Quantitative Triacylglycerol Analysis of Whole Vegetable Seeds by ^{1}H and ^{13}C Magic Angle Sample Spinning NMR Spectroscopy

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High-resolution ^{13}C and ^{1}H magic angle sample spinning nuclear magnetic resonance (NMR) spectra have been obtained and used to define the relative unsaturated acyl distribution of triacylglycerols in whole oil seeds. Inverse gated proton decoupled ^{13}C and ^{1}H NMR spectra permit the quantitative analysis of seeds containing simple oils, *e.g.*, sunflower seeds containing oleyl and linoleyl unsaturates only. More sensitive ^{13}C NMR techniques are necessary for the analysis of specific seed classes. One such class is the rapeseed, which is especially difficult due to its low oil content (≈ 2 mg oil/seed) and complex unsaturated acyl profile of oleyl, linoleyl, linolenyl, erucyl, and eicosenoyl. The Distortionless Enhancement by Polarization Transfer technique significantly improves sensitivity to the extent that single rapeseeds can be examined within an hour of acquisition time. Furthermore, some positional (1,3- or 2-glycerol attachment) groups can be identified leading to a partial estimation of the 1,3-, 2-acyl distribution.

KEY WORDS: ^{13}C ^{1}H NMR, DEPT, MASS, quantitative, rapeseeds, sunflower, triacylglycerols, triglycerides.

In response to the ever increasing importance of the triacylglycerol composition of edible oils produced for human consumption, agricultural research has been moving toward edible oil seed varieties which possess higher ratios of unsaturated to saturated triacylglycerols. There is also a growing need for nonedible oils which are environmentally friendly and possess the unique qualities that are required for existing markets using petroleum-derived oils. Consequently, new seed varieties have been developed to produce oils which will yield the desirable acyl profile that will ensure a specific variety a viable position in the commercial marketplace. These new seed varieties are the result of considerable research in the genetic improvement of seeds, either through plant breeding and/or genetic engineering programs. In order to evaluate valuable hybrid seeds produced in breeding programs, truly nondestructive methods are necessary for the analysis of a seed's oil composition to insure that valuable resources are not wasted on nonviable seeds. One method which has shown considerable promise to this end is ^{13}C nuclear magnetic resonance (NMR) (1,2). More recently, both spectral resolution and sensitivity have been significantly improved for ^{13}C nuclei and, to a greater extent, ^{1}H nuclei with the implementation of Magic Angle Sample Spinning (MASS) NMR for the analysis of a whole seed's oil composition (3,4). At the present time there has been little documentation as to the quantitative integrity of MASS NMR spectra of whole seed triacylglycerols. Furthermore, to our knowledge, the use of polarization transfer techniques have been discussed as possible methods for the examination of whole seed triacylglycerols (5), but have not been implemented as of yet.

This present study is divided into two sections. The first is to compare the unsaturated acyl distribution, derived by ^{1}H MASS NMR and inverse gated ^{13}C MASS NMR, of randomly selected early hybrid high oleyl sunflower seeds. The quantitative integrity of the NMR derived data is examined by gas chromatographic (GC) analysis of the methyl esters derived from the corresponding seeds from which the NMR data are obtained. The second section is to examine the Distortionless Enhancement by Polarization Transfer (DEPT) (6) technique as a method which can increase sensitivity of the ^{13}C nuclei to the extent that semi-quantitative information about the acyl distribution of seeds, with very low oil content, *i.e.*, single rapeseeds, can be obtained within a practical time frame.

EXPERIMENTAL PROCEDURES

The NMR μ-image was obtained using a Bruker NMR μ-imaging unit on a Bruker MSL 400 spectrometer (Bruker Instruments, Inc., Karlsruhe, Germany). All seeds were examined on a Bruker MSL 400 at the magic angle using a CP/MAS ^{13}C solids probe. The seeds were packed with an alumina/silica (75:25) powder (American Cyanamide Co., Michigan City, MI) in a 7-mm spinner. The spectra were acquired at spinning rates of 3 KHz with low power decoupling and low power excitation pulses. The proton spectra were acquired at 400.13 MHz and the carbon spectra at 100.63 MHz. The ^{13}C and ^{1}H 90° pulses were 10 μsec and 16 μsec, respectively. The ^{13}C inverse gated spectra of sunflower seeds were acquired with a 5-second relaxation delay, 90° excitation pulse, 32 KHz sweep width, 0.5 second acquisition time, and cyclops phase cycling. The DEPT spectra were obtained using the DEPT sequence described in the text, 90° variable proton pulse (Ω_H), τ = 1/2J (J = 160 Hz) polarization transfer/refocusing delay, and 2-second relaxation delay. The proton 90° pulse was calibrated by nulling the internal CH_2's of the triacylglycerol backbone with the DEPT pulse sequence. The Free Induction Decay (FID) was acquired with 32K points and an acquisition time of 0.5 seconds. The FID was zero filled to 128K points before Fourier transformation. There were no resolution nor sensitivity enhancement filters applied to the FID before Fourier transformation.

The proton spectra were acquired with a 1 second acquisition time, 8-second relaxation delay time, and 4 KHz sweep width. A Gaussian filter (LB = −2 and GB = 0.4) was applied to some of the proton FIDs before Fourier transformation. The carbon spectra were referenced to the β OCH = 68.94 ppm unless otherwise specified, and proton spectra to C=C−CH_2−C=C=2.79 ppm.

The ^{13}C T_1 values were obtained for high oleyl Cascade rapeseeds using a MASS inversion-recovery experiment

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with a 3 KHz spinning speed. The T_1 values were calculated using the supplied DISR88 Bruker software for T_1 calculations. The GC analyses were performed on oils which were hexane extracted from the corresponding seeds that the MASS NMR experiments were performed on. The extracted triacyglycerols were dissolved in 3 mL methanol and transesterified using three drops of sodium methoxide and then heated at 60°C for one hour before the addition of 3 mL water and 3 mL hexane. The extracted hexane layer was analyzed directly with a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) using a Supleco 2330 30 m × 0.25 mm ID (Supleco, Inc., Bellefonte, PA) column.

