TABLE VIII

Triterpene Alcohols (%)

Oil	β-Amyrin	α-Amyrin+ unknown	c-Artenol	Methylene c-artanol
Evening Primrose	12	64	16	5
Cottonseed	6.5 (7[a])	22 (60[a])	27 (12[a])	40 (21[a])
RRT (acetates)	0.88	0.92–0.98	1.00	1.14

[a]Data from Kornfeldt & Croon (14).

TABLE IX

Tocopherols (ug/g of Oil)

Oil	α-Tocopherol	γ-Tocopherol	δ-Tocopherol
Evening primrose	76.0	187.0	–
Cottonseed	102.0	216.9	2.2
RRT[a]	0.78	0.64	0.49

[a]Stigmasterol, used as an internal standard, had RRT 1.00.

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Oil and Water Analysis of Sunflower Seed by Near-Infrared Reflectance Spectroscopy

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ABSTRACT

The applicability of NIR for oil and moisture analyses of sunflower seed was determined using a NIR spectrocomputer system. The method was compared with the wide-line NMR method for oil analysis and with the A.O.C.S. oven method for moisture analysis. The NIR was calibrated with 120 samples for oil (96 for calibration, 24 for prediction) and 63 samples for moisture (55 for calibration, 8 for prediction). Twenty-two sunflower seed samples were analyzed for oil and moisture by NIR and by methods used by industry. The oil contents of the samples by NMR and NIR were not significantly different. The overall mean oil contents and mean of the standard deviations for the samples were: NMR, 44.2% ± 0.35% and NIR, 44.34% ± 0.74%. A significant difference was found between the moisture values obtained by the oven-drying method and NIR. The average standard deviation for moisture by NIR was 0.57% compared with 0.07% for the oven-drying method. The variability of the oil content in one of the commercial seed samples was 1.52% oil as determined by NMR and 2.52% as determined by NIR. The advantages and disadvantages of both methods are discussed.

INTRODUCTION

The standard method for the determination of oil content of oilseeds since about the 1880's has been the direct solvent extraction method. This is a time-consuming process involving the use of flammable solvents. Moreover, the sample is destroyed, which is an inconvenience, particularly for plant breeders who often have only a few seed available for planting and analysis. These serious drawbacks resulted in the development of wide-line nuclear magnetic resonance (NMR) and near-infrared reflectance (NIR) spectroscopy techniques.

In 1960, Conway (1) first used NMR to analyze whole seed for oil content. Since the process is nondestructive and feasible even with single seeds, plant breeders have used the technique extensively (2–4). NMR provides a rapid, accurate means of measuring the oil content of oilseeds (5–6) and has been found to be more reproducible and statistically more reliable than A.O.C.S. and other extraction methods (5, 7–9).

Robertson and Morrison (6) reported that NMR gave accurate estimates of the oil content of sunflower seed, but they found that the NMR response varied depending on the linoleic acid content. In addition, NMR analysis required a predrying step to remove moisture interference before the oil content was determined.

The NIR technique, developed by Norris (10) has become firmly estabilished as a simple, rapid, effective analytical tool for the simultaneous prediction of oil, protein and moisture content of grains and oilseeds (11-13).

Robertson and Windham (9), in a comparative study of the A.O.C.S. extraction method with NMR and NIR for determining the oil content of sunflower seed, reported that the NMR method was more precise and reproducible than the other 2 methods. Although the NIR mean oil contents were not significantly different from the A.O.C.S. and NMR values, the NIR results were quite variable. This variability was believed to be caused by an inadequate number of calibration samples and instrument problems. In addition, the precision of the A.O.C.S. method limited the precision of the NIR analyses because A.O.C.S. data were used to calibrate the NIR.

As in the case of oil extraction methods, the conventional oven-drying methods for moisture determinations are also time-consuming. The A.O.C.S. Official Method for moisture in sunflower seed specifies that 130 C for 3 hr be used (14). Moisture content in sunflower seed also may be rapidly determined with electronic mositure meters (15), however, for highest accuracy the sample should be allowed to temper for 24 hr after combining or drying (16).

