapidly and, if possible, nondestructively. Many procedures are available for oil determination in oilseeds, some of which have been described in detail recently (4). The most common instrumental methods are nuclear magnetic resonance (NMR), pulsed NMR, near-infrared reflectance (NIR) spectroscopy or near-infrared transmission spectroscopy. A number of instrument- and seed-related parameters have been described for nondestructive measurement of oil in Brassica, peanut and sunflower seeds with pulsed NMR (5), while NIR has been used in measuring protein, oil, chlorophyll and glucosinolate contents of whole rapeseed (6). Panford *et al.* (7) used NIR to determine protein, oil, fiber and moisture in nine species of ground oilseeds.

The present paper reports a comparison of oil determination in whole and ground flaxseed by the NIR technique.

MATERIALS AND METHODS

Samples. Samples of flaxseed (*Linum usitatissimum* L.) of the 1986, 1987 and 1989 crops were taken from the flax nursery grown annually at the Kernen Crop Research Farm, University of Saskatchewan, Saskatoon, Canada. The nursery contained different cultivars and genotypes of flaxseed available in our collection. In addition, 20 samples of flaxseed (different cultivars) grown at two separate locations in Saskatchewan, Canada — Elrose (location 1) and Hagen (location 2) — were included in the study to determine the effect of location on oil content of whole flaxseed obtained with NIR. The air-dried, clean seed, with a moisture content between 4.7 to 5.4%, was used as is in all experiments except one. In this experiment, the effect of seed moisture on oil content in whole flaxseed was investigated. An equal volume of each sample as measured with a marked container (seed weight about 20 g) was ground, while continuously shaken, in a Krups coffee grinder for 45 sec; the ground seed was transferred to a 4-oz glass jar which was tightly capped and stored at room temperature (25°C). The ground samples were used for oil determination, and the whole seed and the ground seed samples for calibration of the NIR Infralyzer 500 (Technicon Canada Inc., Mississauga, ON).

Oil determination. Oil content of the ground samples was determined, in duplicate, by extraction with petroleum ether in a Goldfisch extraction apparatus. Details of the procedure have been described elsewhere (8). Percent oil in the sample was calculated as: weight of oil in sample divided by sample weight (as is or moisture-free) × 100. Moisture plus volatile matter was determined by drying ground flaxseed at 130°C for one hour.

Calibration of NIR 500. Thirty samples of whole or ground flaxseed were used for the calibrations. Each sample was poured into the holding cup (open for the whole seed, glass-covered for the ground seed), and scanned from 1,100 to 2,500 nm in the Infralyzer 500. The oil value for each sample was entered into the Hewlett-Packard (HP) 1000 minicomputer interfaced with the Infralyzer. The data analysis program identified the best wavelength combinations and calculated regression coefficients. These were transferred to the Infralyzer. After the calibration, the unknown samples were poured into the appropriate cup and read for percent oil.

Statistical analyses. The standard deviation of duplicate determination (laboratory method) was calculated as $\Sigma S_i/n$, where S_i was the standard deviation for duplicate determination of samples $_i$, and the standard deviation of laboratory analysis (SD_x) as $[\{\Sigma(X_i - X)\}/(n-1)]^{0.5}$, where X_i was the mean of duplicate determination for sample $_i$. Best wavelength combination, multiple correlation coefficients, standard error of estimate (SEE) and standard error of prediction (SEP) were obtained from multiple linear regression analysis data generated by the HP 1000 minicomputer interfaced with the infralyzer. The generation of these data is described in *Technicon Infralyzer 500 Operator Manual* available from the Technicon Industrial Systems, Tarrytown, NY.

RESULTS AND DISCUSSION

For the past several years, oil content of ground flaxseed has been determined in our laboratory by NIR. NIR

TABLE 1

Analytical and Multiple Regression Data of Ground and Whole Flaxseed Oil Determined by a Laboratory Method and NIR Analysis on Flaxseed Grown in 1986, 1987 and 1989