RESULTS AND DISCUSSION

The ability of NMR to determine the acyl distribution of triacylglycerols within whole seeds is possible as a result of the liquid like nature of the oil. The liquid oil dispersed in the solid seed matrix produces an inhomogeneous sample and considerable line broadening of the non-MASS ^{13}C and ^{1}H NMR spectra of intact seeds. The line broadening effect originates from the heterogeneous nature of the seed and ultimately from induced dipolar fields produced by differences of bulk magnetic susceptibility (BMS) (7) between the oil and solid matrix. The heterogeneous nature of seeds is evident from an NMR μ-image (0.5-mm thick horizontal slice) of a high oleyl sunflower seed produced by the Agrigenetics Company (Fig. 1). The light shades define the geometric location of the oil and the dark shades the voids or solid masses. This particular sunflower seed was soaked in water for 12 hr. The water, which was absorbed exclusively into the shell, shows a chemical shift image 3 ppm downfield from the oil (shifted ring around seed represents the shell). The irregular voids or solid masses shown in this image have a different BMS than the oil causing the induced dipolar fields and observed line broadening effect. Fortunately, the induced dipolar fields vanish to zero under MASS conditions ($\theta = 54.7°$; θ = angle between the axis of rotation and the applied magnetic field) since the oil has flexible mobility and the induced dipolar fields have a geometric relation of $3\cos^2\Theta-1$. Consequently, MASS NMR exploits these factors to reduce significantly inhomogeneous line broadening and susceptibility shifts (8) due to differences in magnetic susceptibility. This ultimately increases both sensitivity and resolution over non-MASS experiments.

We have used both ^{1}H and ^{13}C MASS NMR to examine the relative unsaturate acyl content (oleyl:linoleyl) of a variety of our high oleyl sunflower seeds, as well as some early hybrid high oleyl sunflower seeds. The conventional quantitative technique for obtaining ^{13}C NMR spectra, *i.e.*, inverse gated proton decoupling experiment, provides adequate sensitivity for the quantitative analysis of sunflower seeds (≈ 20 mg oil/seed) with less than an hour of acquisition time. This technique, however, does not provide sufficient sensitivity to analyze seeds with a low oil content. Some examples of seeds which fall into this category are corn seeds, which are relatively large but contain only 4% (≈ 7 mg oil/seed) to 8% (≈ 14 mg oil/seed) oil/seed, and rape seeds, which contain 40% (≈ 1.5 mg oil/seed) to 50% (≈ 2 mg oil/seed) oil/seed, but are quite small. In the case of corn seeds, the unsaturated acyl distribution is readily defined by ^{1}H NMR if the linolenyl content is assumed to be negligible. In many cases the linolenyl content can even be obtained by ^{1}H NMR if additional time is allotted for more accurate shimming or ultimately a more homogenous field. This, in turn, permits one to resolve and quantify the terminal linolenyl CH_3 from the remaining terminal CH_3s. The increased resolution from accurate shimming is critical even at

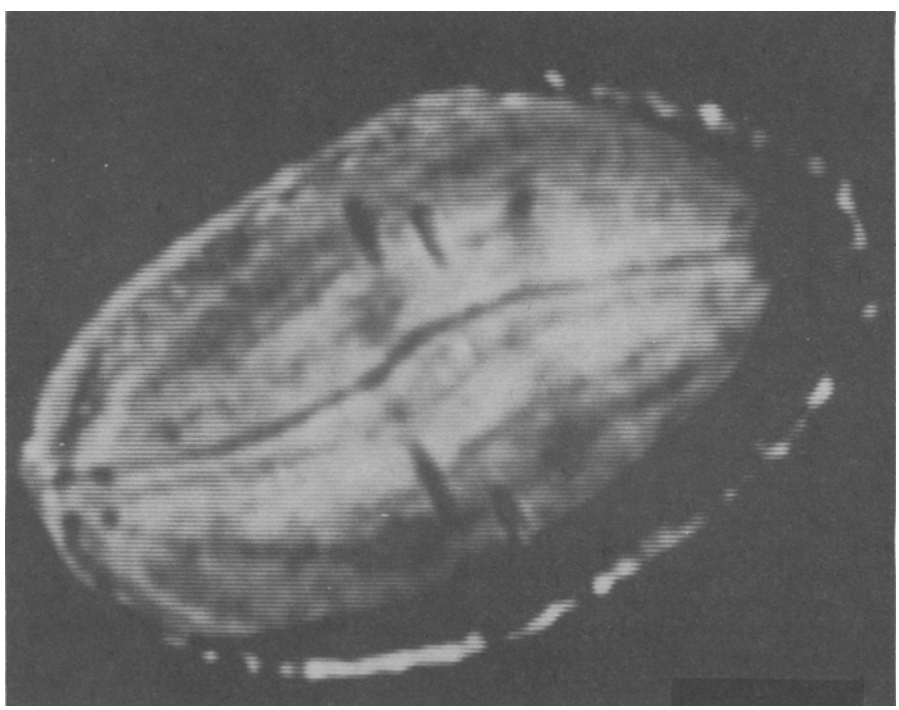

FIG. 1. NMR μ-image of a 0.5-mm horizontal cross section of a sunflower seed at 400 MHz.

400 MHz, since there is only a 7Hz difference between the outer line of the linolenyl CH_3 triplet and that of the overlapping CH_3 triplets from the remaining acyl groups. This scenario also holds for rapeseeds if the rapeseed variety does not contain significant quantities of either erucyl or eicosenoyl. Unfortunately, not all rapeseed oil is that simple. Some rapeseed varieties contain not only oleyl (O), linoley (L), and linolenyl (Ln), but also substantial quantities of erucyl (Er) and eicosenoyl (E). In fact, certain varieties, such as Bridger, are bred to be high-erucyl-containing rapeseeds. The problem here is that the different monoenes (N:1) do not display any distinguishable resonances in the ^{1}H spectrum. Therefore, if knowledge of the oleyl or erucyl content is to be obtained, the only alternative is to acquire the less sensitive ^{13}C spectrum.

The obvious benefit of using the ^{13}C nuclei is a 20-fold increase in the chemical shift range relative to protons and the elimination of scalar coupling through the decoupling of protons during ^{13}C acquisition. Each of the olefinic carbons associated with rape oil can be identified (Fig. 2a) with the exception of L_9, $E_{11,12}$, and $Er_{13,14}$. The major disadvantage of the ^{13}C nuclei is the low sensitivity and longer spin-lattice relaxation times (T_1), which are given in Table 1 (peak assignments are given in Fig. 3) for high oleyl Cascade seeds.

