Quantitative Analysis of Partial Acylglycerols and Free Fatty Acids in Palm Oil by ^{13}C Nuclear Magnetic Resonance Spectroscopy

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ABSTRACT: The chemical shifts, spin-lattice relaxation times (T_1), and one-bond C–H coupling constants of the glycerol carbons of mono-, di-, and triacylglycerols in $CDCl_3$ solution are presented and discussed. The glycerol carbons have low T_1 values (<1.0 s) and full nuclear Overhauser effect and also exhibit broader linewidths than the aliphatic carbons, suggesting that the glycerol carbons are at or near the T_1 minimum for the dipole-dipole relaxation mechanism. Therefore, for quantitative measurement of the composition of partial acylglycerols (relative to the triacylglycerols) in palm oil, the nuclear magnetic resonance (NMR) spectrum of the β-carbons, which lie exclusively in the region δ68.3–72.1 ppm, should preferably be acquired at medium or low magnetic fields and at an elevated temperature in order to ensure that the condition for extremely narrow spectral lines is satisfied. The chemical shifts and spin-lattice relaxation times of the aliphatic C-2 and C-3 carbons and of the carbonyl carbons (C-1) of acyl groups present in palm oil are also presented and discussed. The presence of free fatty acid in the palm oil is easily detected and quantified in the spectrum of the aliphatic carbons. The presence of partial acylglycerols in palm oil can also be detected and/or quantified in the NMR spectra of the C-2 and the carbonyl carbons. The quantitative analysis of the glycerol carbons of a known mixture of acylglycerols obtained by using this method is presented.

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KEY WORDS: ^{13}C quantitative NMR, free fatty acid, NOE, palm oil, partial acylglycerols, T_1.

Partial acylglycerols are present in significant quantities in palm oil (1) and are known to have significant effects on the physical properties of the oil and of products containing palm oil and on the fractionation of palm oil. Mono- and diacylglycerols (MG and DG) are the most commonly used emulsifiers in the food industry (2). They are also used extensively in various applications in the pharmaceutical, cosmetic, and textile industries. A convenient method for the detection and quantitative determination of these partial acylglycerols in palm oil or in processes involving palm oil is therefore important in the industry.

Conventional methods used in the analyses of partial acylglycerols in vegetable oils are gas–liquid chromatography (GLC) and thin-layer chromatography (TLC). Each method has its drawbacks. In the GLC method, to circumvent the rapid deterioration of column efficiency due to the presence in palm oil of polar groups, free fatty acids, and other minor polar compounds (3), trimethylsilyl derivatives are prepared prior to GLC analysis (4). In the tedious TLC method the separation of the partial acylglycerols in bands is followed by quantitative determination with dichromate oxidation of the acylglycerols in the respective bands.

^{13}C nuclear magnetic resonance (NMR) spectroscopy has been found to have advantages for the quantitative analysis of MG isomers in a mixture (5) and of mixtures of acylglycerols in which the enantiomeric purity can be determined by using a shift reagent (6). MG, DG, and triacylglycerols (TG) have characteristic chemical shifts for the three glycerol carbons and for C-1 and C-2 carbons in each acyl chain, and this information can lead to qualitative and semiquantitative information about mixtures of these glycerol esters (7,8). The quantitative analysis of TG in vegetable oils has also been reported (9–11). ^{13}C NMR has the unique ability to detect *cis*-vaccenic acid or other monoenic acyl groups (12).

This paper describes the detection and quantitative determination of partial acylglycerols and free fatty acids (FFA) in palm oil by ^{13}C NMR spectroscopy. In particular this involves consideration of the resonances of the glycerol carbons, the carbonyl carbon (C-1), and the two adjacent carbons (C-2 and C-3) of the acyl chain.

EXPERIMENTAL PROCEDURES

The sample of natural palm oil used in this work was obtained from a local palm oil factory and was homogenized before use. The synthetic samples of simple TG and partial acylglycerols were obtained from Sigma Chemical Co. (St. Louis, MO) and were used without further purification.