	Ground flaxseed			Whole flaxseed		
Analytical/Regression data	1986	1987	1989	1986	1987	1989
Sample number[a]	29	30	30	28	25	28
Oil range, % (as is)[b]	37.4-45.3	36.7-44.8	35.7-41.6	37.4-45.3	36.7-44.8	35.9-41.6
Mean oil, %	40.6	40.1	38.0	40.6	40.1	38.0
Standard deviations of duplicate determination (laboratory method)	0.32	0.23	0.15	0.32[c]	0.20[c]	0.16[c]
Standard deviation of laboratory analysis (SD_x)	2.1	2.2	1.5	2.1	2.2	1.5
Best wavelength combination, nm	1674	2024	1436	1772	1198	1198
	1702	2388	1478	2052	1240	1268
	2136	2472	1730	2430	1492	1520
Multiple correlation coefficient[d]	0.99	0.97	0.98	0.98	0.98	0.98
Standard error of estimate (SEE)	0.29	0.57	0.33	0.41	0.41	0.31
Standard error of prediction (SEP)	0.34	0.53	0.31	0.44	0.59	0.36
RPD[e]	6.2	4.2	4.8	4.8	3.7	4.2
Check sample (numbers)	6	6	20	6	5	20
Oil % (laboratory method, as is)	39.4	39.5	37.9	39.4	39.5	37.9
SD_x	0.4	0.3	1.6	0.6	0.3	1.6
Oil, % (NIR, as is)	39.3	39.1	38.1	39.4	39.5	37.7
SD_x	1.0	0.3	1.4	0.4	0.2	1.5

[a]Used in NIR calibration.

[b]Oil determined in duplicate by Goldfisch extraction (laboratory method).

[c]Values taken from ground flaxseed data for number of samples in each year.

[d]Between laboratory method and NIR.

[e]Ratio of standard deviation of laboratory analysis (SD_x) and standard error of prediction (SEP) of NIR data (i.e., SD_x/SEP).

offered several advantages over pulsed NMR, another instrumental method commonly used for oil determination in oilseeds (5,9). The sample need not be dried, which is mandatory in NMR due to interference of hydrogen nuclei in the signal. However, grinding of flaxseed samples to a uniform particle size was tedious, time-consuming and influenced results to a large extent. Furthermore, the ground samples may not be stored for long periods as the oil may be absorbed by the container or separated from the meal. The availability of NIR 500, interfaced with Hewlett-Packard 1000 minicomputer, allowed comparison of oil determination by using whole and ground flaxseed calibrations.

In each of the three years, thirty samples of flaxseed were selected for calibration of the Infralyzer. The calibration samples included a number of flaxseed cultivars and genotypes that were grown on a single location and had seed oil (ground seed) ranges of 37.4 to 45.3%, 36.7 to 44.8% and 35.7 to 41.6%, with means of 40.6%, 40.1% and 38.0% in 1986, 1987 and 1989, respectively (Table 1). The actual number of samples used in each calibration varied from 25 to 30 due to elimination of samples in which the residual (difference) between the actual and predicted oil values was unacceptable. Since percent oil of the calibration samples was determined in the laboratory on ground flaxseed, these values were used both for whole and ground flaxseed calibrations. The standard deviation of duplicate oil determination in the laboratory, a measure of precision of the method, varied from 0.15 to 0.32% and was less than 1% of the mean oil content of the samples in each of the three years (Table 1).

In the use of NIR, obtaining optimal wavelengths for the constituent to be determined is most critical. This is done by converting reflectance data for wavelengths obtained with a scanning monochromator to apparent absorbance ($A = \log 1/R$ where R is the reflected energy), followed by multiple linear regression analysis of actual (laboratory analysis) and predicted (NIR) values. Wavelengths that give the best correlation between the actual and predicted values are finally selected. The NIR 500 is fitted with six interference filters and the software available with the minicomputer selects the best wavelengths and calculates correlation coefficients. Figure 1 shows the absorbance spectra of one sample each of whole and ground flaxseed of the 1987 and 1989 crops. These spectra represent the absorbance of the total samples at various wavelengths in the NIR. The absorbance patterns of the whole and ground flaxseed were generally similar except between 2,200 and 2,400 nm, a region of oil reflectance in the NIR spectrum. This region was more diffuse in whole flaxseed, both in 1987 and 1989 (Fig. 1), than in ground flaxseed. Fatty acid 1st, 2nd and 3rd overtones, implying C-H stretch, C=0/0-H stretch or coupled to $C(CH_3)_3$ and CH_2 groups have been tentatively assigned in this region in rapeseed, soybean and sunflower (7). The absorbance was greater for the whole than for the ground flaxseed, suggesting that more light was reflected from ground flaxseed, probably because of its larger surface area. However, relative absorbance is not critical in NIR analysis. In both years, wavelength maxima ranged in the entire region of the spectrum. This was reflected, as well, in the best wavelength combinations selected by the computer. The first combination selected in each case was used in the calibrations and is given in Table 1. There were few common or nearly common wavelengths except in 1987 and 1989 whole flaxseed calibrations, suggesting that different constituents were measured but these

