Quantitative High Resolution ^{13}C Nuclear Magnetic Resonance of the Olefinic and Carbonyl Carbons of Edible Vegetable Oils

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The acyl distribution and acyl positional distribution (1,3-acyl and 2-acyl) of triacylglycerols derived from edible vegetable oils has been examined by ^{13}C nuclear magnetic resonance (NMR) spectroscopy. The acyl profile of three natural oils (corn, peanut, canola) and one specialty oil (high oleic sunflower oil, Trisun® 80) has been defined from the high resolution (medium field 75.4 MHz) spectrum of the carbonyl and olefinic regions. The quantitative integrity of the NMR derived acyl profile is substantiated by gas chromatographic (GC) analysis. The positional distribution data of the three natural oils indicates that polyunsaturates are replaced in the 1,3-glycerol position exclusively by saturates, while the oleyl distribution remains, for the most part, randomly distributed. The same is not true for Trisun® 80, which shows a more random distribution of the linoleyl groups as well.

KEY WORDS: Quantitative ^{13}C NMR high resolution, positional distribution, acyl distribution, triglyceride.

High resolution ^{13}C nuclear magnetic resonance (NMR) spectra of vegetable oils can provide valuable information about the acyl distribution and acyl positional distribution for a mixture of triacylglycerols. The position of each acyl group is defined by its placement on the glycerol backbone. The terminal methylenes are the 1,3-glycerol position (α), and the internal methine is the 2-glycerol (β) position. The position of the acyl groups can be defined as attached to the α (1,3-acyl) or β (2-acyl) glycerol carbons. The acyl distribution and acyl positional distribution, which is defined by the triacylglycerol mixture, can vary greatly between different oil varieties. This is expected, since the oil composition is quite dependent on the seed from which the oil is derived.

Plant breeding and genetic engineering of vegetable seeds is presently being used in the development of specialty vegetable oils. Agricultural researchers are interested in altering the oil composition or, more specifically, the carboxylic acid profile so as to enhance certain properties of an oil. Clearly there is a great need for reliable techniques to define both the acyl distribution and position of the acyl groups on the glycerol backbone of triacylglycerols.

NMR has been shown to be an adequate method when defining both the acyl distribution and positional placement on the glycerol backbone. Early quantitative analysis of vegetable oils by NMR was first performed by Shoolery (1). More recently, Ng has shown that the acyl positional distribution of palm oil can be defined from the carbonyl ^{13}C NMR spectral region (2,3). Furthermore, Ng has been able to discern the unsaturate fatty acid positions on the glycerol backbone of palm oil (4) from the high field olefinic carbon spectrum.

In this paper we discuss the type of structural information that can be obtained via high resolution ^{13}C NMR for three edible vegetable oils (canola, peanut, corn) and one edible specialty oil (high oleic sunflower oil, Trisun® 80). The quantitative integrity of the NMR derived acyl distribution has been verified by gas chromatographic (GC) analysis on the corresponding oils examined by NMR. Similarly, the quantitative accuracy of the acyl positional distribution, as measured by NMR, has been assessed with the use of a known positional distribution standard.

EXPERIMENTAL

^{13}C NMR spectrometry was performed on a Bruker AM 300WB (Bruker Instruments, Inc., Karlsruhe, Germany) operating at 75.4 MHz. All oils examined were prepared to approximately a 10 wt% solution in $CDCl_3$. The quantitative ^{13}C spectra were obtained with a 10 mm broadband probe using the NOE-suppressed, inverse-gated, proton decoupled (Waltz-16) technique. The free induction decay (FID) of each oil was acquired at 27°C with a 20 second acquisition time, using a sweep width of 4545 Hz, and 181K acquisition points zero filled to 256K points to yield a digital resolution of 0.035 Hz/pt. Typically, 1000 scans were collected using a 90° excitation pulse and a 20 second relaxation delay for a 40 second recycle time. Three of the oils examined are commercially available—canola (Puritan®), corn (Mazola®), and peanut (Planters®)—the fourth is a high oleic sunflower oil trade named Trisun® 80, which was obtained from SVO Enterprises, a division of the Agrigenetics Company.

The model triacylglycerol examined was prepared from triolein, trilinolein, and trilinolenin obtained from the Sigma Chemical Co., St. Louis, MO. The spin-lattice (T_1) relaxation values were acquired on the model triglyceride mixture using the standard inversion-recovery T_1 pulse sequence provided in the Bruker NMR software version DISR88. The T_1 data were acquired after degassing the sample with six freeze-pump-thaw cycles. A Gaussian filter of 0.15 Hz Lorentzian narrowing and 0.18 Hz Gaussian broadening was applied to the FID before Fourier transformation.

GC analysis of the methyl carboxylates derived from the triacylglycerols was carried out on a Hewlett-Packard 5890 (Hewlett-Packard Co., Palo Alto, CA). The GC procedure used to define the carboxylic acid profile of each oil was AOCS Official Method Ce 1-62. One modification of procedure Ce 1-62 was the use of a capillary column instead of a packed column. The capillary column used was a Supleco SP 2330 30 m × 0.25 mm ID (Supelco, Inc., Bellefonte, PA). The methyl esters were prepared using AOCS Official Procedure Ce 2-66.

