# Determination of Seed Oil Content and Fatty Acid Composition in Sunflower Through the Analysis of Intact Seeds, Husked Seeds, Meal and Oil by Near-Infrared Reflectance Spectroscopy

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**ABSTRACT:** A methodological study was conducted to test the potential of near-infrared reflectance spectroscopy (NIRS) to estimate the oil content and fatty acid composition of sunflower seeds. A set of 387 intact-seed samples, each from a single plant, were scanned by NIRS, and 120 of them were selected and further scanned as husked seed, meal, and oil. All samples were analyzed for oil content (nuclear magnetic resonance) and fatty acid composition (gas chromatography), and calibration equations for oil content and individual fatty acids ($C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$) were developed for intact seed, husked seed, meal, and oil. For intact seed, the performance of the calibration equations was evaluated through both cross- and external validation, while cross-validation was used in the rest. The results showed that NIRS is a reliable and accurate technique to estimate these traits in sunflower oil (validation $r^2$ ranged from 0.97 to 0.99), meal ($r^2$ from 0.92 to 0.98), and husked seeds ($r^2$ from 0.90 to 0.97). According to these results, there is no need to grind the seeds to scan the meal; similarly accurate results are obtained by analyzing husked seeds. The analysis of intact seeds was less accurate ($r^2$ from 0.76 to 0.85), although it is reliable enough to use for pre-screening purposes to identify variants with significantly different fatty acid compositions from standard phenotypes. Screening of intact sunflower seeds by NIRS represents a rapid, simple, and cost-effective alternative that may be of great utility for users who need to analyze a large number of samples.

*JAOCS 75*, 547–555 (1998).

**KEY WORDS:** Fatty acid composition, *Helianthus annuus*, husked-seed samples, intact-seed samples, NIRS, near-infrared reflectance spectroscopy, meal samples, oil content, oil samples, sunflower.

Gas–liquid chromatography (GLC) is widely used to determine the fatty acid composition of the oil in sunflower (*Helianthus annuus* L.) seeds. This technique is reliable and accurate, but also expensive and time-consuming, and requires the use of toxic, flammable, and polluting reagents and gases (1). For many applications, e.g., screening for fatty acid composition in plant breeding programs, a more rapid, simple, and nondestructive method is needed (2).

Near-infrared reflectance spectroscopy (NIRS) is a rapid and nondestructive multitrait technique that is widely used to analyze an increasing number of seed quality traits in food and agricultural products (3). Holman and Edmondson (4) and Murray (5) studied the near-infrared spectra of homologous series of fatty acids and reported spectral differences associated with both chainlength and unsaturation level. Based on these studies, NIRS has been used to discriminate between different oil types (6–8) and to estimate the fatty acid composition of the oil in intact-seed rapeseed (9–11), intact-seed mustard (1,12,13), and husked sunflower seeds (2).

In sunflower, NIRS was first applied to determine oil, protein, water, and fiber contents in meal samples (14–16). Sato *et al.* (2) studied the ability of NIRS to estimate the fatty acid composition in husked sunflower seeds. They found that husked seeds that differed in linoleic acid content could be accurately discriminated by NIRS. However, no results have been reported for the other major fatty acids. Furthermore, if NIRS is to be used as a rapid screening technique for this species, intact seed should be analyzed to avoid the extra time and additional cost of husking seeds.

The objective of this work was to study the potential of NIRS to estimate the seed oil content and its fatty acid composition in sunflower by analyzing intact seed, husked seed, meal, and oil.

## MATERIALS AND METHODS

*Sunflower material*. A total of 387 individual sunflower plants were used in this study. The plants were chosen from different lines that showed a wide range of fatty acid composition of the seed oil. They included several lines with standard fatty acid composition of the oil, a mutant line with high oleic acid content (17), three mutant lines with increased stearic acid content (18), and two mutant lines with increased palmitic acid content (18,19). Table 1 shows the number of plants used from each line. All plants were grown and self-pollinated in the greenhouse in 1995.

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**TABLE 1**
**Sunflower Lines Used in This Study**

| Line | Phenotype | Number of samples | Reference |
|---|---|---|---|
| CAS-3 | Very high $C_{18:0}$ | 26 | 18 |
| CAS-4 | High $C_{18:0}$ | 26 | 18 |
| CAS-5 | High $C_{16:0}$ | 30 | 18 |
| CAS-8 | High $C_{18:0}$ | 22 | 18 |
| CAS-12 | High $C_{16:0}$/high $C_{18:1}$ | 21 | 19 |
| HAOL-9 | High $C_{18:1}$ | 30 | 17 |
| Several[a] | Standard | 232 | |

[a]Plants from 20 different inbred lines with standard fatty acid composition of the seed oil were used.

*Global design of the experiment*. Samples of intact seeds from the 387 individual plants were scanned by NIRS. In a second step, 120 samples out of the initial 387 were selected, and the seeds were first husked, then ground, and finally the oil was extracted from the meal. The husked seed, the meal, and the oil from these 120 selected samples were scanned by NIRS. These samples were selected out with the main objective of having a uniform representation of all different fatty acid profiles. However, this work was performed with plant material from a plant breeding program, and several samples from valuable genotypes, which produced very few seeds, had to be excluded owing to the destructive character of the subsequent process.

The NIRS study to estimate oil content and fatty acid composition by analyzing intact seeds included all 387 samples. From them, a total of 100 randomly selected samples were reserved for external validation, and NIRS calibration equations for oil content and individual fatty acids were developed by using the remaining 287 samples. For husked seed, meal and oil, NIRS calibration equations were developed by using the selected set of 120 samples. The performance of these equations was evaluated through cross-validation.

*Sample preparation*. The seeds were husked manually with a scalpel. The husked seeds were ground with an IKA A10 grinder (Janke & Kunkel GmbH & Co. KG, Staufen, Germany). For oil extraction, 1 g of meal was placed into a vial, and 5 mL diethyl ether was added. The vial was shaken periodically over 5 h, and then the solvent was evaporated.