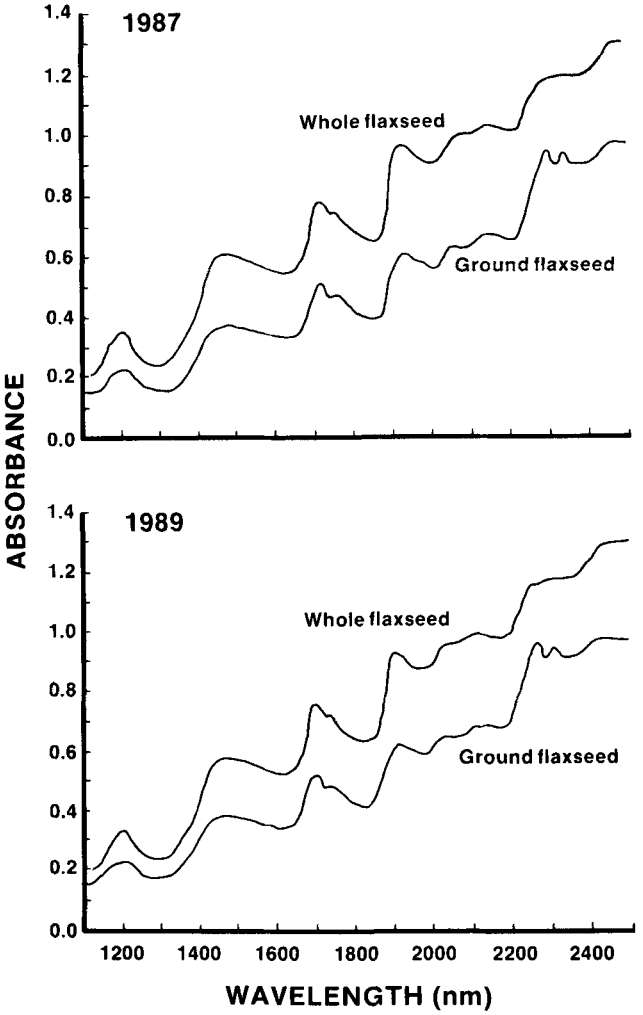

FIG. 1. Absorbance spectra of one sample each of whole and ground flaxseed of the 1987 and 1989 crops.

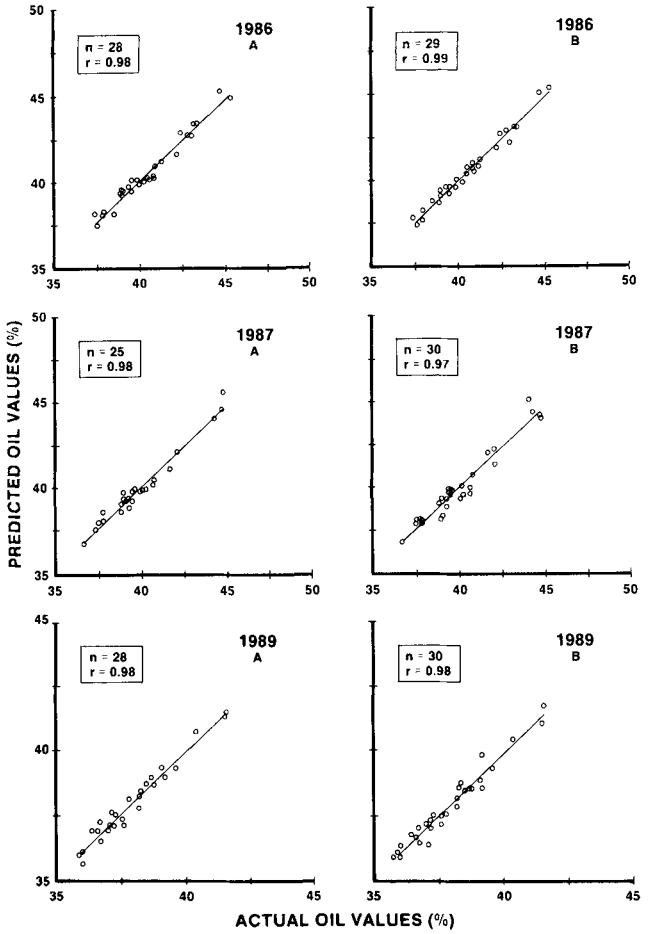

FIG. 2. Distributions of correlation data between actual (laboratory method) and predicted (NIR) oil values of whole (A) and ground (B) flaxseed of 1986, 1987 and 1989 crops.

constituents were apparently present both in ground and in whole flaxseed grown over the three years. This may suggest that one year's calibration may not be used the next year. However, this finding is irrelevant as we routinely calibrate the Infralyzer every year.