Standard procedures were followed in obtaining the NMR data and spectra. The 67.94 MHz ^{13}C NMR spectra were recorded on a JEOL GSX270 spectrometer, and the 25.05 MHz ^{13}C NMR spectra were recorded on a JEOL FX100 spectrometer (Tokyo, Japan). The chemical shifts were measured with the GSX270 or the JEOL LA400 spectrometers, the latter operating at 100.52 MHz. The spin-lattice relaxation

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times and the associated nuclear Overhauser effects (NOE) were measured on the same spectrometer, either the FX100 or the JEOL EX90A spectrometer, the latter operating at 22.50 MHz. The inversion-recovery method was used for the T_1 measurements. The samples in $CDCl_3$ solution were degassed through five freeze-pump-thaw cycles. The integrated intensity of a peak for the NOE measurement was obtained on the expanded spectrum in which the total area under the curve was calculated using Simpson's rule. The NOE is the ratio of the peak area obtained under broadband proton-decoupled condition to that under gated-decoupling condition in which the NOE was suppressed. The relaxation delay used in the former condition was $7T_1$ and in the latter condition was $10T_1$.

A mixture consisting of the following acylglycerols was made by weighing to form 2.5 mol% solution of acylglycerols in $CDCl_3$: tripalmitin 6.71×10^{-5} mol, tristearin 1.04×10^{-5} mol, triolein 6.45×10^{-5} mol, 1-monopalmitin 4.26×10^{-5} mol, and 1,3-dipalmitin 4.20×10^{-5} mol. The total amount of TG was 14.20×10^{-5} mol, and mole ratio of TG to DG and MG as TG/DG/MG is 1.00:0.296:0.301. The NOE-suppressed NMR spectrum of the glycerol carbons in this mixture was obtained at 100.52 MHz at two temperatures (28 and 50°C) with the following parameters: acquisition time 1.517 s, pulse delay 9.0 s (or relaxation delay = 10 T_1), pulse angle 89°, spectral resolution 0.33 Hz (before zero-filling), and 3300 accumulations. The broadband proton-decoupled spectrum was also obtained at the same frequency at 28°C with the following parameters: acquisition time 1.517 s, pulse delay 3.0 s, pulse angle 25°, spectral resolution 0.33 Hz, and 6000 accumulations. The total integrated intensity of a NMR signal was obtained by measuring the area under the band using Simpson's rule.

RESULTS AND DISCUSSION

The detection and/or quantitative analysis of the partial acylglycerols in palm oil can be performed on three regions of the ^{13}C NMR spectrum: (i) that of the glycerol carbons, (ii) that of the C-2 carbons at high field in the region of δ34.0–34.5 ppm in $CDCl_3$ solution, and (iii) that of the carbonyl carbons. The spectrum of C-3 at δ24.7 ppm shows the presence of free fatty acids in the oil. Where appropriate this method can be applied to any other vegetable oil.

Glycerol carbons. The ^{13}C NMR spectrum of the glycerol carbons of a sample of palm oil containing 1,3- and 1,2-DG is shown in Figure 1. The peaks are well separated, except for α-C (G-1) of the 1,2-DG, which overlaps with the peak at δ62.07 ppm for the α-C of the TG. The chemical shifts and spin-lattice relaxation times T_1 of the glycerol carbons in MG, DG, and TG are shown in Table 1. The chemical shift data of the glycerol carbons in palm oil are independent of the nature of the fatty acid esterified to them. In a mixture of MG and DG in $CDCl_3$ the peak for the esterified G-1 in the MG is shifted by 0.12 ppm to high frequency of that of the esterified carbons of 1,3-DG at δ65.05 ppm. The β-C (G-2) in MG and 1,3-DG have the highest T_1 (0.90–0.99 s in $CDCl_3$ at 28°C and 22.5 MHz). The TG have the lowest T_1 [0.22 (α-C) and 0.35 s (β-C) at 22.5 MHz]. The NOE of the glycerol carbons of the four species of acylglycerols is found, at 22.5 MHz, to have maximal values of 2.98, that is, $\eta = 1.98$ and NOE = $1 + \eta$. The low T_1 values and full NOE reflect the fact that the glycerol carbons are in the relatively immobile region of the acylglycerol molecule, having long reorientational correlation times relative to the carbons in the flexible acyl chain; that is, the glycerol carbons are at or near the T_1 minimum for the dipole-dipole relaxation mechanism (13,14). Therefore, the short T_1 values and full NOE enable the quantitative spectrum to be obtained efficiently with the broadband proton-decoupling mode. For a more accurate quantitative spectrum, low pulse angles (<30°) should be used. The full NOE eliminates the necessity of employing the time-consuming gated-decoupling technique in which the proton-decoupler is required to be gated off for a period of 8–10 times the longest T_1 value in order to suppress the NOE.