*Chemical measurements*. The oil content was determined on previously desiccated intact seeds by nuclear magnetic resonance (NMR) on an Oxford 4000 analyzer (Oxford Analytical Instruments Ltd., Abingdon, OX, United Kingdom). The fatty acid composition of the oil was analyzed by methyl esterification (20), followed by GLC on a Perkin-Elmer Autosystem gas-liquid chromatograph (Perkin-Elmer Corporation, Norwalk, CT), equipped with a 2-m long column packed with 3% SP-2310/2% SP-2300 on Chromosorb WAW (Supelco Inc., Bellefonte, PA). The oven, injector, and flame-ionization detector were held at 195, 275, and 250°C, respectively. The carrier gas was nitrogen at a flow of 20 mL $min^{-1}$. The analysis time was 12 min.

*NIRS scanning: intact and husked seeds*. About 10 to 15 intact seeds from each sample were randomly selected and scanned on a monochromator NIR Systems model 6500 (NIR Systems, Inc., Silver Springs, MD) by using a small ring cup (ref. IH-0307, NIR Systems), equipped with a microsample insert (ref. IH-0337, Ø 18.5 mm). The reflectance spectra (log 1/*R*) from 400 to 2500 nm were recorded at 2-nm intervals. The seeds were husked and scanned again.

*NIRS scanning: meal*. The meal was scanned as described for the seeds but with a different microsample insert (ref. IH-0337, Ø12.0 mm).

*NIRS scanning: oil*. The oil was scanned by using glass fiber disks (ref. number AP4004705; Millipore Iberica S.A., Madrid, Spain) impregnated with 2–3 drops of oil.

*NIRS calibration*. NIRS calibration equations were developed for oil content (except for oil samples), palmitic acid ($C_{16:0}$), palmitoleic acid ($C_{16:1}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), and linoleic acid ($C_{18:2}$). Fatty acids were expressed as the percentage of total fatty acids. For calibration, only the spectral data from 1100 to 2500 nm were used because inclusion of the segment from 400 to 1100 nm led to poorer results. Second-derivative transformation, De-trend, and standard normal variate (SNV) scatter corrections (21) were applied to the log (1/*R*) spectra, and calibration equations were developed by using modified partial least squares (MPLS) regression (ISI v. 3.10, Infrasoft International, Port Matilda, PA). Cross-validation was used in MPLS regression to avoid overfitting (3). The statistic "1 minus variance ratio (1 − VR)," calculated in cross-validation as an estimate of $r^2$, is called "$r^2$ of cross-validation" throughout the manuscript, to facilitate its interpretation. Table 2 shows the oil content and fatty acid composition of the calibration and validation sets for intact seeds, and the calibration set for husked seed, meal, and oil.

*Spectral analysis*. To detect spectral differences that are associated with different fatty acid profiles in the oil, the oil samples were classified according to the fatty acid composition in the following groups: low palmitic ($C_{16:0} < 10\%$), high palmitic ($C_{16:0} > 25\%$), low stearic ($C_{18:0} < 8\%$), middle stearic ($8\% < C_{18:0} < 15\%$), high stearic ($C_{18:0} > 20\%$), low oleic-to-linoleic ratio (OLR) (%C18:1/%C18:2 < 1.5), middle OLR ($13 < OLR < 19$, $C_{18:1} < 60\%$), and high OLR (OLR > 20, $C_{18:1} > 80\%$), and the average spectrum was calculated within each group. The second-derivative transformation and scatter corrections were applied to the new spectra, and the standard deviations between the average spectra for each fatty acid were studied.

## RESULTS AND DISCUSSION

*Spectral analysis.* Figure 1 shows the average spectra from a set of 120 sunflower samples scanned by NIRS as whole seeds, husked seeds, meal, and oil. The average spectrum of the oil samples had maximum absorption values at 1210, 1412, 1720, 1760, 2144, 2306, and 2348 nm, similar to those reported previously (2,22), except for the peak at 1210 nm. The maximum absorption peaks at 1210, 1720, 1760, 2306, and 2348 nm were still sharp in the average spectrum of the

**TABLE 2**
**Seed Oil Content (%) and Fatty Acid Composition (% of total fatty acids) in the Different Data Sets Included in This Study**

| | Intact seed, calibration | | Intact seed, validation | | Husked seed, meal, and oil, calibration | |
|---|---|---|---|---|---|---|
| | Mean | Range | Mean | Range | Mean | Range |
| Oil | 37.8 | 24.7–51.0 | 37.8 | 23.7–51.6 | 37.0 | 23.7–49.1 |
| $C_{16:0}$ | 9.0 | 3.0–35.5 | 10.0 | 3.5–33.8 | 10.7 | 3.9–35.8 |
| $C_{16:1}$ | 0.7 | 0.0–8.6 | 1.0 | 0.0–8.1 | 1.4 | 0.0–8.6 |
| $C_{16:2}$ | 0.1 | 0.0–2.5 | 0.1 | 0.0–2.3 | 0.2 | 0.0–2.5 |
| $C_{18:0}$ | 6.6 | 1.4–30.3 | 6.2 | 1.3–29.2 | 9.5 | 1.7–28.5 |
| $C_{18:1}$ | 41.5 | 7.7–90.7 | 41.0 | 7.6–90.2 | 42.8 | 9.1–90.5 |
| $C_{18:2}$ | 41.5 | 1.8–74.5 | 41.0 | 1.8–72.2 | 35.2 | 1.9–64.4 |

meal samples and also detectable in the average spectrum of husked seeds. However, in both spectra, the maximum at 1412 nm was not detectable, and a maximum at 2174 nm was found instead of the 2144 nm peak in the oil spectrum. None of the characteristic absorption peaks of the oil spectrum was noticeable in the average spectrum of intact seed, which was poor in absorption characteristics, as was also reported by Sato *et al.* (2).

Spectral differences associated with different fatty acid profiles (Fig. 2) showed that such differences were mainly located within five spectral regions: 1150 to 1250, 1350 to 1400, 1600 to 1800, 2100 to 2200, and 2250 to 2370 nm. Similar spectral regions were reported in a previous study carried out with intact-seed mustard (13), and all of them corresponded to absorption bands associated with fatty acid absorbers (4,5,13,23,24). Moreover, these regions were closely associated with the maximum absorption peaks found in oil samples (Fig. 1). For $C_{16:0}$, the spectral region between 2100 and 2200 nm was especially important, as compared to the other fatty acids (Fig. 2). This may be due to the simultaneous presence of significant levels of $C_{16:1}$ and $C_{16:2}$ (accounting together for up to about 10% of total fatty acids) in the oils with high $C_{16:0}$ levels; previous studies have reported this region as being the most characteristic to detect changes in the level of *cis*-unsaturation (5,22).

