Nondestructive NMR Determination of Oil Composition in Transformed Canola Seeds

William C. Hutton[a,*], Joel R. Garbow[b], and Thomas R. Hayes[c]

[a]Nutrition and Consumer Sector, Monsanto, St. Louis, Missouri 63167, [b]Monsanto Corporate Research, St. Louis, Missouri 63167, and [c]Nutrition and Consumer Sector, Calgene, Davis, California 95616

ABSTRACT: Magic-angle spinning (MAS) ^{13}C nuclear magnetic resonance (NMR) spectroscopy is a convenient method for nondestructive, quantitative characterization of seed oil composition. We describe results for intact hybrid and transformed canola seeds. The MAS ^{13}C NMR technique complements and agrees with gas chromatography results. The spectral resolution approaches that of neat, liquid oils. MAS ^{13}C NMR data allow quantitative analysis of major oil components, including saturates and oleic, linoleic, and linolenic acyl chains. ^{13}C NMR directly and quantitatively elucidates, triglyceride regiochemistry and acyl chain *cis-trans* isomers that cannot be quickly detected by other methods. MAS ^{13}C NMR can serve as the primary method for development of near-infrared seed oil calibrations. These NMR methods are nondestructive and attractive for plant-breeding programs or other studies (e.g., functional genomics) where loss of seed viability is inconvenient.

Paper no. L8308 in *Lipids 34*, 1339–1346 (December 1999).

The analysis of plant seed oils is critical to assess the value of individual seeds as well as entire crops. Numerous methods are used to determine seed oil composition (1,2). Methods based on gas chromatography (GC), GC–mass spectrometry (GC–MS), and high-performance liquid chromatography–MS (HPLC–MS) are destructive and require chemical manipulation. GC analysis—the most common method—is inexpensive, precise, and efficient. By contrast, optical spectroscopy (near-infrared, NIR) and nuclear magnetic resonance (NMR) methods are nondestructive and attractive for plant-breeding programs or other studies where loss of seed viability is inconvenient. For example, an understanding of gene function relevant to oil composition could be gained from mutant plant screening. Single oilseeds would be screened to identify unique triglyceride (TAG) compositions. Linking the change back to the mutation could then lead to an understanding of gene function.

Whole seed NIR analysis is attractive from a cost per seed perspective. In addition, the information content of NIR is high. Total oil content and oil iodine number are measured with NIR. TAG compositions for multiple seed samples of *Brassica carinata* (mustard) are reported (3). However, NIR analysis is a secondary method and requires careful and comprehensive calibrations. In addition, seed variables, such as hull thickness, can complicate NIR data interpretation.

Low-resolution (wide-line) ^{1}H NMR spectroscopy is well known in seed oil analytical chemistry. Wide-line NMR measures total oil and moisture content for groups of seeds, but cannot determine oil composition. Low-resolution NMR is inexpensive; instrument calibration is straightforward and measurements are automated. High-resolution seed NMR studies to observe individual peaks from structurally distinct nuclei are less well known. The first high-resolution ^{13}C measurements on intact seeds appeared in 1974 (4). Reports on the application of ^{13}C NMR with a variety of intact seeds followed (5–12). Magic-angle spinning (MAS) methods give the highest spectral resolution (13–18). To date, published MAS seed spectra were acquired at moderate magnetic field strengths (^{1}H frequency ≤400 MHz) with sample probes designed to characterize amorphous solids. These probes are constructed with materials important for solid-state spectroscopy and do not emphasize high resolution. Solid-state probes are not readily adapted to provide high sample throughput. We report here results of initial seed oil studies using high-field NMR and a ^{13}C MAS probe designed specifically to obtain high-resolution spectra (19). Our experiments show high-resolution NMR quickly and accurately determines oil composition in whole seeds. This method can be automated to provide detailed seed oil compositions without the need for chemical extractions.

MATERIALS AND METHODS

Seeds. Canola seeds are from the Calgene hybrid and transgenic library. After NMR analysis, a sample of canola seeds germinated normally.

Sample preparation. Canola seeds were placed directly into wide-mouth MAS NMR sample tubes.

NMR spectroscopy. High-resolution spectra are measured at 11.7 tesla. (^{1}H = 500 MHz, ^{13}C = 125 MHz) using Varian NMR Instruments (Palo Alto, CA) Inova™ NMR spectrometers equipped with carbon-observe MAS Nanoprobes™. The ^{13}C spectra were acquired without a field-frequency lock at ambient

*To whom correspondence should be addressed at Monsanto, 800 N. Lindbergh Blvd., St. Louis, MO 63167. E-mail: william.c.hutton@monsanto.com

Abbreviations: CPMAS, cross-polarization, magic-angle spinning; GC, gas chromatography; GC–FID, GC–flame-ionization detection; GC–MS, GC–mass spectrometry; HEAR, high erucic; HPLC–MS: high-performance liquid chromatography–MS; LEAR, low erucic; NIR, near infrared; NOE, nuclear Overhauser enhancement; NMR, nuclear magnetic resonance; MAS, magic-angle spinning; sat, saturated; TAG, triglyceride; TMS, tetramethylsilane.