The proportion of the TG species in the oil is represented by the total integrated intensity of the peak for its G-2 at δ68.90 ppm. The MG species is easily quantified with the peak for G-2 at δ70.25 or G-3 at δ63.40 ppm. The DG species are easily quantified with the peak for G-2 at δ72.10 ppm in the case of the 1,2-DG and for G-2 at δ68.30 ppm in the case of the 1,3-DG. The peaks of the G-2 of the four acylglycerol species lie exclusively in the region δ68.3–72.1 ppm, and as there is one G-2 in each acylglycerol molecule, the integrated intensities of the peaks of the G-2 carbons provide directly the mole fractions of the acylglycerol species in the oil. The G-2 has another advantage in that it has a narrower linewidth than the G-1/G-3, although the latter have shorter T_1 (0.40–0.70 s). The linewidth problem and suitable conditions for acquiring the ^{13}C NMR spectrum are discussed below.

In the ^{13}C spectrum of palm oil or a neat simple TG in $CDCl_3$ solution the peak of the α-C is found to be broader than that of the β-C. In one sample of palm oil at 28°C and

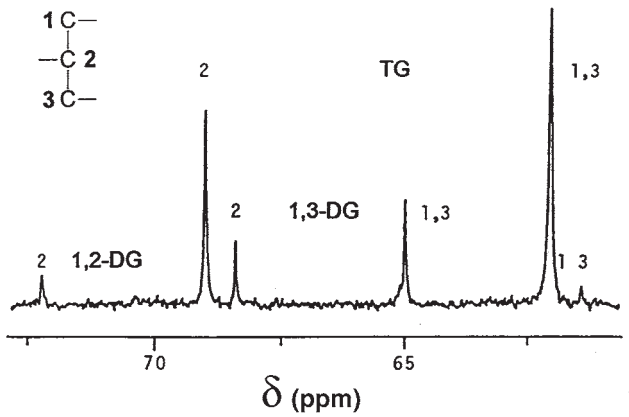

FIG. 1. ^{13}C Nuclear magnetic resonance (NMR) spectrum (25.05 MHz) of the glycerol carbons of a sample of palm oil recovered from the sterilizer condensate in a palm oil mill, in $CDCl_3$ solution at 28°C (concentration 1:3, vol/vol). The peaks for the glycerol carbons (designated as 1, 2, and 3) of each species of acylglycerol are labeled horizontally with TG = triacylglycerol and DG = diacylglycerol.

TABLE 1
Chemical Shifts (δ), Spin-Lattice Relaxation Times (T_1), and C–H Coupling Constants ($^1J_{CH}$) of the Glycerol Carbons[a] of Mono-, Di-, and Triacylglycerols in $CDCl_3$ Solution[b]

Acylglycerol	δ (ppm)	T_1 (s)	$^1J_{CH}$ (Hz)
1-Monopalmitin			
G-1	65.15	0.54	147.6
G-2	70.25	0.99	144.2
G-3	63.39	0.64	142.7
1-Monoolein			
G-1	65.15	0.56	147.5
G-2	70.26	0.98	144.0
G-3	63.38	0.68	142.5
1,3-Dilaurin			
G-1/G-3	64.94	0.53	147.7
G-2	68.13	0.92	145.2
1,2-Dilaurin			
G-1	62.16	0.43	147.6
G-2	72.11	0.79	147.4
G-3	61.31	0.57	143.7
1,3-Dipalmitin			
G-1/G-3	65.08	0.57	148.2
G-2	68.34	0.95	145.8
1,2-Dipalmitin			
G-1	62.10	0.46	147.8
G-2	72.18	0.84	147.9
G-3	61.59	0.64	143.4
1,3-Diolein			
G-1/G-3	64.96	0.58	148.0
G-2	68.26	0.90	145.8
1,2-Diolein			
G-1	62.14	0.44	148.1
G-2	72.09	0.76	148.3
G-3	61.32	0.60	143.7
1,3-Dilinolein			
G-1/G-3	65.08	0.56	148.1
G/2	68.28	0.99	145.8
1,2-Dilinolein			
G-1	62.11	0.58	148.2
G-2	72.13	0.84	148.1
G-3	61.40	0.65	143.7
Tripalmitin/triolein			
G-1/G-3	62.07	0.22	148.4
G-2	68.93	0.35	148.2
Palm oil			
G-1/G-3	62.07	0.24	148.3
G-2	68.94	0.38	148.2
Palm oil (48°C)			
G-1/G-3		0.34	
G-2		0.54	