*Calibration equations from oil samples.* Table 3 shows the calibration and cross-validation statistics obtained in the development of NIRS calibration equations to estimate the fatty acid composition of sunflower oil samples. The $r^2$ of cross-validation ranged from 0.97 ($C_{18:0}$) to 0.99 ($C_{18:1}$ and $C_{18:2}$), which indicates that a rapid and accurate prediction of the fatty acid composition of sunflower oil samples can be easily obtained by NIRS

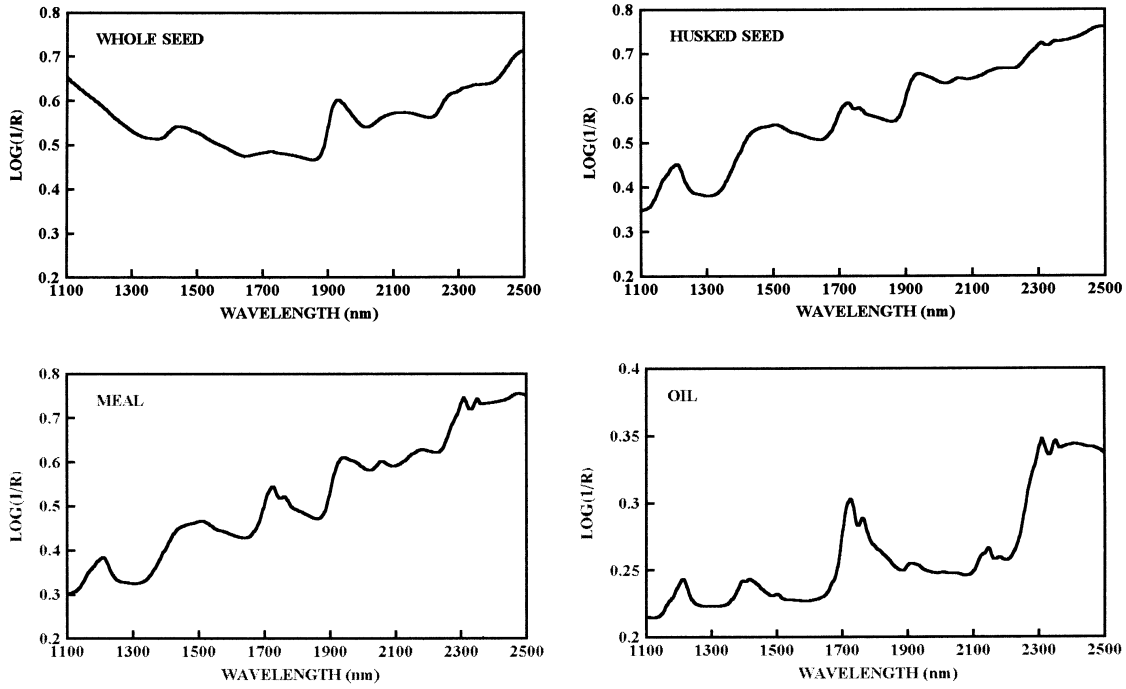


**FIG. 1.** Average near-infrared reflectance spectroscopy (NIRS) spectra of a set of 120 sunflower samples scanned as intact seed, husked seed, meal, and oil.

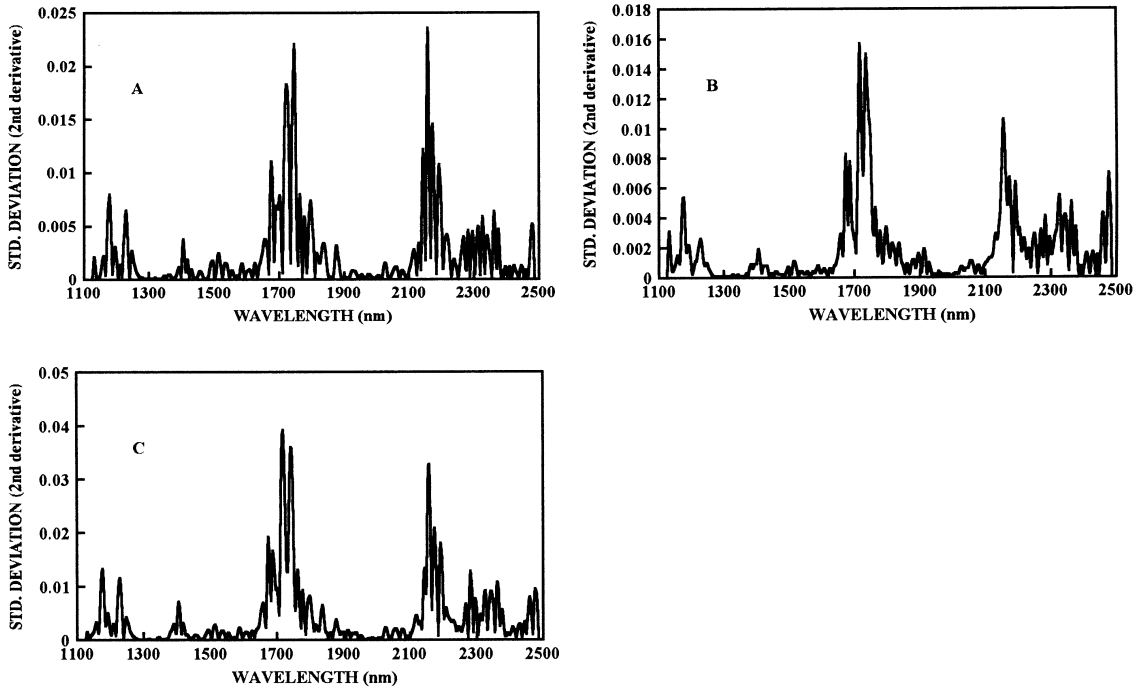


**FIG. 2.** Standard deviations among second-derivative average spectra (low, middle, high fatty acid content). A = $C_{16:0}$, B = $C_{18:0}$, C = ratio $C_{18:1}/C_{18:2}$.

analysis. Figure 3 shows the correlation plot between GLC and NIRS values in the oil samples from the calibration set.