Kaffka et al. (17) reported that NIR measurements can be related to the oil, protein, water and fiber content in sunflower seed. Their calibration results were not tested against unknown samples and standard methods, and the reproducibility and repeatability values were not very good.

The objectives of this study were to determine the applicability of an NIR spectrocomputer system for oil and moisture analyses of sunflower seed and to compare NIR oil analysis with wide-line NMR values.

MATERIALS AND METHODS

Hybrid oil sunflower seed (*Helianthus annuus* L) with different oil contents were obtained from National Sunflower Performance Trial plantings from 36 different locations in the U.S. The oil and moisture content of the samples used for calibration of the NIR were determined in triplicate by NMR (6) and A.O.C.S. Ai 2-75 (14) methods, respectively. In addition, 22 commercially mixed and 23 different hybrid sunflower-seed samples were obtained after grading from the North Dakota Grain Inspection Service (FGIS), Fargo, ND, and from Attaboy Co. Inc., Carrollton, IL, respectively. These samples were analyzed in duplicate for oil and moisture contents as previously described and used to validate NIR prediction equations.

The variability of the oil content within a single commercial sunflower-seed sample was determined. The seed sample was carefully cleaned by picking out all trash held on a 8/64 round-hold sieve by hand. The cleaned seed was mixed by passing it through a Jones riffle 3 times and was riffled to 40 aliquots of ca. 15 g. Then, 20 aliquots (10 g) were analyzed by NIR and 20 aliquots (11 to 12 g) by wide-line NMR.

NMR Analysis

The wide-line NMR instrument used for these studies was the Newport Analyzer Mk III equipped with 150 mL coil assembly. The NMR was standardized by use of a sunflower seed sample of known oil content distributed by the USDA, FGIS. Seed samples were dried in a forced draft oven for 1 hr at 130 C and equilibrated to room temperature in a desiccator with Drierite calcium sulfate desiccant. Readings were taken on approximately 50 g of seed, except as indicated, and the oil contents calculated (6).

NIR Sample Preparation and Analysis

Seed samples were prepared for near infrared reflectance (NIR) analysis by grinding 10 g seed with 10 g Hyflo Super Cel for 2-1/2 min with a Varco Type 228 high-speed grinder. The ground mixture was quantitatively transferred into an air-tight jar, mixed well, and then an aliquot was packed into a Neotec sample cup. NIR analysis of the ground sunflower seed was conducted with a Neotec Model 6100 Spectrocomputer System equipped with a Digital Equipment Corporation (DEC) PDP 11/34 minicomputer and associated peripherals. The Pennsylvania State University/USDA/Neotec spectrocomputer software system developed by Shenk et al. (18) was used to operate the instrument. The spectral data recorded as log reciprocal reflectance (log 1/R) was obtained from triplicate samples and the 3 sets of 64 scans were averaged in the computer with a software program called FILE (18). The NIR was calibrated with a 120-sample set for oil (96 for calibration, 24 for prediction) and a 63-sample set for moisture (55 for calibration, 8 for prediction). Five of the moisture samples were dried in a vacuum oven to 0.5% moisture, scanned a second time and the value of 0.5% moisture was used to force the moisture intercept to zero.

Once a file with 700 data points from the spectral scan of each sample and the analytical values have been placed on the computer, the calibration of the instrument can be made by generating a prediction equation. The usual way of accomplishing this is by making the appropriate mathematical conversion, i.e., first or second derivatives and performing a multiple stepwise linear regression analysis. The best regression equation, which gives the lowest standard error of prediction (SEP), is chosen as the prediction equation. This procedure requires that the operator know the "best" mathematical data treatment. Since this is not a known factor, another means of maximizing the data treatment is needed. A program called "CAL", developed at Pennsylvania State University, considers all possible data treatments and does the linear regression analyses (19). This program was used to obtain the optimal equations for oil and moisture.

Two other features of this program are unique when compared with the earlier software (18). First every *i*th sample may be set aside for an internal prediction set. These samples will not be part of the calibration itself. The second feature is that using the currently computed equation, the predicted versus laboratory data will be plotted in the computer and the slope printed as output. The closer to the slope is, the smaller the bias, and the more accurate the equation can be presumed to be. The precision will not necessarily be the best, but the least bias between predicted and laboratory analytical values will be found.