Different wavelengths have been assigned to oil bands in different oilseeds, and these assignments may be due to C-H stretch, C=0/C-H stretch combinations, 0-H combination, different frequency of the same band as well as to weaker absorption bands or overtones (7). The wavelength assignment may be quite different in extracted or purified oil when compared to oil present in the seed. The differences may be further accentuated by interaction between constituents, particle size in ground seed, and chain length and degree of saturation of fatty acids. By using an NIR scanning monochrometer, Panford *et al.* (7) assigned the following wavelengths to a freshly ground sample of flaxseed: 1,400, 2,000, 2,312 and 2,390 nm. These were within the best wavelength combinations obtained in the present study both in ground and whole flaxseed (Table 1).

Nevertheless, an indication of good NIR calibration is the high multiple correlation between oil values obtained by a laboratory method (actual) and predicted by the NIR. In this study, the correlations obtained between the two methods varied from 0.97 to 0.99 for the ground flaxseed and were identical (0.98) for the whole flaxseed in each of the three years. These values justified determination of oil on whole flaxseed by NIR. Figure 2 shows distribution of the correlation data (25 to 30 samples in each case) in whole and ground flaxseed for the three years.

Another indication of an acceptable calibration is the standard error of estimate (SEE), which measures the agreement between the NIR and the laboratory method of analysis. The SEE should be lower than the standard deviation (SD_x) of the laboratory method (7). The regression data show (Table 1) that this was the case in all calibrations. In ground flaxseed calibrations, the SEE values varied from 0.29 to 0.57% and in whole flaxseed from 0.31 to 0.41%. The SD_x values varied from 1.5 to 2.2% in ground and whole flaxseed. The 1986 ground flaxseed and 1989 whole flaxseed calibrations gave the lowest SEE values.

Another statistic (RPD), the ratio of standard deviation of laboratory analysis and standard error of prediction (SD_x/SEP), should ideally be ten or higher in an acceptable calibration (7). A lower ratio may indicate a poor calibration, largely as a result of the narrow range of the

TABLE 2

Influence of Seed Moisture on Oil Content of Whole Flaxseed Determined by Goldfisch (laboratory method) and Near-Infrared (NIR)

	Oil content %			
	Goldfisch		NIR (whole seed)	
Sample no.	"as is"[a]	dry basis[b]	"as is"[a]	dry basis[b]
1	37.7	39.5	37.1	39.2
2	34.7	36.7	35.1	37.1
3	35.4	37.3	35.7	37.7
4	37.2	39.1	37.4	39.3
5	38.5	40.4	38.1	40.0
6	36.4	38.2	36.3	38.1
7	37.4	39.4	37.8	39.8
8	38.7	40.7	38.0	39.9
9	36.7	38.6	36.6	38.5
10	37.0	38.9	36.3	38.2
11	37.6	39.6	37.1	39.1
12	39.9	41.8	40.0	42.0
13	40.4	42.5	40.0	42.1
14	40.2	42.3	40.3	42.4
15	39.8	41.9	39.8	41.9
16	38.2	40.3	37.5	39.5
17	36.8	38.7	37.4	39.3
18	39.3	41.3	39.1	41.1
19	38.3	40.0	37.8	39.8
20	37.1	39.0	36.7	38.6
Mean	37.9	39.8	37.7	39.7
SD_x	1.6	1.6	1.5	1.5
Correlation	0.96		0.97	

[a,a;b,b]Means were not statistically significant by a t-test in these columns.

constituent (oil in this case) in the calibration samples. In the present study, RPD values obtained varied from 4.2 to 6.2 in the ground flaxseed and from 3.7 to 4.8 in the whole flaxseed; the lowest value was for the 1987 ground flaxseed (Table 1). Variation in RPD values was greater in ground flaxseed calibrations than in whole flaxseed calibrations. The major reason for lower RPD, obtained in this study, was the higher SEP values which were three to five times greater than the value reported for flax (ground) calibration by Panford *et al.* (7). This may reflect, in part, a narrower oil range of the calibration samples, although it accommodated oil content normally encountered in flaxseed grown under our conditions. Nevertheless, in spite of lower RPD values, NIR calibrations obtained with whole flaxseed were not inferior to those obtained with ground flaxseed.