*Calibration equations from meal samples*. The calibration equation developed to estimate total oil content in sunflower meal showed calibration and cross-validation $r^2$ of 0.97 and 0.92, respectively (Table 4), which indicates the high reliability of the NIRS technique to estimate oil content in sunflower meal. These results represent an improvement in comparison to those reported by Robertson and Barton (16), who developed a calibration equation with values of $r^2$ of 0.94 and 0.75 for calibration and validation, respectively. With regard to individual fatty acids, the value of $r^2$ in cross-validation was always higher than 0.90 (Table 4). Therefore, the NIRS technique can provide a simultaneous and accurate estimate of both oil content and oil composition in sunflower meal. Figure 4 shows the correlation plots of NIRS vs. reference method data for these calibration equations.

*Calibration equations from husked-seed samples*. The results obtained with husked seeds (Table 5) were similar to those obtained with meal, which indicates that the additional time and cost effort to grind sunflower seeds is not necessary to obtain an accurate estimate of oil content and oil composition of sunflower seed. Figure 5 shows the correlation plots of NIRS data vs. reference method data for husked seeds. Sato *et al.* (2) reported the reliability of NIRS to predict $C_{18:2}$ content in husked sunflower seeds. The results of our work show that not only $C_{18:2}$ but also the total oil content and the other major fatty acids, i.e., $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, and $C_{18:1}$, can be accurately estimated in husked sunflower seed by NIRS. Furthermore, and in contrast to the aforementioned work (2)

**TABLE 3**
**Calibration and Cross-Validation Statistics in the Development of Calibration Equations for Individual Fatty Acids in Sunflower Oil Samples**

| | Percentage of total fatty acids | | | |
|---|---|---|---|---|
| | Calibration | | Cross-validation | |
| | $r^2$ | SEC[a] | SECV[b] | $r^2$ |
| $C_{16:0}$ | 0.99 | 0.81 | 1.26 | 0.98 |
| $C_{16:1}$ | 0.99 | 0.25 | 0.35 | 0.98 |
| $C_{18:0}$ | 0.99 | 0.79 | 1.22 | 0.97 |
| $C_{18:1}$ | 0.99 | 0.67 | 1.09 | 0.99 |
| $C_{18:2}$ | 0.99 | 0.56 | 0.78 | 0.99 |

[a]SEC, standard error of calibration.
[b]SECV, standard error of cross-validation.

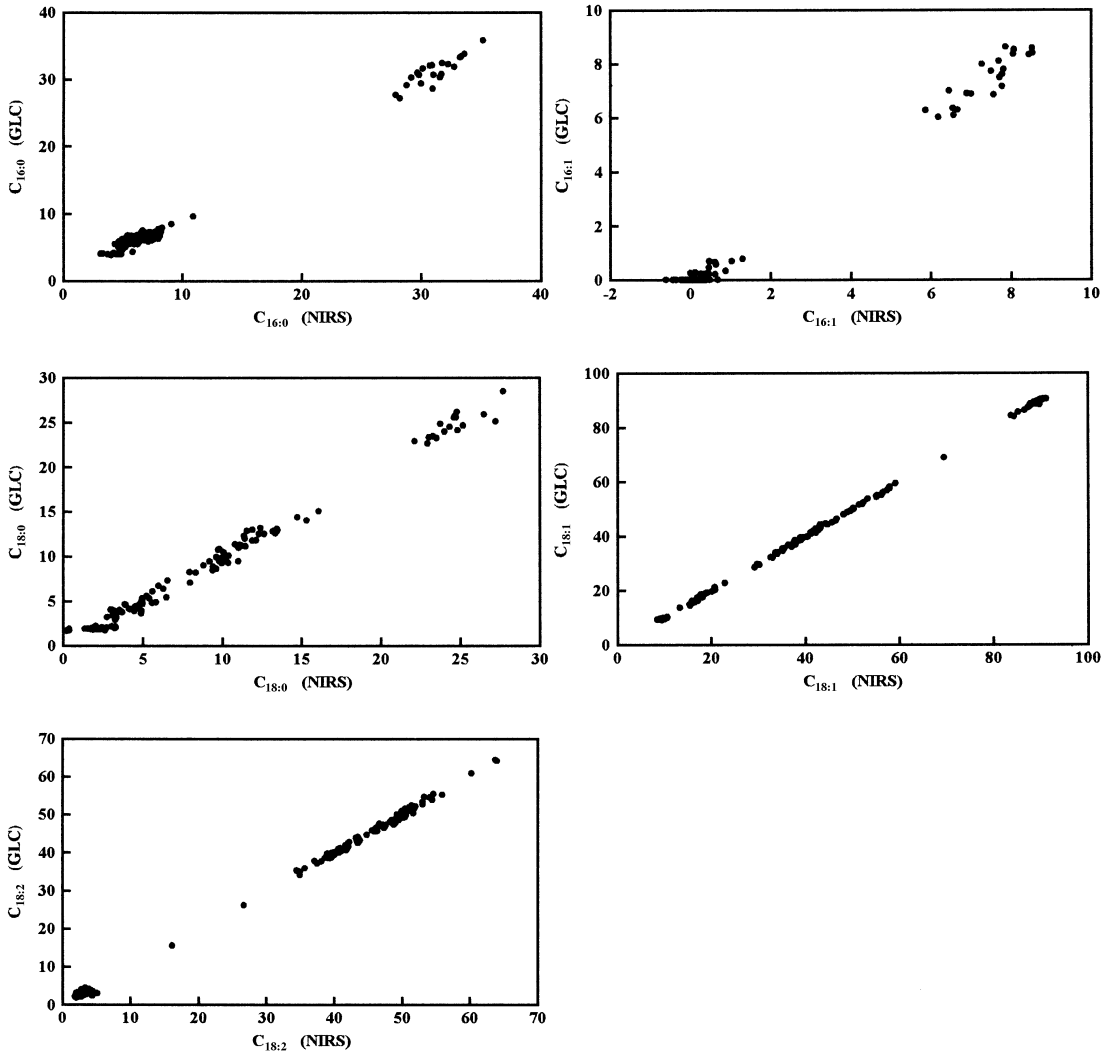


**FIG. 3.** Calibration plots for $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$ contents (NIRS estimated vs. reference method calculated) in a set of 120 oil samples of sunflower. Individual fatty acids are expressed as the percentage of total fatty acids. GLC, gas–liquid chromatography; for other abbreviations see Figure 1.

in which only the spectral region from 1696 to 1724 nm was used to predict the $C_{18:2}$ content, we have developed whole-spectrum (1100–2500 nm) calibration equations, which are of great interest because NIRS information of fatty acids is not concentrated in a single NIRS region but distributed in several regions along the NIRS spectrum (13,25).