Statistical Analysis

Data were analyzed to identify the main effects by a 2-way analysis of variance and for differences between means by the Duncan multiple-range test, using the statistical analysis system described by Barr et al. (20).

RESULTS AND DISCUSSION

Regression equation coefficients, F values, wavelengths and details of the mathematical treatment of the spectral data are shown in Table I. The values in parenthesis are the number of nm in the moving average smoothing, nm per derivative segment, and nm between segments, respectively. Each equation contains 1 division term, a mathematical data treatment in which the signal-to-noise ratio is improved. The calibration parameters are shown in Table II. The H and T statistics refer to the number of samples

TABLE I

Coefficients, Wavelengths, Mathematical, and Statistical Parameters in the Equations for Oil and Moisture Analyses of Sunflower Seed by NIR.

Term	Coefficient	F value	Wavelength	Math treatment derivative (segments)
Oil				
b_0	53.41			
b_1	−4,585.72	19.61	1,284	1(8, 4, 4)
b_2	−2,897.09	28.62	2,340	2(4, 4, 4)
b_3	12.71	146.64	2,010/1,258	2(16, 8, 4/16, 4, 4)
Moisture (5 term)				
b_0	4.94			
b_1	−3,006.95	66.78	1,806	1(4, 4, 4)
b_2	0.45	260.57	1,382/2,004	2(36, 24, 4/16, 16, 2)
b_3	−749.44	179.29	1,964	2(16, 16, 4)
b_4	1,019.87	39.25	1,186	2(8, 8, 4)
b_5	541.89	33.98	1,398	2(16, 4, 2)

TABLE II

Calibration of Sunflower for Percentage of Oil and Moisture

Variable	N	No. terms	SD[a]	SEC[b]	No.* Hc	No.* Tc	R^2
Oil	96	3	3.74	0.94	1	3	0.937
Moisture	55	5	4.29	0.26	2	2	0.996

[a]Standard deviation.

[b]Standard error of calibration.

[c]See reference 18 for discussion of statistical parameters.

TABLE III

Prediction of Oil and Moisture with Sunflower-Seed Calibration Set

Variable	N	SD	SEP	SEP_c[a]	Bias	No.* H	No.* T	R^2	Slope
Oil	24	4.16	2.04	2.08	0.10	1	2	0.75	0.96
Moisture	8	5.70	0.62	0.66	−0.06	1	1	0.994	0.92
Moisture	8	5.70	0.46	0.49	−0.02	1	1	0.966	0.94

[a]SEP_c − SEP corrected for bias.

rejected by the computer in the set that either have a spectrum different from the set (H) or the predicted oil and moisture would be different, as determined by t test, from the value obtained by NMR (18). These samples were left in the calibration file. The range of percentage of oil was 30.52–53.88% and percentage of moisture was 0.50–19.70%. These were broad ranges into which commercial samples would be expected to fall.

The CAL program allowed prediction within the calibration set so that every fifth sample was used for the prediction of oil and every eighth sample for moisture. The results of the predictions are shown in Table III. The SEP was large (2.08) for oil, but was also larger than the SEP for other sets. The slope (0.96), however, was quite good. The SEP was large because of 2 samples, 1 of which was a gray stripe. The inclusion of gray-stripe samples presented a problem of appearance and affected the statistics as well as the predicted values. While color, per se, should not influence the absorption of NIR energy, the amount of specular or nonreradiated reflectance will be affected. A ceramic standard is used to correct for this term, but it cannot correct for color changes that may slightly affect the reflectance properties. The gray stripe samples are lighter in color and, when ground, are easily distinguishable from the usual oil hybrids. The bias for the predicted values was quite low and well within the SEP. In general, these samples validate the equation.

Further validation of the equation with different sets of samples was accomplished with 2 unknown sample sets. The first was a set of 23 different hybrid sunflower-seed samples. The range of oil content was 36.44–42.91% by NMR and 37.16–45.39% by NIR. The sample set means and standard deviations are given in Table IV. The SEP for the set was 0.98 with a very small bias (−0.026). The results for 2 samples differed by more than 2 percentage units. Both were high in moisture. When samples that were air equilibrated were predicted, they differed by about 0.5 percentage units.