[a]Designated as G-1 and G-3 for the α-carbons and G-2 for the β-carbon.
[b]Concentration at 5 mol% or less. Magnetic field at 25.05 or 22.50 MHz. Temperature at 28°C unless otherwise stated.

low magnetic field (22.508 MHz), the half-width of the β-C is 1.18 Hz whereas that of the α-C is 1.66 Hz (or α/β ratio of 1.41). At medium field (100.535 MHz), the corresponding value for the β-C is 1.26 Hz and for the α-C is 2.44 Hz (ratio 1.94), and the half-width of the peak (unoverlapped) of an aliphatic or olefinic carbon is 0.92 Hz. At 48°C at the low field the peaks are slightly narrower, with an α/β ratio 1.33, while at the medium field the α-C peak is considerably narrower (1.84 Hz) but the β-C is only slightly narrower (1.21 Hz), giving an α/β ratio of 1.52. As the glycerol carbons are relatively immobile, the broadening of the peaks is attributed to the relatively long reorientational correlation times, τ_c, not satisfying the condition for extremely narrow spectral lines, $\omega^2\tau_c^2 << 1$, where ω is the resonance frequency (13,14). This condition specifies the range of molecular mobility where the effective linewidths are determined by instrumental rather than fundamental factors and where extremely narrow spectral lines occur in the linear region of the correlation between T_1 and τ_c. The observed linewidths indicate that the α-C deviate more than the β-C from the condition, such that α-C is

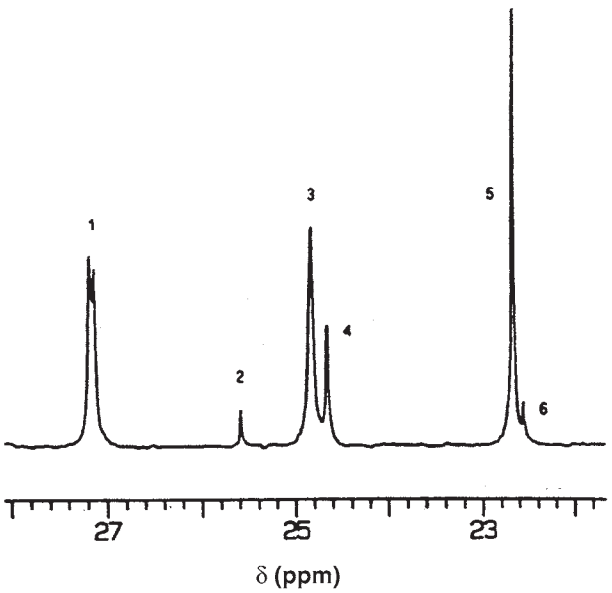

FIG. 2. ^{13}C NMR spectrum (25.05 MHz) of the aliphatic carbons in palm oil containing free fatty acids (FFA), in $CDCl_3$ solution at 28°C (concentration 1:3, vol/vol). Assignments: Peak 1, multiplet for the allylic carbons of oleic and linoleic acyls in both TG and FFA; peak 2, C-11 of linoleic; peak 3, C-3 of all acyl chains in TG; peak 4, C-3 of FFA; peak 5, C15 of palmitic, C17 of stearic, and C17 of oleic acids in TG; peak 6, C17 of linoleic acid in TG. For abbreviation see Figure 1.

TABLE 2
Chemical Shifts (δ) and Spin-Lattice Relaxation Times (T_1) of C-2 and C-3 Carbons of Acyl Groups in Palm Oil in $CDCl_3$ Solution[a] at 28°C

	C-2		C-3[b]	
Acyl group	δ (ppm)[c]	T_1 (s)[d]	δ (ppm)	T_1 (s)[d]
Palm oil (triacylglycerols)				
Palmitic/stearic (1,3-acyl)	34.020 (x)	0.48	24.855 (m)	0.57
Oleic				
(1,3-acyl)	34.000		24.835	
(2-acyl)	34.165	0.43	24.870	
Palmitic (2-acyl)	34.190		24.900	
Free fatty acids				
Lauric	x + 0.075	0.85	24.690	1.10
Stearic/oleic/linoleic	x + 0.075	0.70	24.690	0.90
Monoacylglycerols				
Mono-palmitin/-olein	x + 0.105	0.71	m + 0.050	0.83
Diacylglycerols				
1,3-Dipalmitin	x + 0.055	0.95	m + 0.027	1.00
1,3-Diolein	x + 0.040	0.90	m + 0.020	1.00
1,3-Dilinolein	x + 0.035	0.90	m + 0.050	1.00
1,2-Dipalmitin				
(1-acyl)	x + 0.055		m + 0.050	
(2-acyl)	x + 0.245		m + 0.080	
1,2-Diolein				
(1-acyl)	x + 0.045			
(2-acyl)	x + 0.210			
Triacylglycerols				
Trilaurin				
(1,3-acyl)	34.074	0.63	24.895	0.87
(2-acyl)	34.246	0.58	24.910	
Tripalmitin				
(1,3-acyl)	34.074	0.58	24.891	0.73
(2-acyl)	34.242	0.53	24.906	
Triolein				
(1,3-acyl)	34.041	0.57	24.876	0.69
(2-acyl)	34.206	0.52	24.891	
Trilinolein				
(1,3-acyl)	34.025	0.53	24.860	0.61
(2-acyl)	34.190	0.47	24.896	