*Calibration equations from intact-seed samples.* The accuracy and reliability of calibration equations for oil content

**TABLE 4**
**Calibration and Cross-Validation Statistics in the Development of Calibration Equations for Oil Content and Individual Fatty Acids in Sunflower Meal Samples[a]**

| | Calibration | | Cross-validation | |
|---|---|---|---|---|
| | $r^2$ | SEC[a] | SECV[b] | $r^2$ |
| | | Percentage | | |
| Oil content | 0.97 | 1.02 | 1.57 | 0.92 |
| | | Percentage of total fatty acids | | |
| $C_{16:0}$ | 0.97 | 1.63 | 2.73 | 0.93 |
| $C_{16:1}$ | 0.99 | 0.40 | 0.83 | 0.95 |
| $C_{18:0}$ | 0.96 | 1.44 | 2.36 | 0.92 |
| $C_{18:1}$ | 0.99 | 2.10 | 3.12 | 0.98 |
| $C_{18:2}$ | 0.99 | 2.21 | 2.78 | 0.98 |

[a]See Table 3 for abbreviations.

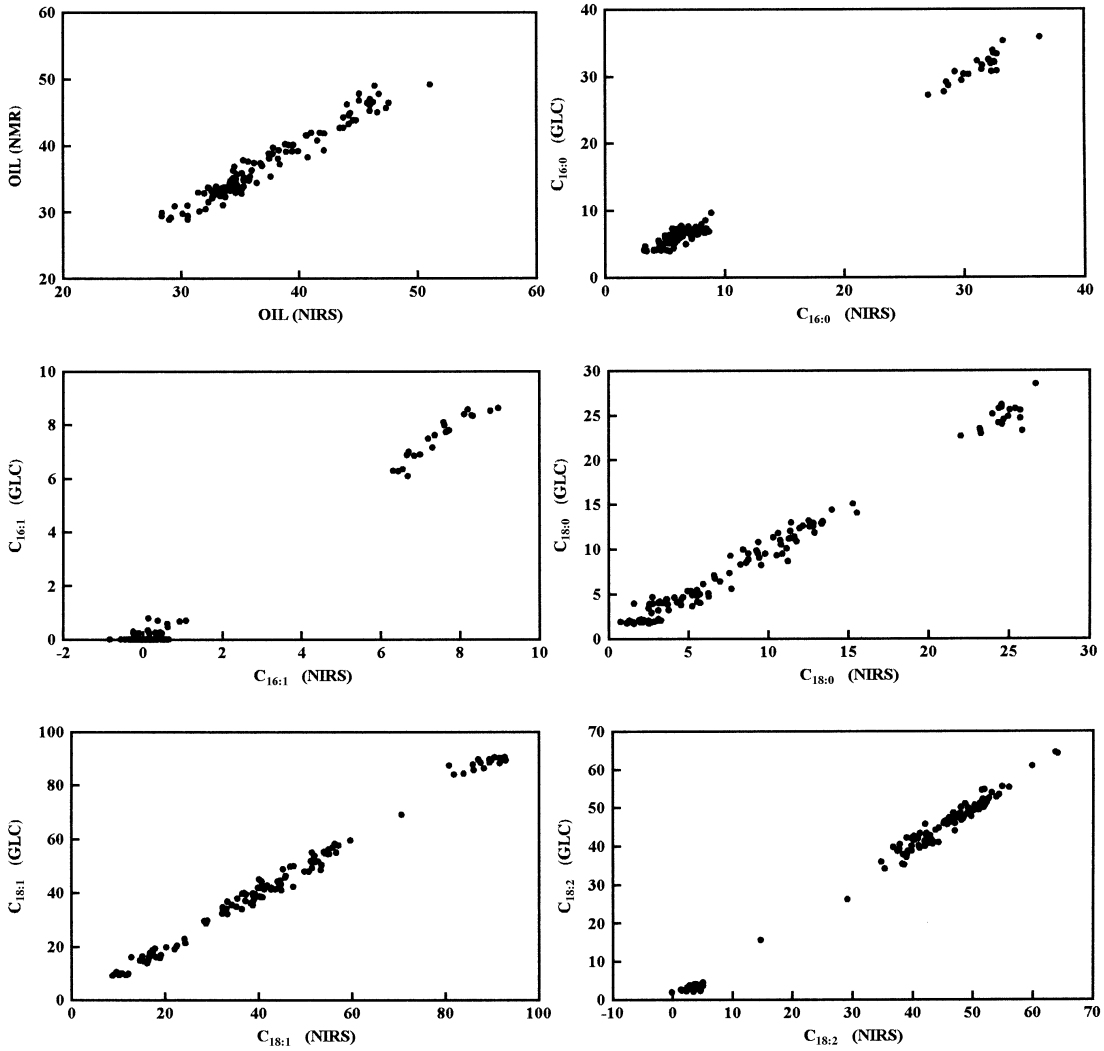


**FIG. 4.** Calibration plots for seed oil content, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$ contents (NIRS estimated vs. reference method calculated) in a set of 120 meal samples of sunflower. Oil content is expressed in percentage, and individual fatty acids as percentage of total fatty acids. NMR, nuclear magnetic resonance; for other abbreviations see Figures 1 and 3.

and individual fatty acids, developed from intact-seed samples, were lower than those obtained with husked seed, meal, and oil, as was expected from the attenuation of the characteristic absorption peaks in the spectrum of intact seed (Fig. 1). The $r^2$ values, calculated by using an external validation file, ranged from 0.76 ($C_{18:1}$) to 0.85 ($C_{18:0}$). These values were not high enough to support a recommendation to routinely analyze oil and fatty acid composition in intact sun-

**TABLE 5**
**Calibration and Cross-Validation Statistics in the Development of Calibration Equations for Oil Content and Individual Fatty Acids in Sunflower Husked-Seed Samples[a]**

| | Calibration | | Cross-validation | |
|---|---|---|---|---|
| | $r^2$ | SEC | SECV | $r^2$ |
| | | Percentage | | |
| Oil content | 0.95 | 1.19 | 1.69 | 0.90 |
| | | Percentage of total fatty acids | | |
| $C_{16:0}$ | 0.96 | 1.89 | 2.81 | 0.92 |
| $C_{16:1}$ | 0.99 | 0.14 | 0.63 | 0.97 |
| $C_{18:0}$ | 0.96 | 1.40 | 2.10 | 0.92 |
| $C_{18:1}$ | 0.99 | 2.59 | 3.98 | 0.97 |
| $C_{18:2}$ | 0.99 | 2.15 | 3.55 | 0.97 |

[a]See Table 3 for abbreviations.