RESULTS AND DISCUSSION

The advantages and disadvantages of the carbonyl and olefinic ^{13}C regions as they pertain to defining the acyl distribution and acyl positional distribution is examined in this study. The carbonyl region alone can be used to define the acyl distribution and acyl positional distribution for fairly simple oils, i.e., sunflower (Fig. 1b), olive, corn, and peanut. In general, these oils contain only

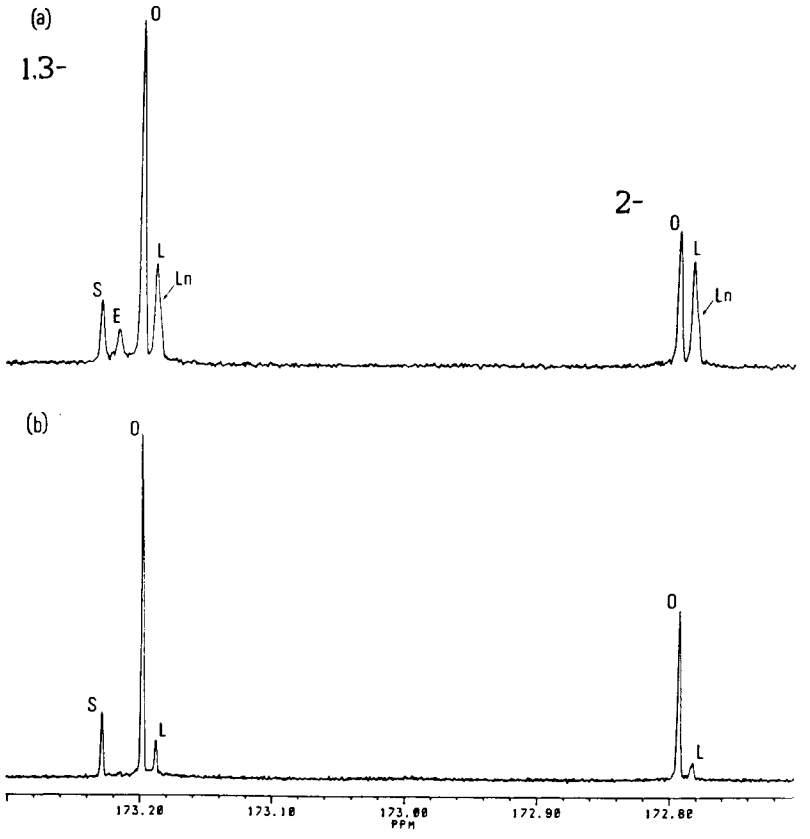

FIG. 1. 75.4 MHz C^{13} NMR carbonyl spectra of two edible vegetable oils made to 10 wt% oil in $CDCl_3$; a) LEAR canola oil (Puritan®); b) high oleic sunflower oil, Trisun® 80. The cabonyl peaks are defined as attached to the 1,3-glycerol (1,3-) or 2-glycerol (2-) positions. The acyl groups are saturates (S), 173.229δ(1,3-); oleyl (O), 173.216δ(1,3) and 172.794δ(2-); linoleyl (L), and linolenyl (Ln), 173.188δ(1,3-) and 172.783δ(2-); and eicosenoyl (E), 173.216δ(1,3). Peaks are referenced to 18:1 C-9 (1,3-) at 129.7215δ. The shoulder on the high field side of the L, Ln 1,3- and 2-position peaks is linolenyl.

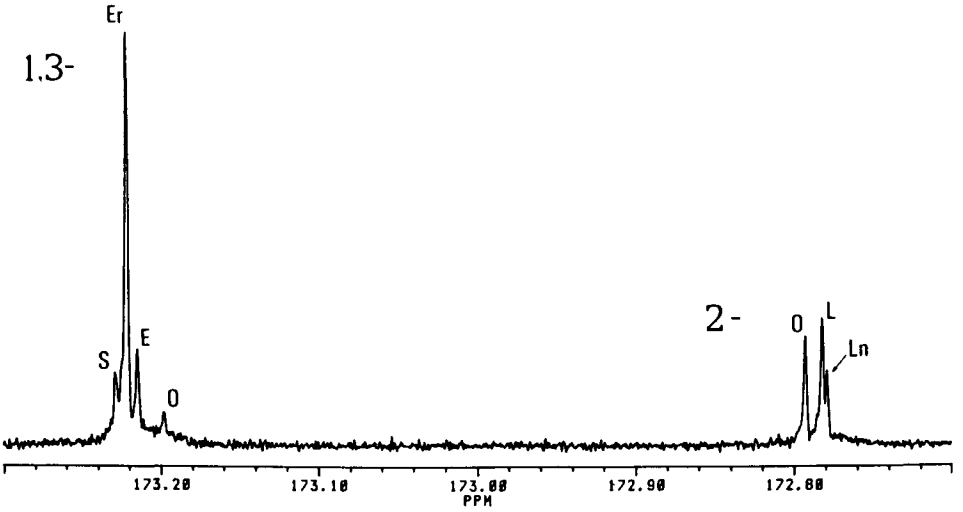

FIG. 2. 75.4 MHz ^{13}C NMR carbonyl spectrum of a nonedible vegetable oil (HEAR rapeseed oil) made to 10 wt% in $CDCl_3$. The carbonyl peaks are defined as attached to the 1,3-glycerol and 2-glycerol positions. The acyl groups are saturates (S), oleyl (O), linoleyl (L), linolenyl (Ln), eicosenoyl (E), and erucyl (Er) 173.221δ(1,3). Note that the saturates, erucyl, and eicosenoyl are exclusively in the 1,3-position and the linoleyl and linolenyl are exclusively in the 2-position. The oleyl is mostly in the 2-position with a small amount in the 1,3-position.