[a]Concentration of palm oil, 3 mol%; for chemical shift measurements, free fatty acids, monoacylglycerols, and diacylglycerols were present in minor quantities.
[b]Overlapping peaks with major peak for palmitic/stearic acyls at 1,3-positions at δ24.855 (denoted as m).
[c]For the free fatty acids and partial acylglycerols, the δ values for C-2 are measured relative to the peak for C-2 of the palmitic/stearic acyl chains in the 1,3-positions of the triacylglycerols in palm oil, at δ34.020 (denoted as x). The δ values are referenced to the middle $CDCl_3$ peak at δ77.05 ppm.
[d]Measured in neat sample of each species in $CDCl_3$ solution (3 mol%) at 28°C and 25.05 MHz.

closer to the T_1 minimum and β-C is in the more linear region. This condition is consistent with the lower rate of increase of T_1 with temperature in α-C (see Table 1). Therefore, the peak broadening of the glycerol carbons is expected to be enhanced at high magnetic fields, but at lower fields it can be eliminated by raising the temperature appropriately. Although the NT_1 value of α-C, where N is the number of attached protons, in TG is slightly larger than that of the β-C, the T_1 values do not indicate that the α-C is more mobile than the β-C as the latter has the advantage of four glycerol protons (vs. one), separated by two bonds, to augment the directly bonded proton in its relaxation. In the case of the MG and DG the esterified α-C also show similar peak broadening, even at low field. In view of the narrower linewidth of the β-C, it is advantageous to evaluate the spectrum of the β-C acquired at medium or low magnetic field (300 MHz or lower) and at an elevated temperature (*ca.* 50°C) to ensure that the condition for extremely narrow lines is satisfied. Sharp spectral peaks are essential for rapid quantitative analysis using electronic integration.

Table 1 also lists the one-bond CH coupling constants of the glycerol carbons. The esterified glycerol carbons in the MG, DG, and TG have $^1J_{CH}$ of the same magnitude (148.0 Hz), but this is larger than those of the unesterified carbons, in which the $^1J_{CH}$ values also vary in the MG and DG. In the 1-MG $^1J_{CH}$ = 144.0 Hz for G-2 and 142.5 Hz for G-3. In the DG $^1J_{CH}$ = 145.8 Hz for G-2 in the case of 1,3-DG, and $^1J_{CH}$ = 143.7 Hz for G-3 for 1,2-DG. The partial acylglycerols and the TG can therefore be distinguished in terms of the magnitude of the $^1J_{CH}$ of the glycerol carbons, although the species of the acyl groups cannot be distinguished.

Aliphatic carbons. The presence of FFA in palm oil is easily detected in the ^{13}C NMR spectrum in the region of the aliphatic carbons, although it may not be possible to identify the particular species of FFA. Figure 2 shows the peak for C-3 of a FFA at δ24.67 ppm, which is 0.16 ppm lower than the frequency of the peak for C-3 of 1,3-acyl chains of the TG in palm oil. This distinct position and T_1 value of 0.90 s (in $CDCl_3$ solution at 28°C) for the C-3 in the FFA enable easy detection and quantitative measurement of the composition

TABLE 3
Chemical Shifts (δ) and Spin-Latticed Relaxation Times (T_1) of the Carbonyl Carbons of Acyl Groups in Palm Oil in $CDCl_3$ Solution[a] at 28°C