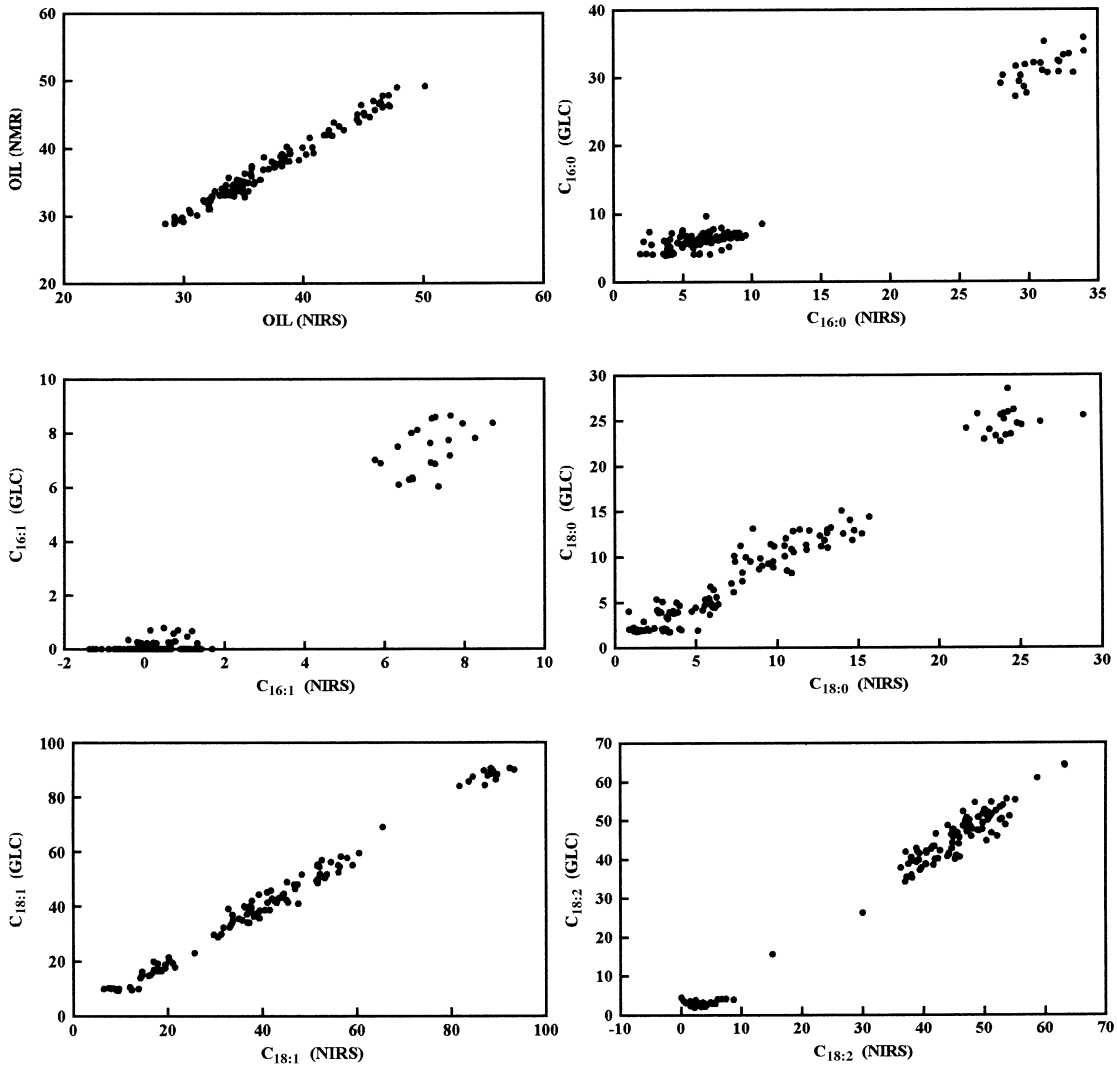


**FIG. 5.** Calibration plots for seed oil content, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$ content (NIRS estimated vs. reference method calculated) in a set of 120 husked-seed samples of sunflower. Oil content is expressed in percentage, and individual fatty acids as percentage of total fatty acids. For abbreviations see Figures 1, 3, and 4.

flower seeds by NIRS. However, they revealed that, although less accurate than the calibration equations for husked seed, NIRS calibration equations for intact seed may be of great use, especially for rapid screening purposes. Figure 6 shows the scatter plots of NIRS data vs. reference data for oil content and fatty acids in the external validation set. Despite the high standard error of performance (Table 6), a preselection of variants by using NIRS data would be very efficient. For

**TABLE 6**
**Calibration, Cross-Validation, and External Validation Statistics in the Development of Calibration Equations for Oil Content and Individual Fatty Acids in Sunflower Intact-Seed Samples**

| | Calibration | | Cross-validation | | External validation | |
|---|---|---|---|---|---|---|
| | $r^2$ | SEC | SECV | $r^2$ | SEP[a] | $r^2$ |
| | | | Percentage | | | |
| Oil content | 0.87 | 1.86 | 2.18 | 0.82 | 2.07 | 0.83 |
| | | | Percentage of total fatty acids | | | |
| $C_{16:0}$ | 0.86 | 2.83 | 3.58 | 0.83 | 3.63 | 0.82 |
| $C_{16:1}$ | 0.85 | 0.73 | 0.91 | 0.76 | 0.85 | 0.79 |
| $C_{18:0}$ | 0.92 | 1.45 | 1.73 | 0.89 | 1.82 | 0.85 |
| $C_{18:1}$ | 0.86 | 6.75 | 8.94 | 0.76 | 8.75 | 0.76 |
| $C_{18:2}$ | 0.85 | 5.83 | 7.25 | 0.77 | 7.58 | 0.78 |

[a]SEP, standard error of performance. See Table 3 for abbreviations.