Copyright © 1999 by AOCS Press

temperature (~21–22°C) in 10 min to 14 h using the following conditions: spectral width = 29.996 kHz, acquisition time = 2.185 s, $\pi/2$ pulse (3.8 μs) with no relaxation delay, $^1H\ \gamma B_2 = 2.5$ kHz with Waltz decoupling. The following conditions were typical of data processing: digital resolution = 0.11 Hz, 0.3 to 1.5 Hz line broadening, and time-reversed linear prediction of the first three data points. Chemical shifts are referenced by adding neat tetramethylsilane (TMS) to canola seeds and using the resulting referencing parameters for subsequent spectra. The ^{13}C resolution is 2–3 Hz for the most narrow seed resonances. Spectral resolution is independent of MAS spinning speeds (2.0–3.5 kHz) and data are typically obtained with 2.2 kHz spinning speeds. Spinning sidebands are <1% of the main resonance. TAG ^{13}C assignments are made from comparison with literature assignments or with shifts computed from a ^{13}C NMR database (20).

Preparation of fatty acid methyl esters and GC analysis. Twenty seeds from each Calgene canola line were crushed in a glass vial (12 × 32 mm) and extracted with toluene (500 μL) at room temperature for 3 h. An aliquot (100 μL) of the extracted seed oil TAG was subjected to methanolysis with 0.5 M sodium methoxide/methanol (500 μL) at 60°C for 20 min in another vial (capped). A saturated solution of NaCl (500 μL) and heptane (100 μL) was added to each sample and then centrifuged at 2,000 × *g* for 5 min. An aliquot (100 μL) of the upper phase containing methyl esters was recovered and subjected to compositional analysis using a GC–flame-ionization detector (FID) (model 6890; Hewlett-Packard, Wilminton, DE). The chromatographic conditions were 25 m × 0.25 mm fused-silica column (Omegawax 250; Supelco, Bellefonte, PA); 250°C injection and 270°C detection port temperatures; He was the carrier gas; H_2 at 40 mL/min; air at 450 mL/min; N_2 makeup at 35 mL/min; column temperature programmed from 172 to 242°C at 40°C/min; holding at 172°C for 1.7 min; column pressure programmed from 25 to 75 psi at 40 psi/min; holding at 25 psi for 1.7 min; 1.0 μL injected at a split ratio of 100. Peak retention time assignments were determined using authentic reference compounds (Sigma Chemical Co., St. Louis, MO) and the weight percent results were calculated from peak areas adjusted by theoretical FID response factors.

RESULTS AND DISCUSSION

High-resolution ^{13}C NMR of seeds. Figure 1 compares a ^{13}C spectrum acquired by placing about 80 canola seeds in a standard 5-mm NMR tube (Fig. 1A) with a spectrum of a single canola seed spinning at the magic angle (Fig. 1B). The resolution improvement is dramatic. The high-resolution MAS spectrum only contains carbon peaks from molecules with rapid molecular motions, i.e., molecules in liquid seed oil storage droplets. Long-chain saturated TAG are solids at room temperature, but they readily dissolve in seed oil droplets containing common amounts of unsaturated TAG. TAG and phospholipids immobilized in cell membranes do not appear in high-resolution MAS spectra. These components, and others with highly restricted global motion (proteins and carbohydrates), are seen in cross-polarization (CP) MAS ^{13}C NMR spectra of similar seeds (21). Figure 1C depicts a 10-min spectrum obtained from a single low-erucic (LEAR) canola seed. The 40-μL probe sample volume is larger than this seed, but the sensitivity is adequate to observe carbons from all oil components. This spectrum indicates that the MAS ^{13}C method can analyze hundreds of seed samples per week. The best compromise between sig-