saturates (S), oleyl (O), and linoleyl (L) groups. Each of these groups can be accurately integrated since they are fully resolved in the carbonyl region. More complex oils, such as low erucic (LEAR) canola (Fig. 1a) and certain nonedible vegetable oils (Fig. 2) contain peaks in the carbonyl region which are overlapping at our observed frequency of 75.4 MHz. The olefinic region, which has a larger dispersion of chemical shifts, can be utilized to quantify those acyl groups which have superimposed carbonyl peaks. In this way, a combination of both the olefinic and carbonyl spectral regions can provide the necessary information to quantify the full acyl positional distribution of more complex oils.

Carbonyl region. The unsaturate carbonyl chemical shifts of the triglycerides examined in this study indicate a shift to higher field (lower frequency) as the double bond is positioned closer to the carbonyl carbon or the number of double bonds increase for acyl groups which are C_{18} or greater. This is illustrated in the carbonyl spectrum of canola oil (Fig. 1a). The carbonyl chemical shift order, going from low field to high field, for some of the common acyl groups which we have examined is shown here: saturates(S) > 22:1δ13;erucyl(Er) > 20:1δ11;eicosenoyl(E) > 18:1δ9;oleyl(O) > 18:2δ9,12;linoleyl(L) > 18:3δ9,12,15; linolenyl(Ln) > 20:1[*cis*]-δ5 > 22:2 [*cis,cis*]-δ5,13.

The assignments of the above carbonyl chemical shift order agrees with the chemical shift order given by Ng *et al.* (5) for *cis*-monoene (oleyl, erucyl, eicosenoyl) carbonyl chemical shifts. The dispersion of chemical shifts in the olefinic region is clearly superior to that of the carbonyl region for the observation of both erucyl and eicosenoyl (Fig. 3). Therefore, the olefinic region can be used to quantify the erucyl and eicosenoyl content at both the 1,3- and 2-positions and compared with that observed in the carbonyl region to substantiate carbonyl assignments. The peak area for the assigned eicosenoyl peak in Figure 1a is in agreement with GC analysis of the oil and that area observed for eicosenoyl in the olefinic region. The comparison of the carbonyl spectra of high erucyl rapeseed and high oleyl rapeseed oil further substantiates the erucyl and eicosenoyl peak assignments. The order of the linoleyl and linolenyl carbonyls was previously indicated by Ng (3) as well, but no spectra were given which displayed distinguishable linoleyl and linolenyl carbonyl peaks. These peaks are clearly resolved in the carbonyl spectrum of high erucyl rapeseed oil displayed in Figure 2. The relative linoleyl and linolenyl peak heights agree with GC analysis, and therefore support both the assignments and above chemical shift order. The additional unsaturate carbonyl peaks (20:1δ5 and 22:2δ5,13) defined above are those derived from meadowfoam oil, and were identified and assigned by NMR on the basis of known unsaturate acyl quantitaies of each of these groups from the GC analysis of meadowfoam oil.

Unfortunately, the different saturates are not fully resolved in the carbonyl region. The saturates as illustrated in Figure 1 are observed as a set of unresolved peaks, and therefore represented as a single acyl group. Additional complexities can occur as the unsaturation is positioned further from the carbonyl carbon, e.g., the δ13 double bond in erucyl. This leads to poorly resolved saturate and erucyl peaks (Fig. 2) due to the extensive bond distance between the erucyl olefinic (δ13) and carbonyl carbons.

The positioning, as well as the number of double bonds, is important in dictating the carbonyl chemical shift. We have observed, as has Ng (5), that the further the monoene unsaturation is removed from the carbonyl the more similar the monoene and saturate carbonyl electronic environments become. This leads to discernible, but poorly resolved, saturate and erucyl peaks.

The 1,3- and 2-linoleyl, linolenyl resonances also overlap in the carbonyl region (Fig. 1a). The 2-linolenyl peak is shown to be 0.003 ppm to the high field side of the 2-linoleyl carbonyl peak in Figure 2. The linoleyl and linolenyl carbonyl peaks therefore permit their identification, but are not suitable for accurate integrals. In order to obtain reliable integrals and thereby the acyl and 1,3-acyl, 2-acyl composition of an oil containing saturates and erucyl and/or linoleyl and linolenyl, the olefinic spectrum must be obtained.

Defining the acyl and 1,3-acyl, 2-acyl distribution from the carbonyl region has many advantages over the olefinic region. The most obvious benefit is that the saturates concentration and 1,3-:2-saturate ratio is obtainable only from the carbonyl spectrum. Two additional benefits are similar NOE (nuclear Overhauser effect) factors which were observed by Ng (2), and similar carbonyl T_1 values (6–8 seconds) as given in Table 1.