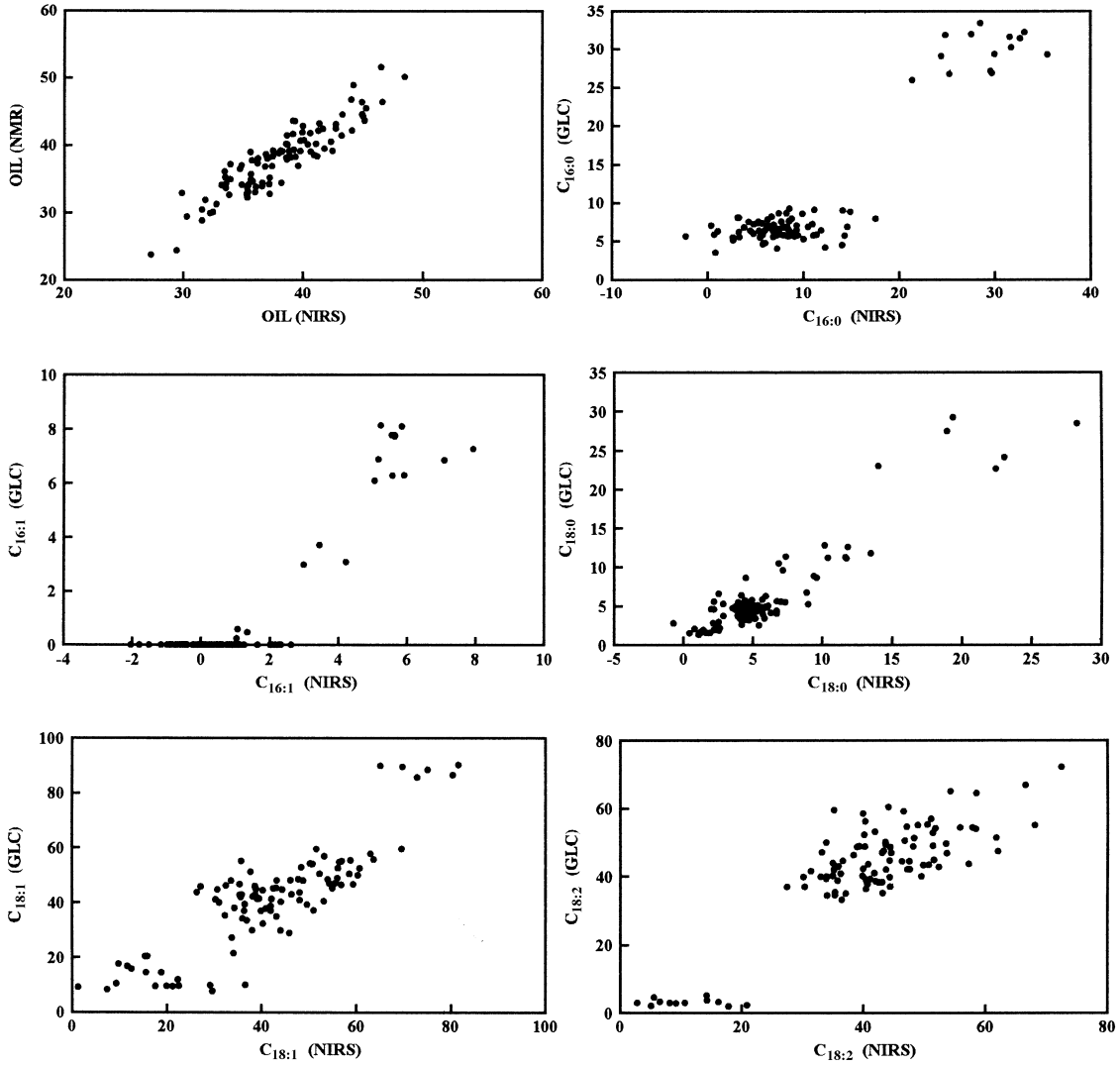


**FIG. 6.** Prediction plots for seed oil content, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$ contents (NIRS predicted vs. reference method calculated) in external validation of NIRS calibration equations for intact sunflower seeds. The validation set consisted of 100 intact-seed samples. Oil content is expressed in percentage, and individual fatty acids as percentage of total fatty acids. For abbreviations see Figures 1, 3, and 4.

$C_{16:0}$, for example, there were two well-differentiated classes: standard seeds, with GLC values of under 15%, and mutant seeds, with GLC values of over 25%, and also with significant levels of $C_{16:1}$. The discrimination of mutant from standard phenotypes by analyzing intact seeds could be carried out by using either the calibration equation for $C_{16:0}$ or the equation for $C_{16:1}$, with high reliability for both. Furthermore, the identification of variants for high values of oil content, $C_{18:0}$, $C_{18:1}$ or $C_{18:2}$ content would also be possible by analyzing intact seeds. Owing to the high standard error of performance, NIRS equations for intact sunflower seed should only be used for prescreening purposes, i.e., to preselect variants to be further analyzed by the corresponding reference method. In this way, NIRS would reduce the number of more expensive and time-consuming GLC analyses. Furthermore, it has the additional advantage that the oil content is estimated simultaneously with the fatty acid composition, which also helps to reduce the number of NMR analyses when a simultaneous selection for oil content and fatty acid composition of the oil is performed.

## ACKNOWLEDGMENT

This study was carried out with an NIRS instrument from the Servicio Centralizado NIRS, University of Córdoba.