Oil and moisture contents of 22 commercial sunflower-seed samples analyzed by the NIR technique and by methods routinely used in industry (oil by NMR and moisture by oven drying) are shown in Table V. The oil

TABLE IV

Determination of Oil Content of Different Hybrid Sunflower Seed by NIR and NMR[a]

Variable	Sample no.	NIR Mean	NIR SD[b]	NIR No. stars. "H" or "T"	NMR Mean	NMR SD	NMR Bias	NMR SEP[c] (method)
Oil	23	40.57	1.86	1	40.43	1.69	−0.026	0.98

[a]Analyses conducted on 10–12 g samples.
[b]Standard deviation.
[c]Standard error of prediction.

TABLE V

Oil and Moisture Analyses of Commercial Sunflower Seed

Sample no.	Oil, % DB N.D.G.I.S.[a]	Oil, % DB NMR[b]	Oil, % DB NIR[b]	Moisture, % A.O.C.S. oven[b]	Moisture, % NIR
1	44.0	45.15 ± 0.09	45.29 ± 0.39	5.80 ± 0.09	5.12 ± 0.74
2	44.3	44.33 ± 0.55	44.41 ± 0.75	5.76 ± 0.08	5.04 ± 0.70
3	44.8	45.02 ± 0.34	45.26 ± 0.37	5.71 ± 0.04	5.05 ± 0.45
4	42.9	42.67 ± 0.52	43.74 ± 1.47	6.78 ± 0.05	6.39 ± 0.17
5	42.4	42.99 ± 0.11	42.67 ± 1.48	6.58 ± 0.08	5.89 ± 0.69
6	44.3	44.37 ± 0.60	44.77 ± 0.36	5.72 ± 0.10	5.55 ± 0.14
7	45.6	45.98 ± 0.04	44.46 ± 1.03	5.72 ± 0.04	5.32 ± 0.26
8	42.8	42.55 ± 0.03	44.05 ± 0.16	6.06 ± 0.05	5.29 ± 0.95
9	44.6	44.73 ± 0.52	44.57 ± 0.32	5.83 ± 0.15	5.20 ± 0.45
10	42.9	43.50 ± 0.54	42.68 ± 0.64	5.75 ± 0.04	5.46 ± 0.05
11	41.6	41.66 ± 0.80	40.61 ± 1.29	8.99 ± 0.03	8.60 ± 0.95
12	47.6	47.65 ± 0.47	46.43 ± 0.62	7.67 ± 0.06	6.99 ± 0.46
13	44.3	44.23 ± 0.56	43.00 ± 0.49	5.92 ± 0.01	5.78 ± 0.17
14	42.2	42.04 ± 0.23	43.16 ± 0.93	11.19 ± 0.08	10.64 ± 0.46
15	42.0	42.73 ± 0.35	44.30 ± 0.08	8.67 ± 0.03	7.95 ± 0.12
16	42.3	42.92 ± 0.19	44.48 ± 1.71	12.02 ± 0.02	11.02 ± 0.63
17	49.3	48.69 ± 0.26	47.37 ± 0.04	7.31 ± 0.15	7.08 ± 1.15
18	44.3	45.12 ± 0.57	45.60 ± 0.98	9.37 ± 0.03	8.86 ± 1.20
19	42.3	43.20 ± 0.14	44.38 ± 0.14	12.05 ± 0.04	11.13 ± 0.04
20	44.1	44.51 ± 0.17	45.21 ± 1.34	8.69 ± 0.21	7.77 ± 0.25
21	43.7	44.03 ± 0.30	43.75 ± 1.63	7.19 ± 0.12	6.38 ± 0.80
22	43.8	44.36 ± 0.30	45.28 ± 0.13	9.32 ± 0.14	8.58 ± 1.62
Mean	43.9	44.29 ± 0.35[c]	44.34 ± 0.74[c]	7.64 ± 0.07[c]	7.05 ± 0.57[c]

[a]North Dakots Grain Inspection Service, oil contents by NMR.
[b]Analyses in duplicate.
[c]Mean standard deviation of 22 duplicate samples.

contents of the samples by NMR and NIR were not significantly different ($P > 0.05$) from the oil contents of the samples determined by the North Dakota Grain Inspection Service (NDGIS) when grading the samples, nor was there any significant difference in the analysis by NMR and NIR. However, a significant difference ($P < 0.05$) was found between the moisture value obtained by the AOCS oven-drying method and NIR (Table V). Standard deviation on duplicate moisture analyses of sunflower seed generally will be ± 0.1% or less and within laboratory precision should be 0.39% or less (14). The average standard deviation of ± 0.57% obtained by NIR would be unacceptable.