As previously pointed out by Ng (2), similar carbonyl T_1 and NOE values suggest that quantitative carbonyl spectra can be obtained with relaxation delay times shorter than the traditional $\geq 5T_1$ necessary for quantitative conditions (see quantitative results section). Very short relaxation delays can be used (1 second) because the carbonyl carbons have nearly identical NOE factors. The long acquisition time (20 seconds), which is essential when acquiring high resolution spectra, can then play a dual role. First and foremost, the spectral resolution is enhanced. This is necessary because the carbonyl peak range is quite narrow. Secondly, the spin system can fully relax back to thermal equilibrium without a long relaxation delay time. The final result is an experimental time which is half that required for the olefinic spectrum. This is a substantial time savings, because the experimental time to acquire the olefinic spectral region is approximately 11 hr (1000 scans).

One notable disadvantage is that the carbonyl chemical shift range is relatively narrow as compared to the olefinic

TABLE 1

Spin-Lattice Relaxation Values (T_1) in Seconds for a Mixture of Trioleyl, Trilinoleyl, and Trilinolenyl Triglycerides Made to 10 wt% in $CDCL_3$ at 300°K

	Triglycerides					
	18:1		18:2		18:3	
Acyl Carbon	1,3-	2-	1,3-	2-	1,3-	2-
C-9	1.9	1.7	2.4	2.1	—	2.4
C-10	1.9	1.8	2.3	2.2	2.4	2.4
C-12			3.8	3.7	4.4	4.4
C-13			3.6	—	4.6	4.5
C-15					8.9	8.6
C-16					8.0	8.1
C=O	7.9	6.5	7.9	6.7	7.7	6.0

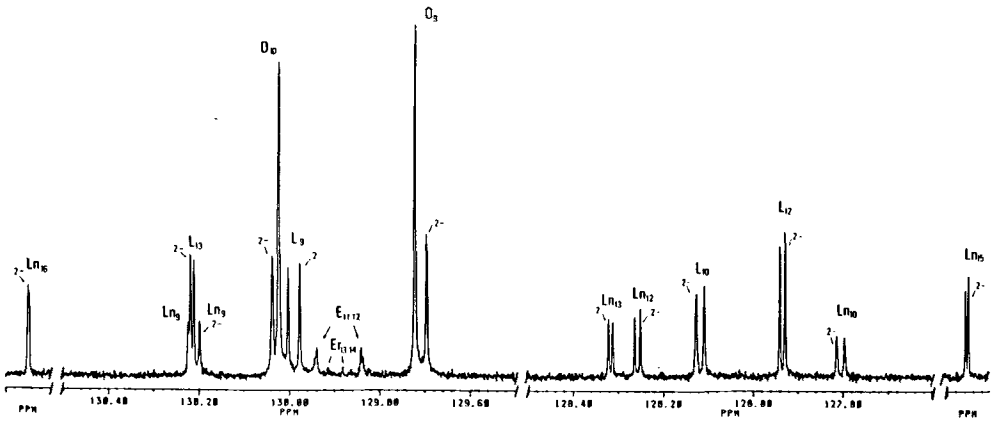

FIG. 3. 75.4 MHz ^{13}C NMR olefinic spectrum of Puritan® canola oil made to 10 wt% in $CDCl_3$. Each olefinic sections shown is scaled to 0.06 ppm/cm. Olefinic carbons are designated by their carbon chain position and acyl type (O = oleyl, L = linoleyl, Ln = linolenyl, E = eicosenoyl, and Er = erucyl). The 2-acyl position is defined for each 1,3- and 2-acyl pair. The O_9, L_{10}, and Ln_{10} 1,3- and 2-acyl peaks are fully resolved and can be used to quantify the unsaturate acyl profile. The eicosenoyl level can be defined, however, the low eicosenoyl level does not permit an accurate integration of the 1,3- and 2-positional peaks. The chemical shifts are given in Table 2.

chemical shift range. The saturates, which differ only by their chain length (usually $\geq C_{16}$), are practically indistinguishable as viewed by the carbonyl carbon. This leads to an indiscernible dispersion of saturate resonances. And as the saturate peaks cannot be resolved, the only quantitative parameter which is definable is the total 1,3- and 2-saturate content. This is not very satisfying because the saturate distribution is an integral part of the triglyceride composition. However, the total saturate content and positional distribution provides generic compositional information of an oil. Certain physical and/or chemical properties of an oil, e.g., enzymatic hydrolysis, specifically at the 1,3-position, is accessible even from the generic saturate data. Furthermore, the feasibility of random distribution (RD) calculations (6,7) to define the actual triglyceride distribution may be deduced from the NMR derived distribution data.

Obviously, the carbonyl region should always be acquired in order to define the saturate concentration, even if the entire unsaturate profile is unattainable from this region.

As mentioned previously, the major drawback of the carbonyl region is the narrow chemical shift range. The triglyceride composition in most edible vegetable oils consists of a handful of acyl groups. These acyl groups are saturates (S), eicosenoyl (E), oleyl (O), linoleyl (L), and linolenyl (Ln). The chemical shift range for acyl groups in the 1,3-position is approximately 0.05 ppm, and in the 2-position 0.015 ppm, because the saturates are exclusively in the 1,3-position. Further spectral crowding (Fig. 2) can be observed in the carbonyl region for nonedible vegetable oils, e.g., high erucic (HEAR) rapeseed oil which contains substantial quantities of erucyl and eicosenoyl as well as linoleyl and linolenyl. One common edible vegetable oil which does contain a significant amount of eicosenoyl, linoleyl, and linolenyl is low erucic (LEAR) canola oil.