A satisfactory whole flaxseed calibration was confirmed by analysis of oil in check samples not included in the calibrations. In 1986 and 1987 calibrations there were only six check samples. In 1989 the number of available samples was increased to 20 with a range in oil from 34.7 to 40.4% (Table 2; column 2). Analysis of the check sample and statistical treatment of the data showed that both laboratory and NIR methods gave almost identical oil values; the largest difference in oil content between the two methods was obtained in the 1987 ground seed calibration (Table 1). Only in one case (1986 ground flaxseed), the SD_x of the NIR determination was larger than that of the laboratory determination. The correlation between the laboratory and NIR oil values for whole seed was highly significant (+0.96) only for the 1989 check samples (n=20), and not for 1986 and 1987 because of fewer samples (n=6) having a smaller range in oil content.

The influence of growth location on the relationship between the two methods of oil determination was established by taking ten samples from each of the two widely separated growth locations. These samples were divided into two lots: one lot was ground and analyzed for oil content by the laboratory method as described before, the second used to determine oil on whole flaxseed by NIR. Data in Table 3 show that the mean oil contents of the samples determined by Goldfisch and NIR were not significantly different (t-test) and that the SD_x for the two methods within each location were generally similar. The correlations between the two methods within each location varied from +0.96 to +0.99. The growth location thus had no effect on oil determination in whole flaxseed by NIR.

In another experiment, the influence of seed moisture content on oil in whole flaxseed was determined by using the 20 samples previously used as check in the 1989 calibration. The samples had a narrow range of moisture (4.7 to 5.4%) due to uniform drying of harvested flax under our conditions. The means of oil content for the Goldfisch and NIR (whole seed) expressed either on "as is" or on "dry basis" were not significantly different (t-test).

In an oilseed breeding program, it is advantageous to determine oil nondestructively on whole seed. For such determination, NIR transmission instruments have been suggested. However, the present data suggest that NIR reflectance instruments, particularly the Infralyzer 500,

TABLE 3

Influence of Growth Location on the Relationship Between Oil Content of Whole Flaxseed Determined by Goldfisch (laboratory method) and Near-Infrared Reflectance (NIR)

	Location 1 (Elrose)		Location 2 (Hagen)	
	Oil, %		Oil, %	
Sample no.	Goldfisch[a]	NIR (whole flaxseed)[b]	Goldfisch[a]	NIR (whole flaxseed)[b]
1	35.3	36.0	40.1	39.1
2	37.9	38.0	39.2	38.3
3	37.0	37.5	39.5	38.2
4	35.9	36.6	42.5	40.9
5	37.8	37.7	37.8	37.6
6	36.0	36.6	37.8	37.1
7	38.0	38.2	38.4	37.8
8	36.9	37.1	37.7	37.1
9	38.5	38.7	41.0	40.9
10	39.9	40.2	41.1	39.9
Mean	37.3	37.7	39.5	38.7
SD_x	1.4	1.2	1.6	1.4
Correlation	0.99		0.96	

[a,b]Means were not significantly different by a t-test in this column.

may be equally satisfactory for flaxseed. This instrument has now been used satisfactorily for oil determination in whole flaxseed for the last three years at a considerable saving of resources. During its operation, the instrument is checked daily, morning and afternoon, with check samples having low, medium and high oil content. By using whole seed calibration, 200 to 250 samples of flaxseed may be analyzed daily in a normal working day.

ACKNOWLEDGMENTS

The excellent technical assistance of D. Hassard is gratefully acknowledged. Dr. G. Rowland generously provided the flaxseed samples.

REFERENCES

1. Green, A.G., and D.R. Marshall, *Aust. J. Agr. Res. 32*:599 (1981).
2. Darrell, D.G., *Fette Seifen Anstrichmittel 77*:258 (1975).
3. Grain Research Laboratory, Canadian Grain Commission, Winnipeg, MB, Canada, Crop Bulletin No. 182 (1989).
4. Thies, W., and D.I. McGregor, in *Oil Crops in the World*, edited by G. Robbelen, R.K. Downey and A. Ashri, McGraw-Hill Publishing Co., New York, 1989, p. 132.
5. Tiwari, P.N., P.N. Gambhir and T.S. Rajan, *JAOCS 51*:104 (1974).
6. Tkachuk, R., *Ibid. 58*:819 (1981).
7. Panford, J.A., P.C. Williams and J.M. deMan, *Ibid. 65*:1627 (1988).
8. Bhatty, R.S., *Can. Inst. Food Sci. Technol J. 18*:181 (1985).
9. Gambhir, P.N., and A.K. Agarwala, *JAOCS 62*:103 (1985).

[Received June 26, 1990; accepted September 17, 1990]