Sources of Sodium Metal

The use of metallic sodium presents certain hazards, particularly with lab personnel who have not been thoroughly trained in its use. In addition, it is not convenient to handle and can lead to hydrocarbon contamination from the protective "oils" or solvents in which sodium metal is usually stored. Therefore, 2 more easily handled forms of sodium were evaluated—sodium methoxide powder and Dri-Na^R, a sodium-lead alloy. With a series of MDBS standards, no significant differences could be detected by GLC using the 3 sources of sodium. Areas obtained by GLC, retention times, the shape of the major peak, and occurrences of minor peaks appeared identical. There appears to be no reason that the 3 sources cannot be used interchangeably and the Dri-Na^R is certainly both the most convenient and safest to use.

Suggested Modifications to the Procedure

Re-esterification of the ester bond would reduce the opportunity for significant losses and decrease the variance between replicate samples; therefore, this step should be added to the procedure. Since both variation in temperatures and localized overheating occurs with the use of mantles, the substitution of controlled-temperature heating blocks is suggested. Third, because of the ease of handling and increased safety, the replacement of metallic sodium with the sodium-lead alloy is recommended.

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Comparative Study of Methods of Determining Oil Content of Sunflower Seed

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ABSTRACT

An extraction-gravimetric method (AOCS Official Method Ai 3-75) was compared with 2 instrumental techniques, near-infrared reflectance (NIR) spectroscopy and wide-line nuclear magnetic resonance (NMR), for the determination of the oil content of oilseed-type hybrid sunflower seed. Eight sunflower seed samples of varying oil contents, replicated 5 times, were analyzed by the 3 procedures. The overall mean oil contents and standard deviations for the 8 samples were: AOCS method, 44.5% ± 0.33%; NMR, 44.8% ± 0.27%; and NIR, 44.2% ± 0.81%. Analysis of variance of the means of the 3 methods of analysis indicated no difference ($p>0.05$) in oil content due to the method. However, there was a difference ($p<0.001$) in total oil content due to replicated analyses of the same sample with the NIR method. With the AOCS and NMR methods, no effect ($p>0.05$) of replicated analyses of the same sample was found. 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 means of the other 2 methods, the coefficient of variations for all samples were consistently higher for the NIR analyses than for the AOCS and NMR analyses.

INTRODUCTION

The standard method for the determination of oil content of oilseeds since about 1880s has been the direct solvent extraction method. All international and most domestic trading of oilseeds are based on this technique and as such is accepted as the "Reference Method" of analysis. The extraction method usually used is a slow and time-consuming process and involves use of flammable solvents. Moreover, it leads to the destruction of the sample, which can be an inconvenience, in particular for the plant breeder. These serious drawbacks resulted in the development of wide-line nuclear magnetic resonance (NMR) and near-infrared reflectance (NIR) spectroscopy techniques.

Wide-line NMR is a term used to describe low resolution nuclear magnetic resonance. The NMR technique measures total hydrogen associated with the oil and water in seed (the only liquid constituents) independent of the hydrogen associated with the non-oil matrix (1). If the measurement is made on dry seed, the response of the apparatus is directly proportional to the quantity of oil present in the seed (2). Accurate estimates of oil content of oilseeds, however, can be made when moisture contents are below 4% (3).

In 1960, Conway (4) first used NMR to analyze whole seed for oil content. Since the process is nondestructive and feasible even on single seeds, geneticists and plant breeders have used the technique extensively (5-7). NMR provides a rapid, accurate means of measuring oil content of oilseeds (3,8) and has been found to be more reproducible and statistically more reliable than AOCS and other extraction methods (2,3,9).

Robertson and Morrison (8) reported that NMR gave accurate estimates of the oil contents of sunflower seed, but they found the NMR response varied, depending on the linoleic acid content. Wide-line NMR now is being used in the domestic trading of sunflower seed.