The eicosenoyl content of canola oil can be measured from both the carbonyl and olefinic region since erucyl, if present, is at a negligible concentration. On the other hand, any appreciable amount of erucyl cannot be fully resolved from the saturates carbonyl peak unless higher fields are employed. Therefore, the saturates and erucyl content of nonedible vegetable oils is not accessible from the carbonyl region. The only alternative in this case is to quantify the erucyl and saturates content using a combination of the olefinic and carbonyl regions. The unsaturate content defined from the olefinic region can then be subtracted from the unresolved carbonyl peaks, with the difference being the 1,3-saturate content.

Olefinic region. The unsaturate acyl and unsaturate 1,3-acyl, 2-acyl triglyceride composition is readably accessible from the olefinic spectrum as well. Many of the unsaturate carbons are baseline resolved (Fig. 4, high oleic sunflower oil) permitting reliable peak integrals. In the case of overlapping olefinic peaks (Fig. 3, canola oil), there are additional olefinic carbons which may be used to define an unsaturate acyl group.

Multiple olefinic carbon peaks of an unsaturate acyl group are the single most important aspect of the olefinic region. Unresolved unsaturate acyl groups in the carbonyl region, such as linoleyl and linolenyl, have four and six olefinic carbons, respectively, to choose from. This allows for many possible combinations to quantify the linoleyl and linolenyl content, as well as the positional distribution when peak regions are congested.

An additional benefit of the olefinic region is the larger dispersion of chemical shifts. This is obvious from the chemical shift assignments (Table 2) of some common unsaturate acyl groups.

The relative peak positions agree with those made by Ng (4) for 1,3-, 2-oleyl and linoleyl peaks. The direct spectral comparison of high oleyl sunflower, high erucyl rapeseed, and low erucyl rapeseed oil substantiates the above assignments. This is possible because the carbonyl spectrum of high erucyl rapeseed oil (Fig. 2) clearly shows linoleyl, linolenyl and the majority of oleyl exclusively in the 2-position and saturates, erucyl and eicosenoyl in the 1,3-position. In contrast, the carbonyl region of high oleyl sunflower and low erucyl rapeseed oil show all the

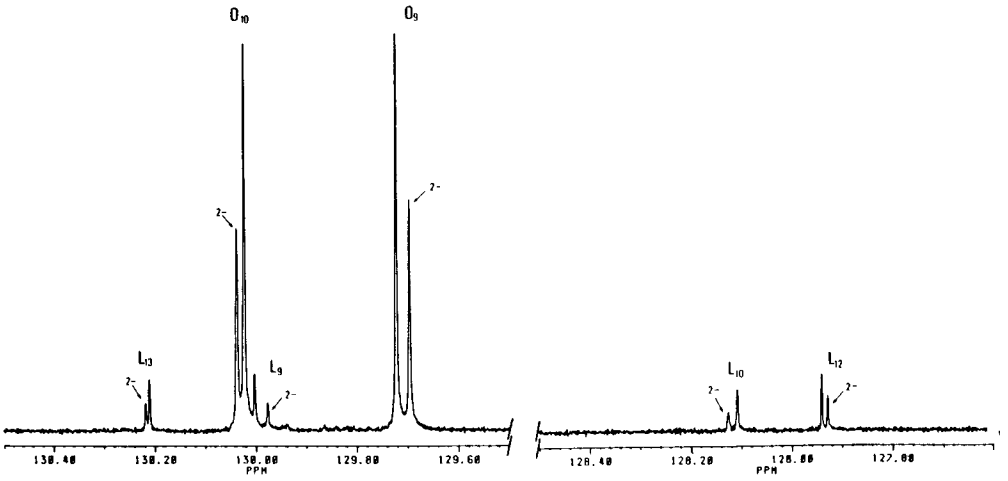

FIG. 4. 75.4 MHz ^{13}C NMR olefinic spectrum of Trisun® 80 high oleic sunflower oil made to 10 wt% in $CDCl_3$. Each olefinic section shown is scaled to 0.06 ppm/cm. Olefinic carbons are designated by their carbon chain position and acyl type (O = oleyl, L = linoleyl). The 2-acyl position is defined for each 1,3- and 2-acyl pair. The O_9 and L_{10} 1,3- and 2-acyl peaks are fully resolved and can be used to quantify the unsaturate acyl profile. The chemical shifts are given in Table 2.

TABLE 2

^{13}C NMR Chemical Shifts in ppm[a] of Common Unsaturate Acyl Groups Found in Edible Vegetable Oils

18:1[*cis*]-*δ*9 (oleic)	
C-9(1,3/2)	129.721/129.695
C-10(1,3/2)	130.022/130.037
18:2[*cis,cis*]-*δ*9,12 (linoleic)	
C-9(1,3/2)	130.002/129.926
C-10(1,3/2)	128.106/128.124
C-12(1,3/2)	127.939/127.927
C-13(1,3/2)	130.210/130.218
18:3[*cis,cis,cis*]-*δ*9,12,15 (linolenic)	
C-9(1,3/2)	130.223/130.198
C-10(1,3/2)	127.794/127.811
C-12(1,3/2)	128.262/128.250
C-13(1,3/2)	128.311/128.321
C-15(1,3/2)	127.157/127.150
C-16(1,3/2)	131.946/131.949
20:1[*cis*]-*δ*11 (eicosenoic)	
C-11(1,3/2)	129.841/129.813
C-12(1,3/2)	129.939/129.948
22:1[*cis*]-*δ*13 (erucic)	
C-13(1,3/2)	129.876/129.856
C-14(1,3/2)	129.912/129.916