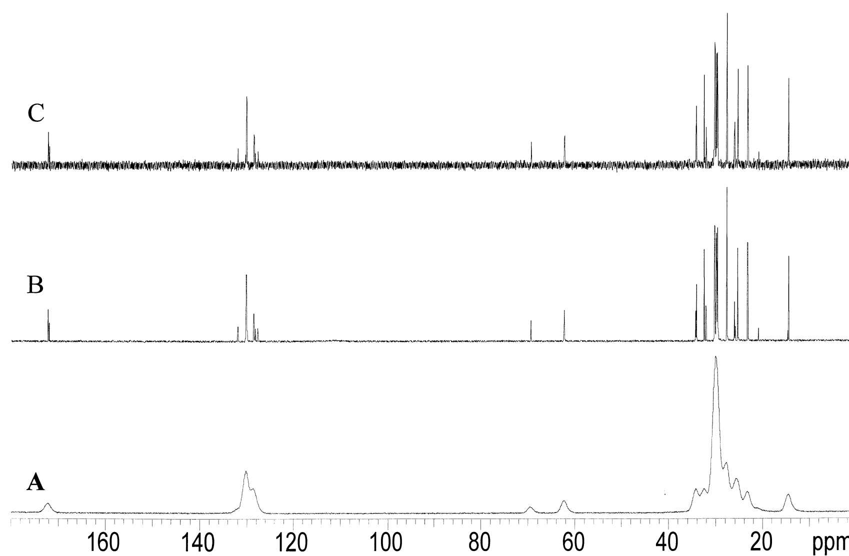

FIG. 1. The 125 MHz ^{13}C spectra of (A) about 80 canola seeds in a 5-mm nuclear magnetic resonance tube and (B) single (7.8 mg) canola seed collected with magic-angle spinning. Both spectra were signal-averaged for 4 h and processed with 1 Hz line broadening. (C) A 10-min (276 scans) single-seed spectrum represents the minimum signal-to-noise ratio suitable for seed screening.

nal-to-noise-ratio and time is achieved after 1 hr of signal averaging.

A set of canola seed and neat canola oil spectra obtained with the MAS probe (data not shown) demonstrates the importance of factors responsible for ^{13}C spectral resolution in seeds. The observed line widths in neat canola oil are about 0.5 Hz sharper than intact seed line widths. When the neat oil is diluted in $CDCl_3$ to ~20% (vol/vol), the line widths sharpen; at ~21–22°C, viscosity broadening in neat oil adds about 0.6 Hz to the sharpest lines. Heating seeds from 22 to 40°C decreases the line width by only 0.3 Hz. Taken together, these results verify earlier conclusions that the primary benefit of MAS is that it averages microscopic magnetic susceptibility gradients inherent in seed tissue (15,16).

Canola seed oil assignments. For initial analysis and carbon assignments, we discuss in detail the 4-h spectrum of a single LEAR canola seed. The terminal methyl, or ω_1, carbon region is well-separated from the other aliphatic resonances. This region (Table 1), along with the ω_2 methylene peaks (Fig. 2), is used to quickly determine the presence of n-3 and n-6 acyl chains. Methyls on saturated chains resonate just downfield of those on n-9 chains and are not resolved. Figure 2 shows how well MAS resolves the other aliphatic carbons at 125 MHz. The bulk methylene resonances (~29.9–30.8 ppm) are highly overlapped and will not be discussed further.

Methylenes near structural moieties relevant to oil composition (e.g., ω_1 methyls, olefin carbons, and ester groups) are readily assigned. Methylenes allylic to *cis* olefin carbons are found at ~26–26.5 ppm and at ~27.6–28 ppm (Figs. 2 and 3). This region is useful to identify mono-, di-, and trienes. The spectral region shown in Figure 3 contains all the peaks required for quantitative TAG analysis (*vide infra*). Identification of *cis* and *trans* isomers is problematic with GC methods. A 38-laboratory study concluded *trans* acyl chain analyses of unhydrogenated seed oils can give variable results (22). This study and another (23) found that canola oil contains 1 to 4% *trans* acyl chains. Allylic methylenes in *trans* olefins appear about 5 ppm downfield of their *cis* counterparts (24). Based on this *cis*-*trans* shift, we assign the small peak at ~32.5 ppm (Fig. 2) found in all spectra to *trans* acyl chain allylic carbons.

The olefin region (Fig. 4) is rich in important information about seed oil composition. The presence of 18:3n-3 acyl chains is determined by inspection. The 18:1n-9 C-10 and 18:2n-6 C-13 peaks overlap in the downfield (130.0–130.4 ppm) region. In the upfield region (128.4–128.8 ppm), the 18:3n-3 C-12 and C-13 peaks overlap. The other olefin methine carbons are well resolved. The 18:1n-9 C-9, 18:2n-6 C-9, C-10, and C-12 carbons, and the 18:3n-3 C-9 and C-10 carbons all separate into *sn*-1,3 and *sn*-2 regioisomer pairs (Fig. 5). The ability to identify all the olefin carbons for a given acyl chain reduces ambiguity due to resonance overlap.

The *sn*-1,3 and *sn*-2 glycerol backbone (~61, ~64 ppm, respectively) and ester carbonyl carbons (~172 ppm) have different chemical shifts, but do not contain information relevant to oil composition in these seeds.

Oil composition in transgenic canola seeds. Figure 6 compares the olefin region for LEAR, high erucic (HEAR) seeds, and seeds transformed to synthesize monoene acyl chains. The intense peak in the HEAR seed spectrum at ~130.2 ppm is from 22:1n-13 acyl chains. Seeds transformed to express 24:1n-15 acyl chains have a comparable resonance. The 24:1n-15 transformed seeds and the HEAR seeds have similar 18:1n-9, 18:2n-6, and 18:3n-3 compositions. Comparison with the LEAR spectrum shows the total amount of 18:3n-3 and 18:2n-6 chains is reduced in seeds with long acyl chains. A more subtle difference in the oil composition is apparent from comparison of allylic peak intensities (data not shown). Both hybrid and transformed seeds have a higher 18:3n-3 to 18:2n-6 molar ratio. Figure 6 indicates seeds transformed to express 18:1n-9 acyl chains do so at the expense of 18:3n-3 synthesis.