The establishment of NIR as a viable procedure for the estimation of protein in simple commodities was first reported in 1973 (10). NIR has since become firmly established as a simple, rapid and effective analytical tool for the simultaneous prediction of oil, protein and moisture content of grains and oilseeds (11-13). However, the technique also is destructive and has not been applied with

much success in the determination of oil content of sunflower seed primarily because of difficulty in sample grinding and calibration of instrument.

The purpose of this study was to investigate the applicability of wide-line NMR and NIR for determining oil content of sunflower seed and to compare their accuracy and reproducibility with the standard AOCS extraction method.

MATERIALS AND METHODS

Samples of hybrid sunflower seed (*Helianthus annuus* L.) with different oil contents were obtained from the 1977 National Sunflower Performance Trial plantings and from 1978 and 1979 experimental plantings of Dr. V.E. Green, Jr., University of Florida, Gainesville. The samples were analyzed for moisture content by AOCS Method Ai 2-75 and for oil content by AOCS Method Ai 3-75 (14).

NMR Analysis

The wide-line nuclear magnetic resonance (NMR) instrument used for these studies was the Newport Analyzer MkIII equipped with a 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 3 hr at 130 C and equilibrated to room temperature in a desiccator. Readings were taken on ca. 50 g of seed and oil contents were calculated (8). Subsequent studies have shown that accurate NMR estimates of oil content of sunflower seed can be obtained by drying seed for 30 min (J.A. Robertson, unpublished data).

NIR Analysis

Near-infrared reflectance (NIR) analysis of ground sunflower seed was conducted with a Neotec Model 51A Feed Quality Analyzer (FQA) which consists of the spectrometer and a microcomputer. The spectral range of the FQA-51A is 1.50-2.36 μm and 6 discrete sections of the range are scanned. Therefore, a discontinuous spectrum over the total range of ca. 0.5 μm is obtained. A total of 2,000 data points is assigned to the filter wheel with 120 points/filter for total of 720 usable data points. The 6 filters and their positions in the commercial FQA-51A are described by Barton and Burdick (15). Slight modifications were made in the fixed filter wavelengths of the instrument. The filter wavelengths (μm) 1.68, 1.97 and 2.33 were replaced with 1.72, 2.14 and 2.36, respectively. Although any filter may be placed in any filter position, each poisition is associated with a specific set of "pulse points" (Neotec's designation of data point or computer memory address). A nomograph provided by Neotec was used to convert data points to wavelengths.

The log reflectance (1/R) vs pulse points (wavelengths) data for 3 sunflower samples of low, medium and high oil content were obtained with a computer program routine called "Versidump." The optical density data obtained were plotted against pulse points (wavelengths) for these samples to determine wavelengths of minimal and maximal absorbance for each filter. After determination of the wavelengths, the spectra of 32 sunflower seed samples with oil contents evenly distributed between 37.2 and 53.6% were taken and stored in the microcomputer. These data were regressed using the Auto-Cal program to obtain a predication equation for oil content. Each sample had been analyzed in triplicate by the AOCS extraction method (14). Two different calibration curves were developed on the spectral data using second derivative (D_2OD) math and the change in optical density (ΔOD). The second derivative method involves correlating a D_2OD function of the absorption spectrum values, whereas the ΔOD method involves correlating the difference between optical density measurements taken at 2 specific points of the spectrum. These spectral data are then regressed with the entered AOCS values. These result in multiple regression equations of the type described by Norris et al. (16), together with the standard deviation of calibration and correlation coefficients for total oil content. For a more thorough explanation of math treatments, see Barton and Burdick (15), Norris et al. (16), and Shenk et al. (17).

Samples were prepared for NIR analysis by grinding 12 g seed with 12 g Hyflo Super Cel for 45 sec with Krups 75 high-speed grinder, mixing well with a spatula, and then grinding for an additional 45 sec. The ground mixture was transferred into an airtight jar. Another 12 g seed-12 g Super Cel mixture was ground in the same manner and added to the jar. The combined, ground sample was then thoroughly mixed and an aliquot packed into a Neotec sample cup.