[a]Chemical shifts are referenced to 18:1[*cis*]-*δ*9 C-9(1,3) = 129.7215 ppm, which agrees with TMS = 0 ppm.

unsaturate acyl groups more randomly distributed in both the 1,3- and 2-positions. This information can be carried over into the olefinic region to aid in the positional assignment of the 1,3- and 2-acyl peaks when comparing these spectra.

The most pronounced disadvantage is that the saturates cannot be defined from the olefinic region. For this reason, both the carbonyl and olefinic regions are acquired as a single experiment. This can lead to quite large data files of 256k bytes or more, but with today's disk storage systems data files of this magnitude are easily handled.

The olefinic region poses additional disadvantages beyond the ability to define the saturate content, the most prominent being the differences in olefinic T_1s. As illustrated in Table 1, the T_1 values increase dramatically as the olefinic carbons are further removed from the glycerol end of the molecule. The most obvious T_1 difference is between the 18:1 C-9,C-10 and 18:3 C-15,C-16 carbons. There is a four- to five-fold increase in the T_1 values going from approximately two seconds for 18:1 C-9,C-10 carbons to 8–9 seconds for 18:3 C-15,C-16 carbons. Therefore, if either the 18:3 C-15 or C-16 peaks are to be used in defining the linolenyl content, the inverse gated experiment would require an increase from 20 seconds (5.5 hr/1000 scans) to 45 seconds (12.5 hr/1000 scans) for each scan if a recycle time of $\geq 5T_1$ of the longest carbon T_1 (18:3 C-15) to be measured is met. This translates into an additional 7 hr of acquisition time for a 1000 scan experiment and does not include the time necessary to quench the NOE signal enhancement resulting from the long acquisition time.

The T_1 values given in Table 1 indicate that the 20 seconds recycle time for the 18:1 C-9,C-10 olefinic carbons is longer than that needed to meet the $\geq 5T_1$ requirement. However, acquisition times (AT) of 20–30 seconds are essential for high spectral resolution because the spectral width was set to 4545 Hz [dwell time (DT) of 110 us] and a total of 181K acquisition points (TD) were acquired to obtain a spectral resolution of 50 mHz. These parameters automatically define the acquisition time to be 20 seconds (AT = TD × DT).

Another quantitative consideration is the NOE enhancement of the carbon signal during the acquisition time. Because NOE is a phenomenon of longitudinal (T_1), not transverse (T_2) magnetization (8), NOE-affected signal enhancement cannot occur during acquisition.

However, the long acquisition times necessary for high resolution spectra can act as a T_1 relaxation period. The return of longitudinal magnetization during this period with the proton decoupler on can induce NOE enhancement of the carbon signal intensity for the ensuing acquisition. This is an unwanted enhancement because not all olefinic carbons necessarily have the same NOE factor, and certainly not the same T_1 values.

Signal enhancement resulting from NOE can be eliminated through longer relaxation delay times because NOE build-up during the acquisition time will decrease as an exponential function of the carbon T_1 once the decoupler is gated off (relaxation delay period). This indicates that carbon nuclei with longer T_1s will need longer relaxation delays due to NOE build-up during the acquisition time, even though the effective time for T_1 relaxation is the acquisition time + relaxation delay time. In other words, the relaxation delay time should be $\geqslant 5T_1$ to allow an effective time period to quench NOE buildup regardless of the total T_1 relaxation time period. Consequently, the T_1 values in Table 1 imply a 45 second relaxation time for 18:3 C-15,C-16, as compared to 20 seconds for olefins $\leqslant$ C-13. Relaxation delay periods of this magnitude, coupled with a 20 second acquisition time translates into experimental times of 18 hr (65 s/scan) and 11 hr (40 s/scan) to quantify 18:3 C-15,C-16 and olefinic carbon $\leqslant$ C-13, respectively.

Fortunately, defining the linolenyl concentration is not limited to using the C-15,C-16 double bond carbons or even C-12,C-13 carbons. Similarly, the linoleyl concentration can be defined by either the C-12,C-13 or C-9,C-10 carbons. In general, the oleyl, linoleyl, and linolenyl composition is best defined using the C-9 or C-10 carbons indicated for canola oil in Figure 3.

There are two major advantages in using the C-9,C-10 carbons, with the first being similar T_1 values. The C-9,C-10 T_1s are relatively short (2–3 seconds) increasing slightly as the number of double bonds for a given acyl group increases. As discussed above, the shorter T_1s allow for a shorter relaxation delay time to quench NOE build-up which, in turn, decreses the overall experimental time. The second major advantage is that the chemical shift difference ($\Delta\delta$) for a positional peak pair (1,3-acyl vs 2-acyl) decreases as the double bond is further removed from the glycerol section of the molecule (4). The $\Delta\delta$ for the 1,3- and 2-positional peaks of 18:3 C-9, C-10, C-12, C-13, C-15, C-16 are 1.906, 1.317, 0.939, 0.711, 0.485, 0.217 Hz, respectively. These $\Delta\delta$ values clearly can aid in the spectral assignments of peaks and illustrate why fully resolved 1,3-acyl and 2-acyl peaks are more easily obtained for the linolenyl group when using the C-9,C-10 olefinic carbons vs the C-15,C-16 carbons. The better resolution from the C-9,C-10 carbons yields more accurate integrals and ultimately better quantification of the linolenyl positional distribution.