TABLE 1
^{13}C NMR Canola Seed Oil Peak Assignments

Carbon(s)	Acyl chain	Chemical shift (ppm)
CH_3, ω_1	Sat, 18:1n-9	14.60
	18:2n-6	14.56
	18:3n-3	14.75
CH_2, ω_2	18:3n-3	21.08
	Sat, 18:1n-6	23.22
	18.2n-6	23.34
CH_2 C-3	All	25.43
C=C–$\underline{C}H_2$–C=C–	C-14, 18:3n-3	26.05
	C-11, 18:3n-3	26.16
	C-11, 18:2n-6	26.19
CH_2–C=C–	Sat, 18:1n-9	27.78
	18:2n-6, 18:3n-3	27.81
n–CH_2	All	29.6–30.6
CH_2, ω_3	18:2n-6	32.18
$\underline{C}H_2$–C=C˜–, *trans* all		32.50
CH_2, ω_3	Sat, 18:1n-9	32.63
CH_2, C-2	All *sn*-1,3	34.24
	All *sn*-2	34.41
Glycerol CH_2	All *sn*-1,3	62.42
Glycerol CH	All *sn*-2	69.55
C=C–H	C-15, 18:3n-3	127.73
	C-10, 18:3n-3, *sn*-1,3	128.31
	C-10, 18:3n-3, *sn*-2	28.33
	C-12, 18:2n-6, *sn*-2	128.48
	C-12, 18:2n-6, *sn*-1,3	128.49
	C-10, 18:2n-6, *sn*-1,3	128.55
	C-10, 18:2n-6, *sn*-2	128.57
	C-13, 18:3n-3	128.61[a]
	C-12, 18:3n-3	128.62[a]
	C-9, 18:1n-9, *sn*-2	130.06
	C-9, 18:1n-9, *sn*-1,3	130.10
	C-9, 18:2n-6, *sn*-2	130.16
	All monoenes, 20:1	130.17
	C-9, 18:2n-6, *sn*-1,3	130.18[a]
	C-10, 18:1n-9, *sn*-1,3	130.23
	C-13, 18:2n-6, *sn*-2[a]	130.24
	C-10, 18:1n-9, *sn*-2[a]	130.26
	C-13, 18:2n-6, *sn*-2[a]	130.27
	C-9, 18:3n-3, *sn*-2	130.36
	C-9, 18:3n-3, *sn*-1,3	130.39
	C-16, 18:3n-3	132.03
C-1	All *sn*-2	171.97
	All *sn*-1,3	172.25

[a]Not resolved due to resonance overlap. NMR, nuclear magnetic resonance; sat, saturated.

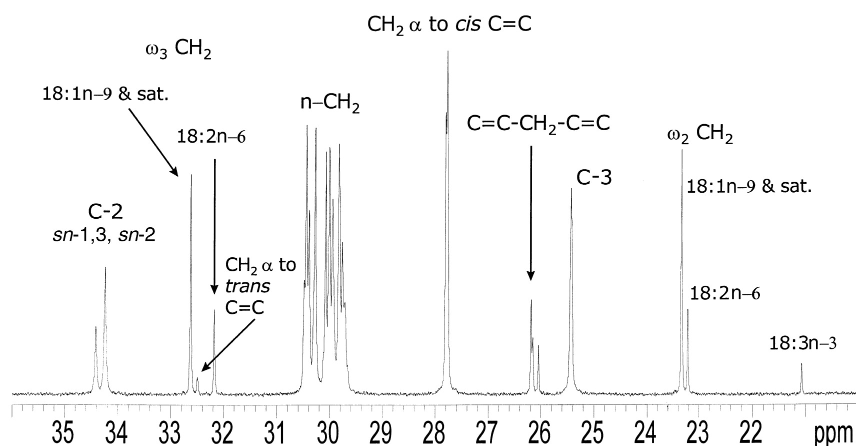

FIG. 2. The methylene-carbon assignments from a 4-h spectrum of a single 7.8-mg low-erucic (LEAR) canola seed.

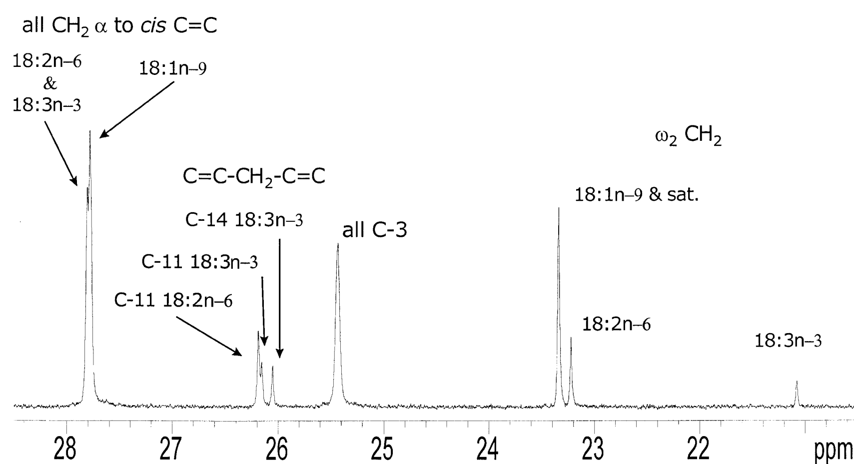

FIG. 3. Expansion of the spectrum in Figure 2 depicting the *cis*-allylic and ω_2 carbons.