To compare the NMR and NIR methods with the standard AOCS extraction method, 8 different hybrid sunflower seed samples were divided into 5 aliquots for each of the 3 methods and analyzed as already described.

RESULTS AND DISCUSSION

The wavelengths chosen for sunflower samples of high, medium and low oil content are shown in Table I. Filters II, V and VI were scanned using Versidump 1 and 2 due to the oil bands being present in the scanning range of these filters. The optimal oil wavelengths chosen are in close agreement with those reported by Norris and Barnes (18) and Rotolo (19) for soybeans and extracted soybean oil. The shifts in the wavelengths that did occur are possibly due to the sunflower sample, sample preparation or differences in instrument optics.

The calibration and equation developments for 32 sunflower samples is shown in Table II. The wavelengths chosen from a "Versidump" output were refined by adjusting the starting data points (wavelengths) close to those

TABLE I

Wavelengths (μm) Chosen by Neotec FQA-51A for Sunflower Seed Samples of High, Medium and Low Oil Content

	Math	Filter II	Filter V	Filter VI
Scanning range (μm)		1.61-1.70	2.24-2.30	2.29-2.35
Wavelength (μm)	$\Delta OD \log (1/R)$[a]	1.672	2.301	2.355
	$D_2OD \log (1/R)$[b]	1.699	2.287	2.349
			2.293	2.357

[a]$\Delta OD \log (1/R)$ = difference in optical density of log reflectance.

[b]$D_2OD \log (1/R)$ = second derivative of log reflectance.

TABLE II

Equation Development for 32 Sunflower Samples

Equation number	Math	Wavelength (μm)	Standard error of calibration	Correlation coefficient
1	ΔOD log (1/R)	1.704, 1.698	1.45	.95
2	ΔOD log (1/R)	2.301, 2.305, 2.290	1.38	.94
3	D_2OD log (1/R)	2.348, 2.349	1.64	.92
4	D_2OD log (1/R)	2.301	1.67	.91

TABLE III

Oil Analysis of Sunflower Seed Samples by NIR Spectroscopy

Sample no.	Reps 1	2	3	4	5	Mean	Std. dev.	C.V.
					%			
1	55.7	54.9	54.1	52.2	52.6	53.9	1.49	2.76
2	42.3	41.5	40.9	40.3	41.0	41.2	0.75	1.82
3	43.5	41.1	42.4	42.4	43.2	42.5	0.93	2.19
4	46.5	46.5	46.7	45.6	45.8	46.2	0.49	1.06
5	43.1	41.8	40.6	40.6	41.2	41.5	1.04	2.51
6	45.2	44.9	43.6	44.9	44.4	44.6	0.63	1.41
7	45.2	43.9	44.5	43.7	43.5	44.2	0.69	1.56
8	40.0	39.8	39.0	39.1	39.2	39.4	0.45	1.14
Overall mean						44.2	0.81	1.81

TABLE IV

Oil Analysis of Sunflower Seed Samples by Wide-Line NMR

Sample no.	Reps 1	2	3	4	5	Mean	Std. dev.	C.V.
					%			
1	53.9	54.0	53.8	53.8	53.8	53.9	0.09	0.17
2	40.9	39.9	41.4	40.1	41.4	40.7	0.71	1.74
3	43.8	43.6	43.7	43.7	43.4	43.6	0.15	0.34
4	47.5	47.7	47.7	47.5	47.6	47.6	0.10	0.21
5	42.2	41.9	42.4	42.1	41.9	42.1	0.21	0.50
6	44.2	44.5	44.9	44.5	45.1	44.6	0.36	0.81
7	46.3	46.4	46.2	46.4	46.4	46.3	0.09	0.19
8	39.7	38.9	39.5	40.1	39.3	39.5	0.45	1.14
Overall mean						44.8	0.27	0.64