One approach to overcoming the detrimental effects associated with the ^{13}C nuclei is to employ a polarization transfer technique, such as DEPT or INEPT (9), which will decrease relaxation delay times and enhance ^{13}C signal intensity. The DEPT technique has the more desirable attribute of a secondary scalar coupling ($^{1}J_{CH}$) effect, where INEPT-derived carbon intensities vary with $^{1}J_{CH}$ as a primary effect. Furthermore, the DEPT sequence contains fewer pulses and is therefore less prone to variations in the carbon signal intensity resulting from inhomogeneous RF or incorrectly set pulses. Consequently, the carbon signal intensities will differ only slightly from theoretical values in the DEPT experiment, relative to the INEPT experiment, for incorrectly set $^{1}J_{CH}$, 90°, and 180° pulses. The DEPT pulse sequence which we employed is shown below with the only modification of the Pegg *et al.* (6) pulse sequence being a composite 180° (90°180°90°) carbon pulse. The composite 180° carbon pulse was utilized to compensate for RF inhomegenity over the spectral width of interest. The delay time (τ) is 1/2J, and the Ω_H pulse is 90°.

The degree of ^{13}C signal enhancement one can expect from the DEPT technique over the more typical inverse gated quantitative technique is exemplified for Cascade rapeseeds in Figure 4. The inverse gated technique required a total of 2260 acquisition transients before Fourier transformation to yield the top olefinic spectrum (Fig. 4a), and the DEPT technique 957 transients to yield the bottom olefinic spectrum (Fig. 4b). Even though the inverse gated spectrum was acquired with twice as many transients as the DEPT spectrum, the signal-to-noise (S/N) of the inverse gated spectrum is approximately half that of the DEPT spectrum. This translates into a three-fold S/N advantage of the DEPT technique over the inverse gated technique, disregarding the additional advantage of shorter relaxation delay times inherent to the DEPT technique.

H:RD	$-90-\tau-180-\tau-$		Ω	$-\tau-$ DECOUPLE
C:		$90-\tau-$	90-180-90	$-\tau-$ FID

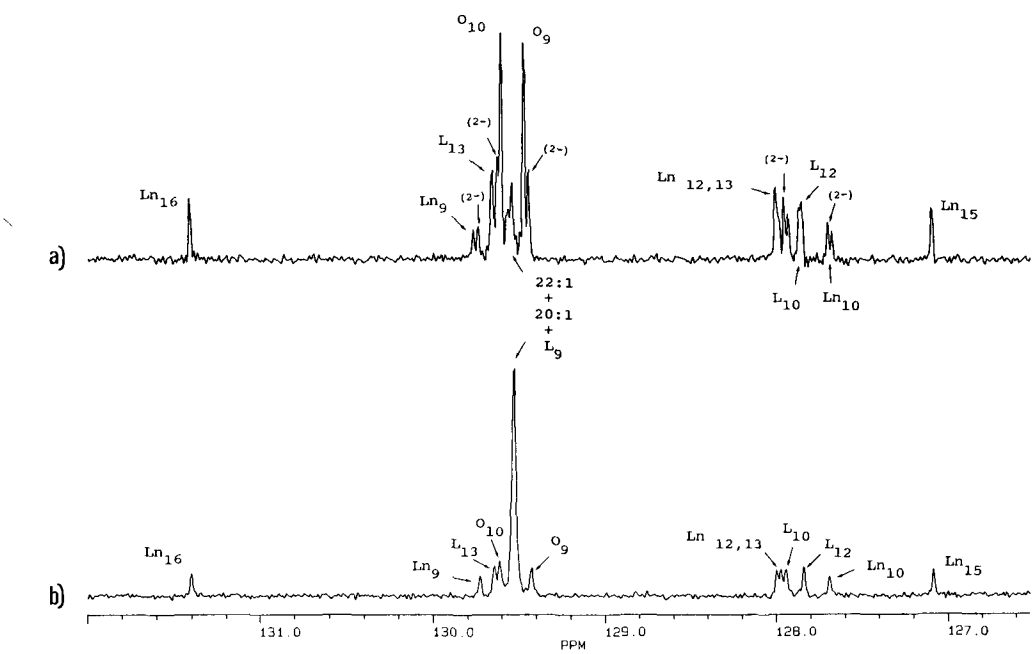

FIG. 2. 100.67 MHz ^{13}C MASS DEPT spectra (variable Ω_H pulse = 90°) of a) four high oleyl Cascade rapeseeds where only the 2-acyl group of each 1,3-,2-acyl peak pair is defined; and b) four high erucyl Bridger rapeseeds with Ln_9, O_9, O_{10}, L_{10}, Ln_{10}, and L_{12} exhibiting exclusively 2-glycerol substitution. The spectra are referenced to β O-CH = 68.94 ppm. Each peak is defined as O = oleyl, L = linoleyl, Ln = linolenyl with the subscript corresponding to the carbon chain position. Unsaturated carbons pertaining to 20:1 (eicosenoyl), 22:1 (erucyl), and L_9 (2-glycerol) are not resolved as indicated in both spectra. Not all peaks appear at the same relative chemical shift as observed in solution spectra of the corresponding oil.

TABLE 1

Approximate T_1 Values for the Assigned Carbons Derived from Triacylglycerols of Whole Cascade Rapeseeds[a]

Peak	Assignment	T_1 (s)
1	C=O (1,3-)	2.9
2	C=O (2-)	2.6
3[b]	Ln_{16}	3.6
4[b]	O_{10}[c]	0.9
5[b]	O_9	0.9
6[b]	$Ln_{12,13}$,L_{10}	1.3
7[b]	L_{12}	0.9
8[b]	Ln_{10}	0.5
9[b]	Ln_{15}	2.2
10	αCH_2O-	0.2
11	βCHO-	0.4
12,13	-$OCOCH_2$-	0.4
14,15	-$CH_2CH_2CH_3$	2.3,2.2
16-20	-$(CH_2)_n$-	0.8-1.1
21	O_8,L_8,Ln_8	0.8
22,23	L_{11},$Ln_{11,14}$	0.8
24	-$OCOCH_2CH_2$-	0.5
25	-CH_2CH_3	2.7
26[d]	Ln_{17}	—
27	-CH_3	6.6

[a]The assigned peaks correspond to the ^{13}C spectrum in Figure 3.

[b]Chemical shift values given in Table 3.