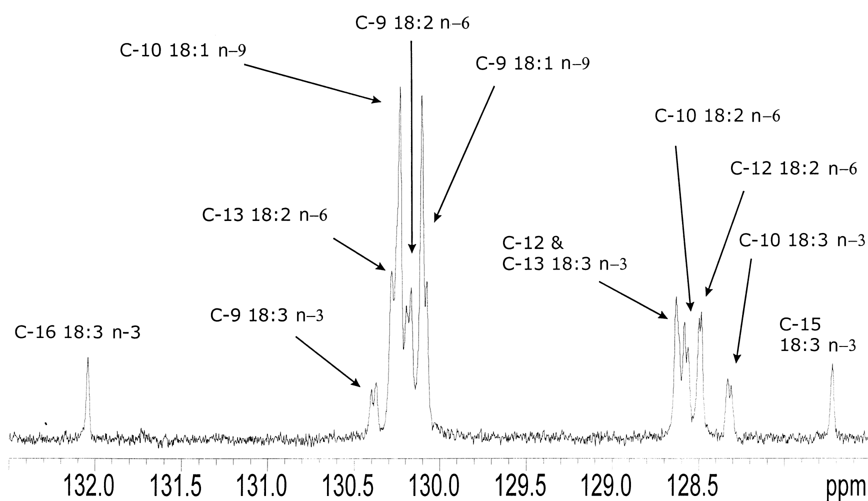

FIG. 4. The olefin-methine carbon assignments for four LEAR canola seeds. This is a 1-h spectrum with 0.3 Hz line broadening. See Figure 2 for abbreviation.

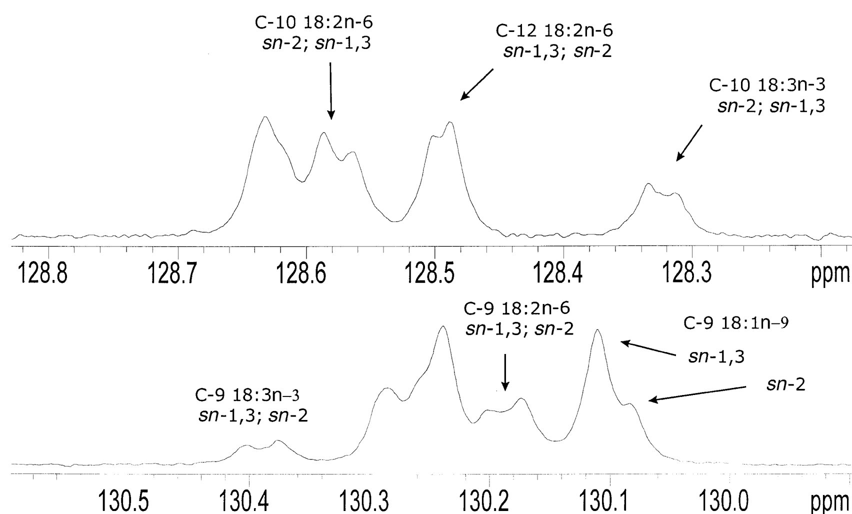

FIG. 5. Olefin-carbon regioisomer assignments for a LEAR canola seed. The *sn*-1,3 and *sn*-2 label order indicates downfield and upfield peak assignments. Unlabeled peaks do not have regioisomer shifts or resonance overlap obscures these shifts. See Figure 2 for abbreviation.

Figure 7 demonstrates how information in the olefin spectral region is used to assess oil composition in a set of closely related seed lines. Here, spectra for transgenic seeds high in 8:0/10:0, 12:0, 14:0, 16:0, and 18:0 acyl chains are shown. Based on the resonance assignments from Figure 5, it is possible to compare the 18:1n-9, 18:2n-6, and 18:3n-3 chain composition among these high-saturate seeds. Detail about saturated chain composition is difficult to extract from these spectra due to resonance overlap from similar methylene carbons in 8:0, 10:0, 12:0, 14:0, 16:0, and 18:0 chains. However, it is possible to compute the sum of all saturated chain concentrations (see below). These experiments demonstrate the potential of ^{13}C NMR in comparing TAG biosynthesis in transformed seed lines.