## REFERENCES

1. Velasco, L., J.M. Fernández-Martínez, and A. De Haro, Screening Ethiopian Mustard for Erucic Acid by Near Infrared Reflectance Spectroscopy, *Crop Sci. 36*:1068–1071 (1996).
2. Sato, T., Y. Takahata, T. Noda, T. Tanagisawa, T. Morishita, and S. Sakai, Nondestructive Determination of Fatty Acid Composition of Husked Sunflower (*Helianthus annuus* L.) Seeds by Near-Infrared Spectroscopy, *J. Am. Oil Chem. Soc. 72*:1177–1183 (1995).
3. Shenk, J., and M.O. Westerhaus, *Analysis of Agriculture and*

*Food Products by Near Infrared Reflectance Spectroscopy*, Infrasoft International, Port Matilda, PA, 1993, pp. 116.
4. Holman, R.T., and P.R. Edmondson, Near-Infrared Spectra of Fatty Acids and Some Related Substances, *Anal. Chem. 28*:1533–1538 (1956).
5. Murray, I., NIR Spectra of Homologous Series of Organic Compounds, in *NIR/NIT Spectroscopy*, edited by J. Hollo *et al.*, Akademiai Kiado, Budapest, Hungary, 1987, pp. 13–28.
6. Sato, T., Application of Principal Component Analysis on Near-Infrared Spectroscopic Data of Vegetable Oils for Their Classification, *J. Am. Oil Chem. Soc. 71*:293–298 (1994).
7. Bewig, K.M., A.D. Clarke, C. Roberts, and N. Unklesbay, Discriminant Analysis of Vegetable Oils by Near-Infrared Reflectance Spectroscopy, *Ibid. 71*:195–200 (1994).
8. Robert, P., M.F. Devaux, and M. Safar, Canonical Correlation Analysis: Application to Near and Mid Infrared Spectroscopy, in *Proceedings of the 6th International NIRS Conference*, Lorne, Australia, 18–22 April 1994, edited by G.D. Batten, P.C. Flinn, L.A. Welsh, and A.B. Blakeney, 1995, pp. 62–65.
9. Koester, S., and C. Paul, Application of Near Infrared Reflectance Spectroscopy (NIRS) in Breeding Rapeseed for Improved Quality, in *Science for Plant Breeding (Book of Poster Abstracts, XII Eucarpia Congress)*, edited by the European Association for Research on Plant Breeding (EUCARPIA), Paul Parey Publishers, Berlin and Hamburg, 1989, vol. 2, posters 31–4.
10. Reinhardt, T.-C., and G. Röbbelen, Quantitative Analysis of Fatty Acids in Intact Rapeseed by Near Infrared Reflectance Spectroscopy, in *Proceedings of the 8th International Rapeseed Conference*, Saskatoon, Canada, 9–11 July 1991, edited by GCIRC, Saskatoon, 1991, pp. 1380–1384.
11. Reinhardt, T.-C., C. Paul, and G. Röbbelen, Quantitative Analysis of Fatty Acids in Intact Rapeseed by NIRS, in *Making Light Work. Advances in Near Infrared Spectroscopy*, edited by I. Murray and I. Cowe, VCH, London, 1992, pp. 1380–1384.
12. Velasco, L., J.M. Fernández-Martínez, M. Del Rio, and A. De Haro, The Applicability of NIRS for Estimating Multiple Seed Quality Components in Ethiopian Mustard, in *Proceedings of the 9th International Rapeseed Congress*, Cambridge, UK, 4–7 July 1995, edited by GCIRC, Henry Ling Limited, Dorchester, 1995, pp. 867–869.
13. Velasco, L., J.M. Fernández-Martínez, and A. De Haro, Determination of the Fatty Acid Composition of the Oil in Intact-Seed Mustard by Near-Infrared Reflectance Spectroscopy, *J. Am. Oil Chem. Soc. 74*:1595–1602 (1997).
14. Robertson, J.A., and W.R. Windham, Comparative Study of Methods of Determining Oil Content of Sunflower Seed, *Ibid. 58*:993–996 (1981).
15. Kaffka, K.J., K.H. Norris, J. Perédi, and A. Balogh, Attempts to Determine Oil, Protein, Water and Fiber Content in Sunflower Seeds by the NIR Technique, *Acta Alimentaria 11*:253–269 (1982).
16. Robertson, J.A., and F.E. Barton II, Oil and Water Analysis of Sunflower Seed by Near-Infrared Reflectance Spectroscopy, *J. Am. Oil Chem. Soc. 61*:543–547 (1984).
17. Fernández-Martínez, J., J. Muñoz, and J. Gómez-Arnau, Performance of Near-Isogenic High and Low Oleic Acid Hybrids of Sunflower, *Crop Sci. 33*:1158–1163 (1993).
18. Osorio, J., J. Fernández-Martínez, M. Mancha, and R. Garcés, Mutant Sunflowers with High Concentration of Saturated Fatty Acids in the Oil, *Ibid. 35*:739–742 (1995).
19. Fernández-Martínez, J., M. Mancha, J. Osorio, and R. Garcés, Sunflower Mutant Containing High Levels of Palmitic Acid in High Oleic Background, *Euphytica 97*:113–116 (1997).
20. Garcés, R., and M. Mancha, One-Step Lipid Extraction and Fatty Acid Methyl Esters Preparation from Fresh Plant Tissues, *Anal. Biochem. 211*:139–143 (1993).
21. Barnes, R.J., M.S. Dhanoa, and S.J. Lister, Standard Normal Variate Transformation and De-Trending of Near-Infrared Diffuse Reflectance Spectra, *Appl. Spectrosc. 43*:772–777 (1989).
22. Sato, T., S. Kawano, and M. Iwamoto, Near Infrared Spectral Patterns of Fatty Acid Analysis from Fats and Oils, *J. Am. Oil Chem. Soc. 68*:827–833 (1991).
23. Murray, I., Forage Analysis by Near Infra-Red Spectroscopy, in *Sward Measurement Handbook,* 2nd edn., edited by A. Davies, R.D. Baker, S.A. Grant, and A.S. Laidlaw, British Grassland Society, 1993, pp. 285–312.
24. Osborne, B.G., T. Fearn, and P.H. Hindle, *Practical NIR Spectroscopy with Applications in Food and Beverage Analysis*, Longman Scientifical & Technical, Harlow, 1993, pp. 13–35.
25. Panford, J.A., and J.M. de Man, Determination of Oil Content of Seeds by NIR: Influence of Fatty Acid Composition on Wavelength Selection, *J. Am. Oil Chem. Soc. 67*:473–482 (1990).

[Received June 19, 1997; accepted November 13, 1997]