[c]Ln_9, L_{13}, and L_9 are superimposed on O_{10}.

[d]S/N is too low for accurate T_1 measurement.

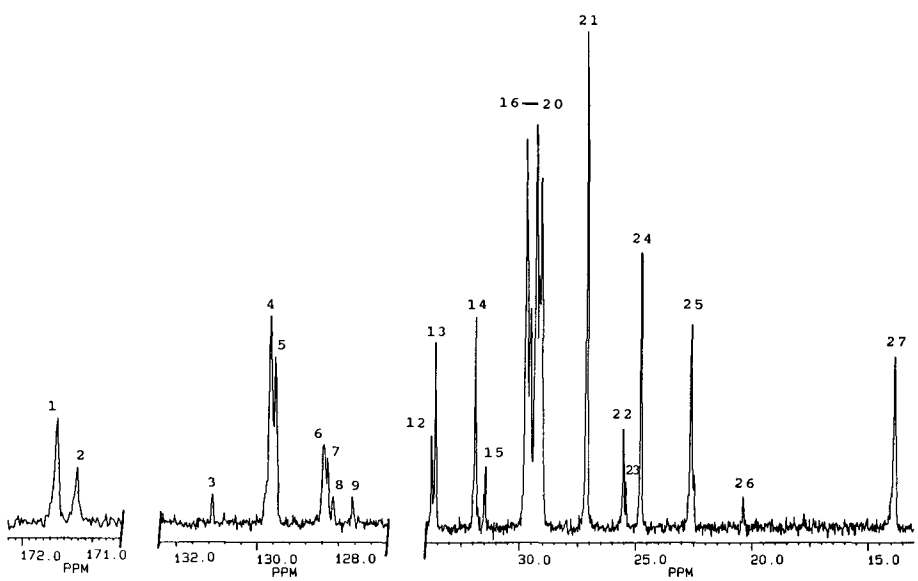

FIG. 3. 100.67 MHz MASS ^{13}C NMR spectrum from an inversion-recovery T_1 spectral set with this spectrum being the last spectrum with an inversion recovery delay time of eight seconds. The peak identities and T_1 values are given in Table 1. In order to increase the plot resolution, peaks 10 and 11 were omitted.

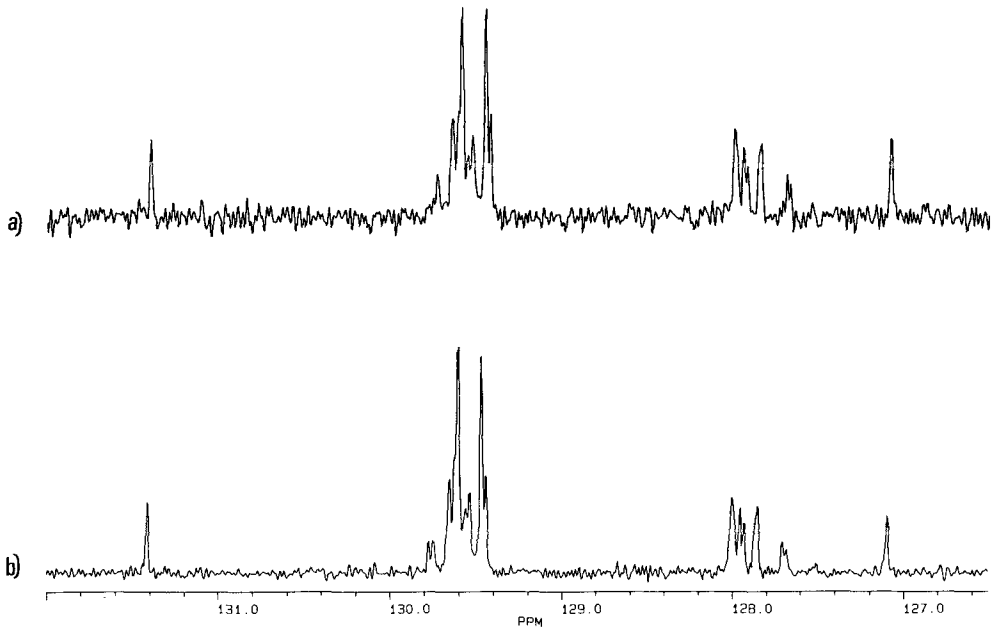

FIG. 4. 100.67 MHz MASS ^{13}C NMR olefinic spectra of Cascade rapeseeds acquired using a) inverse gated technique (2260 transients); and b) DEPT technique (957 transients).

As alluded to above, the DEPT pulse sequence benefits from the transfer of polarization from the more abundant proton spins to the less abundant carbon spins *via* their scalar coupling interaction ($^{1}J_{CH}$ in this case). The non-protonated carbons are eliminated since they contain no protons for polarization transfer. Each acyl olefinic carbon has a single proton attached and the signal intensity modulates as a function of the variable proton pulse (Ω_H) according to $\gamma_H/\gamma_C \sin \Omega_H$, where γ is the magnetogyric ratio of that nucleus. In order to obtain the maximum signal intensity for the olefinic carbons, a Ω_H pulse of 90° is used. An additional advantage of the DEPT technique beyond the carbon signal enhancement is that the carbon signal intensity now depends on the proton T_1 instead of the carbon T_1. This is critical to shortening the experimental time since the difference between the carbon and proton T_1s can be substantial, especially as the carbons are further removed from the glycerol end of the molecule, *e.g.*, the terminal CH_3s of the lipids in sunflower seeds can have ^{13}C T_1s of approximately eight seconds (Table 1) *vs* ^{1}H T_1s of one second (4). This translates to a much shorter relaxation delay time (RD), which for quantitative results should be in the order of $\geq 5T_1$ of the longest carbon or proton T_1 to be measured. Furthermore, the DEPT technique eliminates the hump in the olefinic region resulting from the teflon insert which may be found in solids NMR probes and observed for MASS experiments.

In this later study we were interested in examining the feasibility of utilizing the DEPT technique as both a qualitative and quantitative tool for the analysis of more "difficult" oil seeds. Again, more difficult seeds translates into those seeds which are low oil-containing or have a complex unsaturate acyl distribution. Rapeseeds meet both criteria with an oil content of approximately 2 mg oil/seed and a complex unsaturate acyl distribution of oleyl, linoleyl, linolenyl, erucyl, and eicosenoyl. To this extent we decided to examine two quite different rapeseed varieties using the DEPT technique in order to determine their respective unsaturate acyl distribution. The two rapeseed varieties examined were a high oleyl variety (Cascade) and a high erucyl variety (Bridger).