Canola seed oil quantitative analysis. In principle, the ^{13}C-peak area is directly proportional to the molar concentration of the corresponding carbon(s). In practice, ^{13}C peak areas also depend on relaxation parameters [e.g., nuclear Overhauser effect (NOE), and spin-lattice relaxation rates]. Use of suitable acquisition parameters provides quantitative results, but quantitative experiments require about a 10-fold increase in data collection time. As a result, ^{13}C NMR is often viewed as a qualitative method, well-suited for structure elucidation but inefficient for quantitative applications.

It is possible to correct ^{13}C peak areas to account for relaxation effects (24,25). Then, quantitative results are available with efficient data-collection times. For TAG mixtures in seed

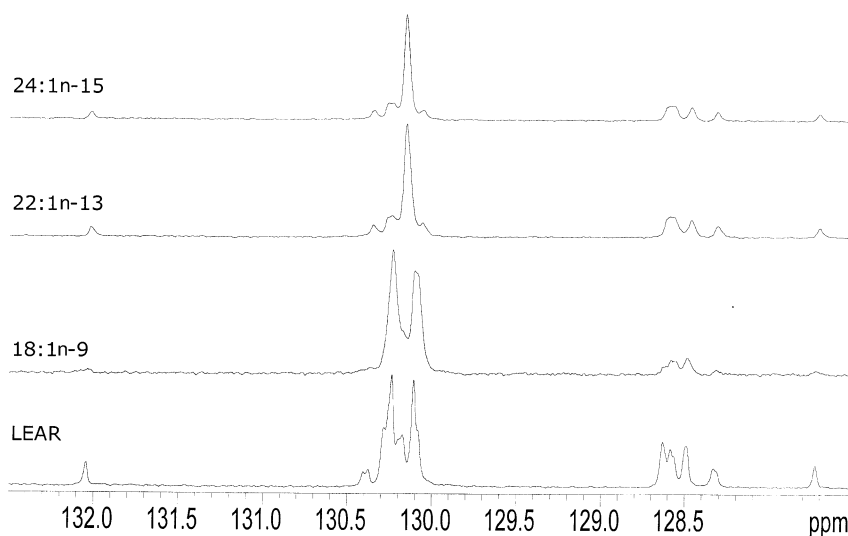

FIG. 6. The olefin region comparison of LEAR and high-erucic (HEAR, 22:1n-13) seeds and seeds transformed to synthesize monoene acyl chains. Assignments are given in Figure 4. For abbreviation see Figure 2.

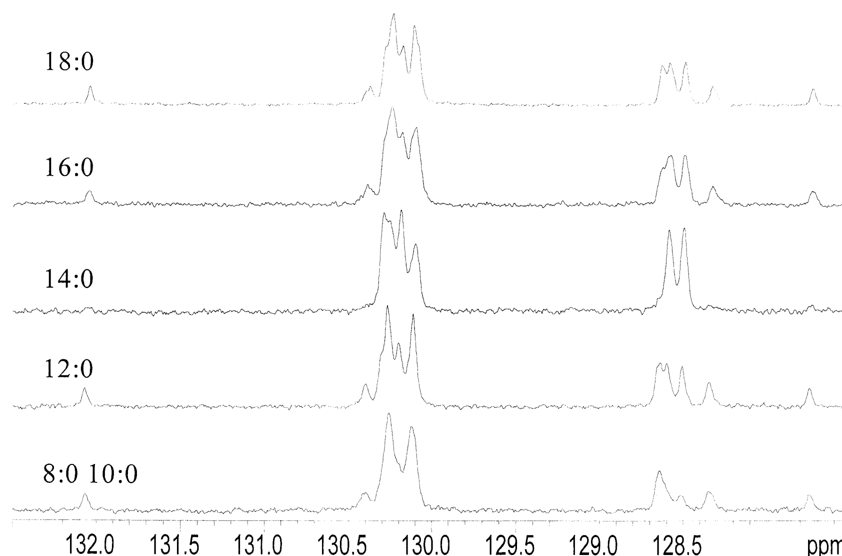

FIG. 7. The olefin region for seeds transformed to enhance saturated acyl chain composition. Assignments are shown in Figure 4.

oil storage droplets, NMR relaxation differences for similar carbons in similar molecules are comparable. If we collect a seed spectrum under both survey and quantitative conditions, we can establish a correction factor for each resonance to apply in survey spectra of similar seeds. Peak area correction factors for seeds for each carbon resonance in LEAR canola seeds are included in Table 2 (see below). The quantitative precision for ^{13}C NMR ranges from 1 to 15% and depends directly on the signal-to-noise ratio. Published studies comparing GC and ^{13}C NMR quantitative analysis indicate agreement between the two methods is 5 to 15% (9,11,16,24).

For intact seed oil composition quantitation, we focus on classes of CH_2 carbons found from 20–29 ppm (see Fig. 3 for assignments). With those assignments, we can describe how the acyl chain types contribute to each resolved peak (Table 3).