There are some C-9,C-10 peaks which are not fully resolved among the different acyl groups. An example of unresolved peaks (Fig. 3) are the 18:2 C-13 (1,3-acyl and 2-acyl) peaks which are superimposed on the 18:3 C-9, positional peaks and the 18:2 C-9 (1,3-acyl) peak overlapping the 18:1 C-10 (1,3-acyl) peak. These unresolved peaks do not hinder the use of the C-9,C-10 olefinic carbons since the 18:1 C-9, 18:2 and 18:3 C-10 1,3-acyl and 2-acyl carbons are baseline resolved.

The presence of 20:1δ11 (eicosenoyl) and/or 22:1δ13 (erucyl) is easily verified in the olefinic region. The 20:1 C-11,C-12 olefinic carbons are well resolved inside the C-10,C-9 oleyl peaks. Likewise, the 22:1 C-13,C-14 olefinic peaks are baseline resolved between the 20:1 C-11,C-12 peaks.

The olefinic carbons therefore permit a facile and unambiguous means of qualitatively assessing the presence of either eicosenoyl or erucyl groups. Furthermore, either olefinic carbon of the eicosenoyl or erucyl group is adequately resolved to define the acyl distribution. The same is not true, however, when defining the 1,3-, 2-acyl positional distribution. The erucyl (C-13) and eicosenoyl (C-11) olefinic carbons that are positioned closer to the carbonyl have $\Delta\delta$s which are approximately 0.5 Hz larger than their low field (22:1 C-14, 20:1 C-12) counterparts. The seemingly small $\Delta\delta$ difference between the 1,3-acyl and 2-acyl carbons of the low field (22:1 C-14; 0.359 Hz and 20:1 C-12; 0.665 Hz) and high field olefinic carbons (22:1 C-13; 0.813 Hz and 20:1 C-11; 1.236 Hz) is quite significant when examining the total chemical shift dispersion between the 1.3- and 2-acyl positions. Obviously, when peak pairs are this closely positioned, any additional peak separation is a crucial factor in the integration process. It is for this reason that we have chosen to integrate only the high field eicosenoyl and erucyl olefinic peaks when quantifying their acyl positional distribution. In general, these spectral regions for most edible vegetable oils can be ignored since the eicosenoyl and erucyl concentration is quite small (<1 mole %). The only exception which we encountered was canola oil (2–4 mole % eicosenoyl).

Contrary to edible oils, nonedible vegetable oils can contain relatively high concentrations of both eicosenoyl and erucyl. Consequently, the olefinic spectrum between 18:1 C-10,C-9 is a critical region when examining nonedible vegetable oils.

Quantitative results. It is important to examine the reliability of the NMR-derived acyl distribution and positional distribution data. The current method commonly used to define the carboxylic acid profile is GC analysis of the corresponding methyl carboxylates derived from the oil or triglyceride mixture. The NMR values, i.e., acyl distribution, have been compared with the GC derived carboxylic acid distribution of the corresponding oils in Table 3. These data show a favorable correlation between the NMR-derived acyl distribution and GC derived carboxylic acid profile. A list of the NMR-derived positional distribution normalized for each acyl type also is given in Table 3.

A standard mixture of triolein, trilinolein, and trilinolenin was prepared to examine the absolute accuracy of the NMR-derived acyl and positional distribution valves. The acyl distribution derived from the carbonyl region is within 2–6% of the actual values and the olefinic region within 2–5% of those values. These error margins are within an acceptable range, using NMR as the quantitative technique.

There was not an additional technique viewed to be superior to NMR for obtaining the positional distribution. However, one possible check on the integrity of these data is the summation of the total 1,3-acyl and 2-acyl concentrations of each oil, which is given in Table 4. Furthermore, a standard triglyceride mixture was prepared and used as a check on the accuracy of the NMR derived positional data.

TABLE 3

NMR/GC derived Acyl Distribution of Triglycerides in Edible Vegetable Oils and a Standard Triglyceride Mixture

Oil	Saturates NMR/GC	Unsaturates 18:1 (1,3:2) NMR/GC	18:2 (1,3:2) NMR/GC	18:3 (1,3:2) NMR/GC
Canola				
Carbonyl	7.7/7.4	—	—	-
Olefin[d,c]		63.3/64.1 (69:31)	21.6/23.0 (47:53)	10.7/11.6 (49:51)
Peanut				
Carbonyl[c]	19.3/18.4	46.3/48.3 (67:33)	30.5/31.5 (45:55)	
Olefin[d,c]		61.5/59.4 (70:30)	38.5/38.8 (45:55)	
Corn				
Carbonyl[c]	13.9/14.0	27.0/27.4 (66:34)	57.0/57.7 (59:41)	
Olefin[d]		31.0/31.9 (66:34)	68.2/67.1 (59:41)	0.8/0.9 (69:31)
Trisun® 80				
Carbonyl[c]	9.5/8.0	80.5/82.1 (64:36)	10.0/9.1 (63:37)	
Olefin[d]		89.3/90.0 (65:35)	10.7/10.0 (63:37)	
Standard				
Carbonyl[a]		31.2/29.5 (66:34)	---------- 68.8/70.5 (67:33)[b] ----------	
olefin[a]		28.7/29.5 (66:34)	34.7/33.2 (66:34)	36.7/37.3 (66:34)

The carbonyl values[c] are normalized to both saturates and unsaturate acyl groups. The olefin values are normalized to only the unsaturate acyl groups. The NMR derived positional distribution is in parentheses and normalized for that acyl group.