Sunflower seeds. The inverse gated technique is adequate when quantifying the oleyl:linoleyl content of sunflower seeds. Sunflower seeds are well suited for a low sensitivity technique due to their high oil content (≈ 20 mg oil/seed) and simple unsaturate acyl distribution. A random examination of an early hybrid of high oleyl sunflower seeds developed by the Agrigenetics Company produced a wide range of results. To verify the quantitative NMR results obtained on the high oleyl sunflower seeds, the intact oil previously examined by MASS NMR was extracted and transesterified to the corresponding methyl ester. The normalized unsaturate acyl distribution data given in Table 2 represent the oleyl:linoleyl distribution derived from both ^{1}H and ^{13}C NMR, as well as GC analysis. The methyl ester distribution calculated by gas chromatography is in excellent agreement with the MASS ^{1}H and ^{13}C NMR results. The MASS ^{13}C NMR olefinic spectra corresponding to these sunflower seeds are illustrated in Figure 5.

The ^{13}C olefinic distribution was obtained from the integration of $O_{9,10}$ relative to the fully resolved $L_{10,12}$ peaks. The molar unsaturate to saturate content can be obtained from the integral difference between the carbonyl and the olefinic regions in the MASS ^{13}C NMR spectrum. However, this approach requires a three-fold increase in acquisition time since the carbonyl carbon T_1s are approximately three times that of the oleyl and linoleyl unsaturate carbons (Table 1).

The ^{1}H olefinic distribution was obtained from the integral of the linoleyl -C=C-CH_2-C=C- protons (G) at 2.79

TABLE 2

Normalized Oleyl and Linoleyl Content of Randomly Selected High Oleyl and Early Hybrid High Oleyl Whole Sunflower Seeds[a]

Seed	18:1[b]	18:2[b]	Technique
Sunflower 1	96.0	4.0	H-1
	95.8	4.2	GC
	100	ND	C-13
Sunflower 2	80.6	19.4	H-1
	80.1	19.9	GC
	80.0	20.0	C-13
Sunflower 3	21.5	78.5	H-1
	21.4	78.6	GC
	17.1	82.9	C-13

[a]The values were derived from MASS ^1H and ^{13}C (inverse gated proton decoupling) NMR spectra of whole seeds. The GC data were derived from the extracted oil of the corresponding seed from which the MASS NMR data were derived.

[b]Normalized to only unsaturate acyl groups.

ND = not detected.

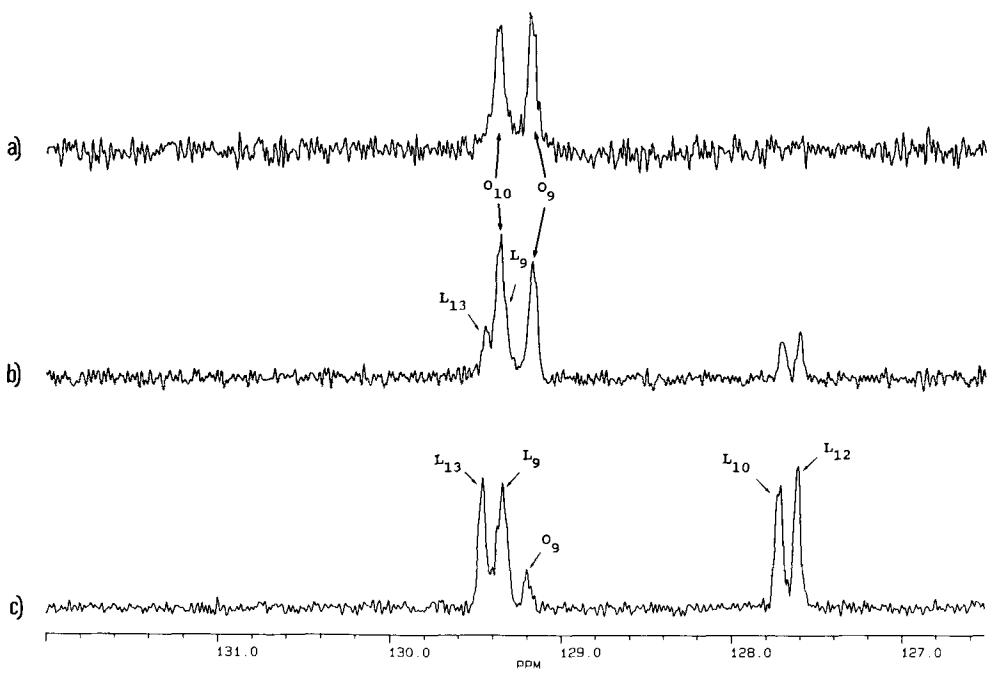

FIG. 5. 100.67 MHz MASS ^{13}C NMR unsaturated spectra of three different sunflower seeds randomly selected from an early hybrid of high oleyl sunflower seeds which correspond to a) sunflower 1; b) sunflower 2; and c) sunflower 3 in Table 2.

ppm (Fig. 6) and the total olefinic protons (J). The three ^1H spectra shown in Figure 7 correspond to the three ^{13}C spectra in Figure 5. The ^1H NMR data are more accurate since the S/N is considerably better, especially when quantifying low levels of an acyl group (< 5 mole%) or seeds with a low oil content, *e.g.*, corn and rapeseeds, as discussed below. One problem which we encountered with MASS ^1H spectra is the more pronounced ^1H spinning sidebands, which must be spun out of the spectral region of interest. This requires spinning speeds of 2-3 KHz at our observed frequency of 400 MHz. These spinning speeds are not necessarily trivial since the seeds do not always have a symmetrical weight distribution. Furthermore, the narrow proton chemical shift range is not suitable for the characterization of seeds which have a more complex monoene acyl distribution, *i.e.*, more than one monounsaturated acyl group.