The average NIR moisture value for the seed was 0.59% lower than the average oven value. Moisture should be the most accurate measurement for NIR since it is the strongest signal and therefore the highest signal-to-noise ratio in the spectra. The NIR was calibrated for moisture content with data obtained by ovendrying whole sunflower seed. In the NIR analysis, the scanned sample was ground with equal weight of Hyflo Super Cel, and the assumption was made that the moisture loss during grinding and filling sample cell was uniform from day to day. Studies have shown that during grinding of sunflower seed, approximately 7.5% of the moisture is lost from seed samples with an average moisture content of 8.9% (i.e., 0.67%). This loss will probably vary, depending on the variety of seed and the temperature and relative humidity of the laboratory during grinding (21). Williams and Sigurdson (22) reported that grains (wheat, oats and barley) lose significant amounts of moisture during the grinding process (sample preparation) for protein determination. Although the oven-drying method is not specific for water and the results are affected by the vaporization of substances other than water, oven moisture values for whole sunflower seed have been found to be very close to Karl Fischer moisture values in the moisture range of 5.4–12.7% (21). The Karl Fischer method is specific for water. Moisture cannot be determined accurately on ground sunflower seed by the oven method, and the time required for the determination of Karl Fischer moisture on the calibration samples would have been prohibitive.

In essence, the differences for the percent of oil between-method (NIR vs NMR) error is 0.89 with a small negative bias (−.38). The between-method and between-laboratory (NIR vs NDGIS) error was little larger (1.09 with −.47 bias), as would be expected. The moisture data reflects the constant ca. 0.5 percentage unit drying effect for grinding in the preparation of samples for NIR analysis. If the 0.5

TABLE VI

Oil Variability in a Single Sunflower-Seed Sample[a]

Parameter	NIR	NMR
Range oil, % dry basis	45.92 – 48.44 (2.52)	46.13 – 47.65 (1.52)
Mean oil, % dry basis	47.54	47.02
Oil standard deviation	0.67	0.32

[a]Results of 20 duplicate analyses.

is subtracted from the 0.62 SEP and bias, the 0.12 percentage unit remaining is very close to the 0.26 SEC for moisture (Table II). The overall SEP of 0.62 is virtually identical to the within set SEP of 0.66 (Table III).

The variability of the oil content in a single commercial sunflower seed sample is shown in Table VI. With NMR analysis, the oil-content range was 1.52% with a mean oil content of 47.02% ± 0.32%, and with NIR analysis the range was 2.52% with a mean oil content of 47.54% ± 0.67%. These data illustrate the great variability of the oil content in individual lots of sunflower seed, even when carefully mixed. Robertson and Morrison (6) reported that by increasing the sample size for NMR analysis from approximately 14 g to 50 g, the average standard deviation (SD) of oil analysis for 10 samples was decreased from 0.37% to 0.17%.

The 0.32% SD for the NMR, based on the 20 aliquots of the same sample, is approximately the same as shown for the mean SD of the 22 different samples shown in Table V (0.35%). This result can be interpreted as much as variation in the sampling as in the method. However, the SD for NIR (0.67%) was less than the set mean SD for the 22 different samples (0.74%). The range for NIR was 1 percentage unit higher than that of NMR. The NIR was calibrated with NMR data and all the variability of NMR data is included in the NIR error term. The salient point is that the variability from sampling is 4 to 5 times the error in either method, and that pproximately 1/2 of the SEP_C is from sampling error in the calibration of the NIR with NMR data. The NMR method is more precise, but requires a dried sample. The NIR method is as fast, but can also give simultaneous data for moisture, fiber, protein and oil as well as other possible constituents of interest.

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