Algebraic combination of these relationships gives solutions for saturate (sat), 18:1n-9, 18:2n-6, and 18:3n-3 chain concentrations:

$$\text{sat} = P_{C\text{-}3} - \frac{P_{\omega 2}}{2} - \frac{P_{\alpha}}{2} \quad [1]$$

$$18{:}1\text{n-}9 = \frac{P_{\alpha}}{2} + \frac{3P_{\omega 2}}{2} \quad [2]$$

$$18{:}2\text{n-}6 = P_v - 2P_{\omega 2} \quad [3]$$

$$18{:}3\text{n-}3 = P_{18:3} \quad [4]$$

TABLE 2
Comparison of ^{13}C NMR and GC–FID Canola Seed Oil Compositions[a]

	Acyl chain ^{13}C NMR (mol%)[b] and GC–FID (wt%)			
Seed line[b]	18:3n-3	18:3n-6	18:1n-9[c]	Saturates
LEAR	9.3 (11.7)	24.0 (19.1)	60.6 (62.3)	6.1 (6.6)
8:0, 10:0	8.1 (9.3)	8.7 (8.4)	49.7 (54.2)	33.5 (27.6)
12:0	6.2 (7.1)	7.6 (9.8)	29.3 (30.8)	56.9 (52.3)
14:0	<1.0 (1.7)	21.8 (22.3)	20.7 (21.4)	57.5 (54.3)
16:0	3.8 (5.0)	21.3 (15.8)	35.0 (37.4)	39.8 (41.6)
18:0	6.8 (8.1)	19.6 (17.5)	46.0 (45.1)	27.6 (28.2)
18:1	<1.0 (1.9)	13.1 (7.8)	82.7 (84.0)	4.2 (6.0)
HEAR	10.8 (10.7)	17.6 (14.7)	69.3 (69.0)	2.3 (4.8)
24:1	9.3 (8.0)	18.2 (14.4)	69.0 (71.2)	3.4 (5.3)

[a]GC–FID, gas chromatography–flame-ionization detection; LEAR, low erucic; HEAR, high erucic; see Table 1 for other abbreviation.
[b]Intact seeds were used. Acyl chain mole percentages from peak area ratios were calibrated with no nuclear Overhauser enhancement. Delay = 7.1 or 12.1 s.
[c]Includes all *cis* monoenes (16:1, 20:1, 22:1, 24.1).

TABLE 3
Acyl Chain Type Contribution to Resolved Peaks

Peak area	ppm	Assignment (acyl chain molar amounts)[a]
$P_{18:3}$	21.1	ω_2 (18:3n-3)
$P_{n\text{-}2}$	23.3	ω_2 [(sat) + (18:1n-9) + (18:2n-6)]
$P_{C\text{-}3}$	25.6	C-3 [(sat) + (18:1n-9) + (18:2n-6) + (18:3n-3)]
P_v	26.2–0.5	vinyl allylic [(18:2n-6) + 2(18:3n-3)]
P_α	28.0	allylic [2(18:1n-9) + 2(18:2n-6) + (18:3n-3)]

[a]sat, saturated.

where each peak area (P_n) is multiplied by its empirical correction factor to correlate quantitative and survey peak areas.

A quantitative summary of canola seed TAG chain compositions from MAS ^{13}C NMR and GC–FID is found in Table 2. Several factors complicate direct comparison of the ^{13}C and GC data. The GC and NMR data are from the same seed lines, but were not done using the same individual seeds. The NMR results are in mole percentages and the GC data are in weight percentages. The most common seed oil TAG (C-18 acyl chains) have similar molecular weights. In this case, mole percentage–weight percentage differences may be neglected. For seed oils with shorter and longer acyl chains, the mole percentage–weight percentage differences become significant. Inspection of the olefin region (Figs. 4 and 6) establishes the importance of long-chain monoenes. The sample handling procedures used in the NMR and chromatography methods could account for some of the differences in Table 2. ^{13}C NMR directly observes liquid TAG in seed oil storage vacuoles. Chromatographic analysis is from whole seed extracts and involves chemical derivatization. Despite these complications, the results affirm that MAS ^{13}C oil characterization is both a qualitative and quantitative method.

ACKNOWLEDGMENTS

The authors thank the Varian NMR Instruments Palo Alto Applications Laboratory for their kind hospitality during our initial experiments. We thank Dr. Paul Keifer for his technical assistance and advice regarding use of the Nano-probe™. We are grateful to Sanjay Panda for his assistance and for growing canola seeds after the NMR measurements.