Rapeseeds. The olefinic DEPT spectra of high oleyl Cascade and high erucyl Bridger rapeseeds are illustrated in Figure 2. The absolute and relative chemical shift positions of the olefinic carbons of the intact oil do not agree exactly with those observed for the extracted oil, *i.e.*, oil

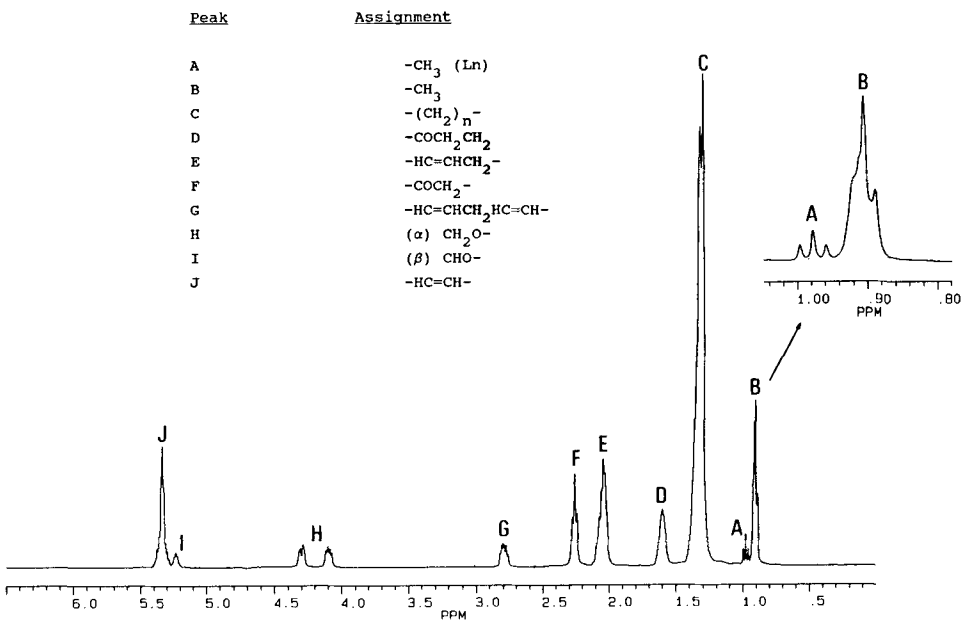

FIG. 6. 400.13 MASS ^{1}H NMR spectrum of high oleyl rapeseed (Cascade). The expanded methyl region displays the type of resolution which can be obtained between the terminal linoleynl CH_3(A) and remaining terminal CH_3s(B). No type of resolution enhancement filter was applied to the FID before Fourier transformation.

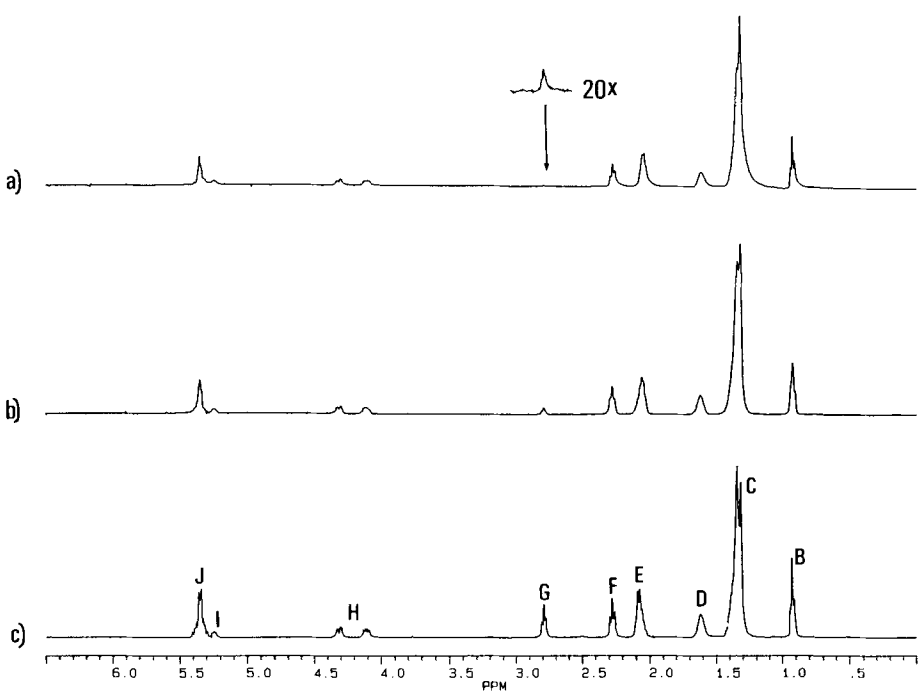

FIG. 7. 400.13 MHz MASS ^{1}H NMR spectra of sunflower seeds randomly selected from an early hybrid of high oleyl sunflower seeds which correspond to a) sunflower 1; b) sunflower 2; and c) sunflower 3 in Table 2.

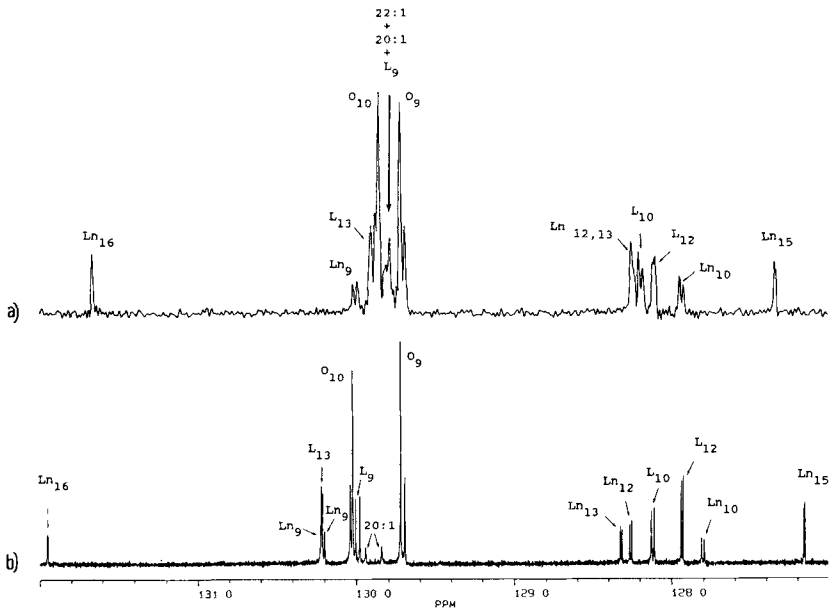

FIG. 8. a) 100.67 MHz MASS DEPT ^{13}C NMR olefinic spectrum of Cascade high oleyl rapeseeds; and b) 75.45 MHz high resolution ^{13}C NMR olefinic spectrum of canola high oleyl rape oil. Both spectra are referenced to O_9 (1,3-) at 129.7224 ppm for convenience to directly compare the spectra. Each peak is defined as O = oleyl, L = linoleyl, and Ln = linolenyl. See Table 3 for assigned chemical shifts of (a) using β OCH = 68.94 ppm.