[a] Values on the right of the slash are known values, not GC values

[b] 18:2 and 18:3 carbonyl peaks are not fully resolved for an accurate integration

[c] The small amount of eicosenoyl and other undefined peaks account for the Σ C=O and Σ C=C peaks < 100%

[d] GC values renormalized without saturates contribution for comparison purposes with NMR olefinic-derived values.

TABLE 4

Summation of the 1,3-Acyl and 2-Acyl Concentrations Derived from the Acyl and Positional Distribution Data Given in Table 3

Oil	Carbonyl Σ 1,3	Carbonyl Σ 2	Olefin Σ 1,3	Olefin Σ 2
Peanut	66.6	33.3	68.0	32.0
Canola	65.4	34.6	—	—
Corn	66.8	33.2	66.5	33.4
Trisun® 80	67.3	32.7	68.1	31.9

The positional distribution data derived for the standard are in good agreement with an exclusively random acyl distribution. This is expected because the triglyceride mixture was prepared from trioleyl, trilinoleyl, and trilinolenyl triacylglycerols. The carbonyl and olefinic derived positional summation values are also reasonably close to the expected values, assuming insignificant quantities of mono- and diacylglycerols, of 66.6% for Σ 1,3-acyl and 33.3% for Σ 2-acyl.

Positional distribution. The ^{13}C NMR spectrum of the carbonyl region (Fig. 1) clearly shows all saturate acyl groups to be in the 1,3-position. The saturate acyl molar concentration ranges from a low of 8% in canola oil to a high of about 20% in peanut oil. The acyl distribution does not appear to affect the saturate positional distribution. Furthermore, the oleyl concentration, which ranges from a high of 82% in Trisun® 80 to a low of approximately 27% in corn oil, is for the most part randomly distributed (where random is defined as 67% α and 33% β) between the 1,3- and 2-glycerol positions. Similar to the saturate positional distribution, large shifts in oleyl content do not appear to affect the normalized oleyl positional distribution. In general, the data in Table 3 indicate that the linoleyl and linolenyl 1,3-acyl:2-acyl ratio of the three natural oils examined in this study depends on the polyunsaturate and saturate content. A change in the level of either one of these acyl groups appeared to have an effect on the polyunsaturate positional distribution.

The seemingly constant oleyl and saturate positional distribution with a fluctuating polyene 1,3-acyl:2-acyl ratio supports the notion that saturates preferentially replace polyunsaturates at the α position on the glycerol backbone for the three natural oils examined. The data indicate that a higher saturates level at a constant polyene level produces a lower polyene 1,3-acyl:2-acyl ratio and, accordingly, a higher polyene level at a constant saturates level yields a higher 1,3-acyl:2-acyl polyene ratio. This is not surprising, since one might expect the polyene positional distribution to approach a random distribution as the polyene level approaches unity. However, at the same time a similar trend is not observed for the oleyl 1,3-acyl:2-acyl ratio at varying levels of oleyl concentration. The apparent insensitivity of the oleyl positional distribution to changes in the acyl distribution for

the three natural oils examined in this study indicate that the oleyl groups are randomly distributed, while the saturates preferentially replace the polyenes at the α position.

In this study the one exception to the preferential replacement of polyene with saturates at the α position is the specialty sunflower oil, Trisun® 80. A closer examination of the data in Table 3 shows Trisun® 80 to have a higher 1,3-acyl:2-acyl linoleyl ratio than corn oil. This is unexpected because corn oil has the highest polyene level and Trisun® 80 the lowest. The similar oleyl and linoleyl 1,3-acyl:2-acyl ratio of Trisun® 80 contradicts the observation for the three natural oils, i.e., that saturates preferentially replace poyunsaturates in the α position, especially when considering the relatively low polyene content of Trisun® 80.

One explanation for the higher linoleyl 1,3-acyl:2-acyl ratio found for Trisun® 80 may be simply the abnormally high oleyl content. Because oleyl groups occupy most of the unsaturate positions, the seemingly random replacement of both the oleyl and linoleyl with saturates might indicate that the saturates begin to loose substitution specificity at elevated oleyl levels.

We hope to further this study by examining additional oils and comparing the triglyceride composition or partial triglyceride composition derived by HPLC analysis with those obtained by NMR-derived random distribution (RD) theory (6,7). The results from this study hopefully will provide some indication as to the amount of random or selective placement of acyl groups at the 1,3- and 2-glycerol positions. A study of this nature should help to clarify the observations described above, as well as provide some insight as to the applicability of using RD theory calculations to define an oil's triglyceride profile.

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