REFERENCES

1. (1991) *Analysis of Oilseeds, Fats and Fatty Foods* (Rossell, J.B., and Pritchard, J.L.R., eds.), Elsevier Science Publishing, New York.
2. (1997) *New Techniques and Applications in Lipid Analysis* (McDonald, R.E., and Mossoba, M.M., eds.), AOCS Press, Champaign.
3. Velasco, L., Fernandez-Martinez, J.M., and De Haro, A. (1997) 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.
4. Schaefer, J., and Stejskal, E.O. (1974) Carbon-13 Nuclear Magnetic Resonance Measurement of Oil Composition in Single Viable Soybeans, *J. Am. Oil Chem. Soc. 51*, 210–213.
5. Schaefer, J., and Stejskal, E.O. (1975) Carbon-13 Nuclear Magnetic Resonance Analysis of Intact Oilseeds, *J. Am. Oil Chem. Soc. 52*, 366–369.
6. Rutar, V., Burgar, M., Blinc, R., and Ehrenberg, L. (1977) Carbon-13 NMR Determination of the Oil Composition in Individual Plant Seeds, *J. Magnet. Reson. 27*, 83–90.
7. Chen, S., Elofson, R.M., and MacTaggert, J.M. (1979) Carbon-13 Nuclear Magnetic Resonance Studies of Lipids and Starch Digestion in Intact Seeds, *J. Agric. Food Chem. 27*, 435–438.
8. Albornoz, F., and Leon, V. (1980) Nondestructive Characterization of Seed Oil Using Carbon-13 FT–NMR, *Acta Cient. Venez. 31*, 20–23.
9. Leal, K.Z., Costa, V.E.U., Siedl, P.R., Campos, M.P.A., and Colnago, L. (1981) Immediate Analysis of the Oil Content of Seeds by Carbon-13 Nuclear Magnetic Resonance, *Cienc. Cult.* (*Sao Paulo*) *33*, 1475–1484.
10. Yoshida, M., Kano, H., Ishida, N., and Yoshida, T. (1989) Nondestructive Analysis of the Oil Composition of Soybean Seeds by Natural Abundance Carbon-13 Nuclear Magnetic Resonance Spectroscopy, *Agric. Biol. Chem. 53*, 1395–1400.
11. Seidl, P.R., and Santos do Nascimento, R. (1990) Analysis of Tropical Oilseeds by Carbon-13 NMR, *Spectrosc. Int. J. 8*, 183–189.
12. Gambhir, P.N. (1994) ^{13}C NMR Spectroscopy of Intact Oilseeds—A Rapid Method for Measurement of Fatty Acid Composition, *Indian Acad. Sci. Proc. Chem. Sci. 106*, 1583–1594.
13. Ye, C., Sun, B., Qui, J., and Yuan, H. (1986) Magic Angle Spinning NMR of Oilseeds, *Kexue Tongbao (foreign lang. ed.) 31*, 16–20.
14. Rutar, V., Kovac, M., and Lahajnar, G. (1988) Improved NMR Spectra of Liquid Components in Heterogeneous Samples, *J. Magnet. Reson. 80*, 133–138.
15. Rutar, V., Kovac, M., and Lahajnar, G. (1989) Nondestructive Study of Liquids in Single Fir Seeds Using Nuclear Magnetic Resonance and Magic Angle Sample Spinning, *J. Am. Oil Chem. Soc. 66*, 961–965.
16. Rutar,V. (1989) Magic Angle Sample Spinning NMR Spectroscopy of Liquids as a Nondestructive Method for Studies of Plant Seeds, *J. Agric. Food Chem. 37*, 67–70.
17. Wollenberg, K. (1991) Quantitative Triacylglycerol Analysis of Whole Vegetable Seeds by Proton and Carbon-13 Magic Angle Sample Spinning NMR Spectroscopy, *J. Am. Oil Chem. Soc. 68*, 391–400.
18. Ye, C., Guo, Y., and Feng, Y. (1991) MAS NMR Measurement of Oil Content in Oilseed, *Chin. Sci. Bull. 36*, 597–601.
19. Keifer, P.A. (1997) High-Resolution NMR Techniques for Solid-Phase Synthesis and Combinatorial Chemistry, *Drug Discovery Today 11*, 468–478.
20. (CNMR DB), Version 3.50, Advanced Chemical Development Inc., Toronto, Canada.
21. Loughman, B.C., and Ratcliffe, R.G. (1984) Nuclar Magnetic Resonance and the Study of Plants, *Avd. Plant Nutr. 1*, 241–283.
22. Van Bruggen, P.C., Dachateau, C.S.M.J.E., Mooren, M.M.W., and van Oosten, H.J. (1998) Precision of Low *Trans* Fatty Acid

Level Determination in Refined Oils. Results of a Collaborative Capillary Gas–Liquid Chromatography Study, *J. Am. Oil Chem. Soc. 75*, 483–488.
23. O'Keefe, S., Gaskins-Wright, S., Wiley, V., and Chen, I.-C. (1994) Levels of *Trans* Geometrical Isomers of Essential Fatty Acids in Some Unhydrogenated U.S. Vegetable Oils, *J. Food Lipids 1*, 165–176.
24. Shoolery, J.N. (1983) Applications of High Resolution Nuclear Magnetic Resonance to the Study of Lipids, *Am. Oil Chem. Soc. Monogr.* 220–240.
25. Gillet, S., and Delpuech, J.J. (1980) Optimum Conditions for Nondestructive Quantitative Analysis by Carbon-13 NMR, *J. Magnet. Reson. 38*, 433–445.

[Received July 7, 1999; revision received and accepted November 24, 1999]