TABLE 3

Assigned Chemical Shifts of Rape Oil Triacylglycerol Olefinic Carbons from a Whole Cascade (High Oleyl) Rapeseed (Fig. 2a)[a]

Peak	Chemical shift (δ ppm)
Ln_{16}	131.42
Ln_9 (1,3-)	129.77
Ln_9 (2-)	129.75
L_{13}	129.66
O_{10} (2-)	129.63
O_{10} (1,3-)	129.61
L_9 (1,3-)[b]	129.57
L_9 (2-)[b]	129.54
O_9 (1,3-)	129.48
O_9 (2-)	129.45
$Ln_{12,13}$	128.01
L_{10} (2-)	127.96
L_{10} (1,3-)	127.94
L_{12} (1,3-)	127.87
L_{12} (2-)	127.86
Ln_{10} (2-)	127.71
Ln_{10} (1,3-)	127.68
Ln_{15}	127.10

[a]The peaks are referenced to the β OCH = 68.94 ppm. The 100.4 MHz MASS ^{13}C NMR spectrum was acquired using the DEPT pulse sequence. The peaks are assigned as Ln (linolenyl), L (linoleyl), and O (oleyl). The (1,3-) and (2-) designations denote the acyl group as being attached to either the α OCH_2 or the β OCH, respectively.

[b]Poorly resolved peaks superimposed on 20:1 and 22:1 olefinic carbon peaks.

dissolved in $CDCl_3$. To illustrate the peak shifts, a high-resolution olefinic spectrum of high oleyl rape oil (canola) is compared directly with a MASS DEPT spectrum of high oleyl Cascade seeds in Figure 8. For convenience, both spectra are referenced to O_9 (1,3-acyl) at 129.7224 ppm. This is a correct reference for the solution spectrum using tetramethylsilane (TMS = 0 ppm) as an internal reference. However, the MASS spectrum was not referenced, except for O_9 (1,3-acyl) at 129.7224 to agree with O_9 of the solution spectrum. Consequently, the MASS spectrum is not displayed with an absolute chemical shift axis. The most striking difference between the two spectra is the compressed chemical shift dispersion of the olefinic carbons for the intact oil relative to the extracted oil. The comparison of each separate cluster of peaks between the two spectra also exhibits a smaller chemical shift difference between the peaks in the MASS spectrum relative to the solution spectrum. In addition to the condensed dispersion of chemical shifts in the MASS spectrum, there is also a shift of the relative peak positions between the MASS and solution spectra. The most obvious relative peak shift for high oleyl rape is an upfield shift of L_{13} in the MASS spectrum relative to the solution spectrum. As shown in Figure 8b, a typical solution spectrum exhibits L_{13} positioned between the 1,3- and 2-Ln_9 carbons, where a typical MASS spectrum shows L_{13} just to the low field side of O_{10}. An additional peak shift of high oleyl rape is L_9, which is positioned just upfield of O_{10} for the extracted oil but resonating equidistant between O_{10} and O_9 for the intact oil. As pointed out by Rutar *et al.* (5), these relative peak shifts may be due to either steric or solvent effects.

The chemical shift assignments for the peaks observed in Figure 2a are given in Table 3. The obtainable resolution is more than adequate to assign the majority of the olefinic carbons as well as the 1,3- and 2-acyl carbons corresponding to $Ln_{9,10}$, L_{10}, and $O_{9,10}$. The Free Induction Decays (FID) corresponding to the spectra in Figure 2 were not manipulated in any manner before Fourier transformation. Therefore, additional resolution may be obtainable with the application of a Gaussian

Filter to the FID before Fourier transformation.

The olefinic carbon peak assignments of the intact triacylglycerols were made on the basis of comparing spectra of different oil seeds as well as high-resolution spectra of extracted oils from the corresponding seeds which were examined by MASS NMR. These seeds included high oleyl sunflower, high oleyl rape, and high erucyl rapeseeds. Peak identities were further substantiated by examining the chemical shift difference ($\Delta\delta$) between the resolved 1,3- and 2-acyl carbons. Solution spectra of extracted triacylglycerols (10,11) have been shown to exhibit decreasing $\Delta\delta$s as the double bond is placed further down the acyl chain from the glycerol end of the triacylglycerol molecule. Since the relative $\Delta\delta$ values of the unsaturated 1,3-, 2-acyl carbons are affected by their carbon position on the acyl chain, these values can be used to help define the unsaturated carbon position. The $\Delta\delta$ values observed in the spectrum of high oleyl Cascade seeds substantiate the assignments of O_9, O_{10}, L_{10}, Ln_9, and Ln_{10} with $\Delta\delta$ values of 2.796, 2.255, 2.398, 2.808, and 2.398 Hz, respectively. In general we have observed that positional peak pairs resulting from the unsaturated carbons at the 1,3- and 2-glycerol positions (at this field strength of 9.4 Tesla) which are further removed than the C_9 or C_{10} position on the acyl chain are not resolved. The only exception is the 1,3-, 2-acyl peak pair of L_{12} with a $\Delta\delta$ of approximately 1.27 Hz. The remaining unsaturated peaks appear as singlets and can be assumed to be at an acyl carbon position which is $\geq C_{12}$. There was some ambiguity as to the peak assignment for Ln_9 and L_{13} due to the relative peak shift between intact and extracted oils. The $\Delta\delta$ values permitted the unambiguous assignment of the doublet to the low field side of O_{10} as Ln_9 and the singlet between Ln_9 and O_{10} as L_{13}. The assignment of Ln_9 and L_{13} is one example where the $\Delta\delta$ values provide additional credibility to peak assignments beyond the comparison of selected seeds with different unsaturated acyl distributions. The ability to identify olefinic carbons at the 1,3- and 2-glycerol position also permits a semi-quantitative analysis of the O, L, and Ln 1,3-, 2-acyl distribution for intact rapeseed triacylglycerols. We previously observed an approximate 1,3-:2-acyl ratio of 1:1 for L and Ln and 2:1 for O in high oleyl rape oil (11), i.e., canola oil. These positional distribution values agree well with those given below, which were derived from the DEPT spectrum of high oleyl Cascade rape seeds (Fig. 2a). Normalized molar ratios for canola oil (extracted) and Cascade seed (intact): 18:1 (1,3-:2-), 69:31, 70:30; 18:2 (1,3-:2-), 47:53, 50:50, and 18:3 (1,3-:2-), 49:51, 52:48; respectively.