originals picked to obtain maximal accuracy. Equations 1 and 2 (Table II) were obtained by regressing the difference between optical density measurements taken at 2 specific points of the spectrum with the AOCS values for the 32 samples. Equations 3 and 4 were obtained by regressing the second derivative of the log 1/R spectrum with the entered AOCS values. Based on the standard error of calibration and the correlation coefficient, equation 2 appears to be the best. This was confirmed by comparing the 4 NIR equations with the AOCS values obtained on 8 sunflower samples (Table V, vide infra). The best results were obtained using equation 2 (Table II). The standard error of prediction and the correlation coefficients were .66 and .97, respectively. The oil analysis of 8 sunflower seed samples by NIR using equation 2 are shown in Table III. The overall mean oil content was 44.2% with a standard deviation (SD) of ±0.81 and coefficient of variation (CV) of 1.81%. Analyses of variance indicated a difference ($p<.001$) in total oil content due to replicated analyses of the same sample. The analyses of the samples by wide-line NMR are shown in Table IV. The overall mean oil content was 44.8% with a SD of ±0.27% and CV of .64%. There was no effect ($p>.05$) of replicated analyses of the same sample with wide-line NMR. In the case of the analyses by the AOCS method (Table V), the overall mean was 44.5% with a SD of ±0.33%, and a CV of .74% and no significant effect ($p>.05$) was obtained due to replicated analysis of the same sample.

Analysis of variance of the means of the 3 methods of analysis (Tables III-V) indicated no difference ($p>.05$) in total oil content due to the method. The overall means of the 3 methods were similar. NMR had the lowest SD and a slightly higher but insignificant oil content than the reference AOCS method. Correlation coefficients between the AOCS method and NMR and NIR were 0.998 and 0.978, respectively. The correlation between NMR and NIR was 0.98. 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 AOCS and NMR values, the NIR results were quite variable (average range of 2% between the reps of the 8 samples) with CV for all samples consistently higher than the other 2 methods.

Even though the NIR oil analysis is very rapid, even

TABLE V

Oil Analysis of Sunflower Seed Samples by AOCS Method Ai 3-75

Sample no.	Reps 1	2	3	4	5	Mean	Std. dev.	C.V.
					%			
1	53.6	53.3	53.9	54.0	54.1	53.8	0.33	0.61
2	39.9	39.2	40.1	40.1	39.7	39.8	0.37	0.93
3	43.2	43.4	42.9	43.2	43.1	43.2	0.18	0.42
4	47.1	47.3	47.6	47.7	47.1	47.4	0.28	0.59
5	41.8	41.0	41.7	42.0	41.9	41.7	0.40	0.96
6	43.7	44.9	44.3	44.3	43.8	44.2	0.48	1.09
7	46.6	45.4	46.0	45.7	46.0	45.9	0.44	0.96
8	39.7	39.8	39.9	39.6	39.6	39.7	0.13	0.33
Overall mean						44.5	0.33	0.74

faster than wide-line NMR, the technique has some obvious disadvantages. In the first place, samples have to be finely ground for NIR analysis. Sunflower seeds have tough, fibrous hulls (20-30%, oilseed type) which makes it very difficult to obtain a uniform fine grind. The NIR instrument response to composition shows significant differences due to mean particle size, particle size distribution, and bulk density (10). Hymowitz et al. (11) reported that particle size or grinding time requires standardization for consistent estimates of protein and oil concentration in corn and soybeans by NIR.

The wide-line NMR is calibrated daily with a single seed sample. The NIR instrument, however, requires large numbers of samples representing the entire range of oil expected in the crop to calibrate the instrument. The 32 samples we used probably were not adequate; however, samples of sunflower seed of widely different oil contents are difficult to obtain. Because the regression curves developed for the FQA-51A are based entirely on chemical analyses, extreme care must be used to ensure accurate chemical analyses of seed samples used for calibration (11). Using a sophisticated NIR spectrocomputer system, we are continuing our investigations of the applicability of NIR for analysis of sunflower seed.

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