When compared with high oleyl rapeseeds, the spectrum of high erucyl rapeseeds (Bridger) depicts an absence of any of the shorter chain O, L, Ln groups in the 1,3-glycerol position. This observation agrees with data derived from solution spectra of extracted high erucyl oils (11). The exclusive substitution of O, L, and Ln at the 2-glycerol position appears to be the result of a preferential substitution of saturates, and longer chain unsaturates (erucyl, and eicosenoyl) at the 1,3-glycerol position. This ultimately leads to the 2-glycerol attachment for the majority of 18:n (n = 1,2,3) acyl groups in oils which contain substantial quantities of longer chain monoenes.

The carbon peaks and equations used to calculate the unsaturated acyl distribution are shown below. The data

TABLE 4

Unsaturate Acyl Composition (Mole%) of Whole Rapeseeds Derived from ^{1}H and ^{13}C DEPT Spectra of Bridger (High Erucyl) and Cascade (High Oleyl) Varieties

	Total						
Seed	18:1[a]	18:2[a]	18:3[a]	20:1+22:1[a]	Monoene[a]	Saturates[b]	Technique
Rape 2	60.1	21.7	12.1	6.0	66.1	—	^{13}C
(Cascade)	—	19.7	11.6	—	68.7	6.6	^{1}H
Four seeds							
Rape 3	54.6	25.1	13.1	7.2	61.8	—	^{13}C
(Cascade)	—	23.3	14.3	—	62.3	9.4	^{1}H
One seed							
Rape 4	16.2	13.2	9.4	61.1	77.3	—	^{13}H
(Bridger)	—	15.2	8.6	—	76.2	5.3	^{1}H
Four seeds							
Rape 5	11.0	13.5	11.2	64.2	75.2	—	^{13}C
(Bridger)	—	15.3	11.9	—	72.7	6.0	^{1}H
One seed							
Rape 6	61.3	18.5	11.9	8.2	69.5	—	^{13}C
(Cascade)	—	22.0	10.1	—	67.9	8.0	^{1}H
Five seeds							
Rape 7	11.7	13.2	11.4	63.7	75.4	—	^{13}C
(Bridger)							^{1}H[c]
Five seeds							

[a]Unsaturates are normalized to unsaturates only.

[b]Saturates are normalized to total acyl composition.

[c]Poorly resolved A and B CH_3s.

derived from the MASS proton spectra can be used as a check on the quantitative integrity of the unsaturated acyl distribution defined from the MASS ^{13}C DEPT spectra (Table 4). The quality of the match can only be compared between the total monoene content and the linoleyl, linolenyl content since there are no distinct monoene peaks in the proton spectra. The monoene values agree quite well, deviating ± 2% on the average between the two methods. The linoleyl values show a higher degree of variation at ± 2.5% and the linolenyl the lowest at ± 1%. Obviously ± 2.5% or even ± 1% for the linoleyl and linolenyl concentrations, respectively, are somewhat deceiving since the polyunsaturates are at a much lower concentration than the total monounsaturates. This actually translates to a variability of ± 10% for both linoleyl and linolenyl when considering the absolute variation of each group separately.

$$[Ln_{15} + Ln_{16}]/2 = 18{:}3'; \qquad 18{:}3 = 18{:}3'/K \qquad [1]$$

$$L_{12} = 18{:}2'; \qquad 18{:}2 = 18{:}2'/K \qquad [2]$$

$$O_9 = 18{:}1'; \qquad 18{:}1 = 18{:}1'/K \qquad [3]$$

$$[\text{region between } O_9 \text{ and } O_{10} - L_{12}]/2 = 20{:}1' + 22{:}1';$$
$$20{:}1 + 22{:}1 = [20{:}1' + 22{:}1']/K \qquad [4]$$

$$K = 18{:}1' + 18{:}2' + 18{:}3' + 20{:}1' + 22{:}1' \qquad [5]$$

The MASS ^1H spectrum shown in Figure 6 is that of a single high oleyl Cascade rapeseed. In order to resolve the terminal linolenyl CH_3 and remaining acyl CH_3s suitably in the ^1H spectra, a Gaussian resolution enhancement filter was applied to many of the FIDs before Fourier transformation. This was not the case for the ^1H spectrum shown in Figure 6. No type of resolution enhancement was necessary for this spectrum which displays baseline resolution between the linolenyl CH_3 and remaining CH_3s as well as a fully resolved triplet resulting from linoleyl CH_3 scalar doupling to the adjacent CH_2. Additional scalar coupling is observed for many of the other resonances in this spectrum as well. The acyl compositional values derived by ^1H NMR in Table 4 were obtained using the following equations:

$$A/3 = 18{:}3'; \; 18{:}3 = 18{:}3'/K \qquad [6]$$

$$[G - 4/3(A)]/2 = 18{:}2'; \; 18{:}2 = 18{:}2'/K \qquad [7]$$

$$[J + I - H/4 - 4(18{:}2') - 2A]/2 = N{:}1'; \; N{:}1 = N{:}1'/K \qquad [8]$$

$$[B - 3(18{:}2') - 3(N{:}1')]/3 = SATS' = SATS'/K' \qquad [9]$$

$$K = N{:}1' + 18{:}2' + 18{:}3' \qquad [10]$$

$$K' = K + SATS' \qquad [11]$$

where N:1 is the total monoene (18:1, 20:1, 22:1) contents, SATS is the total saturates content, and A through I are defined in Figure 6.

MASS NMR techniques certainly cannot replace the routine screening techniques already available for large seed populations. The NMR experiments are too time consuming and costly to be a routine technique for screening large quantities of seeds. On the other hand, most techniques presently used for the "nondestructive" quantitative analysis of the oil composition for large seed quantities also possess a certain risk that some seeds will not germinate after the oil has been isolated from the seed. In cases where seeds are deemed too valuable to be subjected to this type of risk, MASS NMR can be incorporated into the screening process as a truly nondestructive method of analysis.

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