Acyl group	δ (ppm)[b]	T_1 (s)[c]
Triacylglycerols in palm oil		
1,3-acyl: Palmitic/stearic	173.185	10.3
cis-Vaccenic	–0.011	
Oleic	–0.027	
Linoleic	–0.036	
2-acyl: Palmitic/stearic	–0.384	
Oleic	–0.411	9.3
Linoleic	–0.42	
Diacylglycerols in palm oil		
1,3-Dipalmitin	+0.600	16.5
1,3-Diolein	+0.570	15.1
1,3-Dilinolein	+0.560	16.0
1,2-Diacyl		
(1-saturated acyl)	+0.394	
(1-oleic)	+0.365	15.2
(2-saturated acyl)	+0.145/+0.142 (d)[d]	
(2-oleic)	+0.118/+0.115 (d)[d]	11.8
1-Monoacylglycerols in palm oil		
(1-monopalmitin)	+0.955	24.1
(1-monoolein)	+0.905	
Free fatty acids in palm oil	+7.38	

[a]Concentration of palm oil, 3 mol%; other components present in minor quantities.
[b]Relative to δ = 173.185 ppm for the palmitic/stearic acyl groups in the 1,3-positions of triacylglycerols in palm oil.
[c]Measured in neat sample of each species in $CDCl_3$ solution (3 mol%) at 28°C and 22.50 MHz. See text for the respective nuclear Overhauser effect (NOE) values.
[d]d = doublet (unresolved).

of FFA in the palm oil, as the total integrated intensity of the peak at δ24.69 ppm represents one FFA chain relative to that of the complex band for C-3 centered at δ24.85 ppm, which represents three acyl chains of the TG in the oil.

The high-resolution high-field spectrum of the aliphatic C-2 and, to a lesser extent, C-3 carbons of the acyl chains can provide information on the presence of partial acylglycerols in palm oil (7,8). The NMR spectrum of palm oil shows the (major) peak for C-2 in the palmitic/stearic acyl chain in the 1,3-positions shifted to lower frequency by 0.145 ppm (in $CDCl_3$ solution) from the (minor) peak at δ34.165 ppm for C-2 in the oleic acyl chain in the 2-position. The C-2 peaks of 1-MG and 1,3-DG appear between these two peaks from the TG, that is, at a higher frequency compared to the major peak (which is taken to be the reference peak in Table 2). In the case of the 1,2-DG the peak for C-2 in the saturated acyls in the 1-position appears at the same position as that of the saturated acyls in the 1,3-positions of DG, but the peak for C-2 in the acyl in the 2-position appears at a higher frequency compared to the minor peak from the TG, as shown in Table 2. The C-2 peaks from the oleic and linoleic acyls appear at a notably lower frequency than that from the palmitic/stearic acyls in both the 1,3- and 1,2-DG. Table 2 shows that the T_1 values of C-2 in the partial acylglycerols are below 1.0 s, but are nearly double that in the simple TG. The low T_1 values of C-2 in the partial acylglycerols and TG enable efficient quantitative measurements to be made (at high field) of the composition of the partial acylglycerols relative to the TG in the oil.

The spectrum for C-3 in palm oil is a complex band as the peaks from the various acyls are not well shifted, as shown in Table 2. The spectrum for C-3 in the partial acylglycerols appears as overlapped peaks on the high-frequency side of the band for the TG and can be detected in the high-field spectrum, although they are of limited usefulness.

Carbonyl carbons. Table 3 shows the chemical shifts, δ, and spin-lattice relaxation times T_1 of the carbonyl carbons of the acyl groups in palm oil in dilute $CDCl_3$ solution. As the chemical shift differences are small, the δ values are referenced to the peak for palmitic/stearic acyl groups at the 1,3-glycerol positions at δ173.185 ppm, with the minus sign indicating lower frequency and the positive sign, higher frequency. The T_1 values of the carbonyl carbons, measured in the neat samples of each species at 22.5 MHz, show wide variation from the 1-MG (24 s) to the 1,3-DG (15–16 s) and the TG (10 s in the 1,3-positions); in a given species the 2-glycerol position has a T_1 value lower by 10–20%. The NOE values of the carbonyl carbons at 22.5 MHz are high and vary from 2.5 in 1-MG to 2.8–2.9 in 1,3-positions of DG and TG; in the latter, in the 2-position the NOE values are nearly full (~2.98). The NOE values indicate that the carbonyl carbons are at or near the T_1 minimum of the dipolar relaxation mechanism. This condition gives rise to broadening of lines in the NMR spectrum of the carbonyl carbons as the magnetic field increases (10).

In view of the wide variation in the relatively long T_1 values and differences in the NOE, the spectrum of the carbonyl

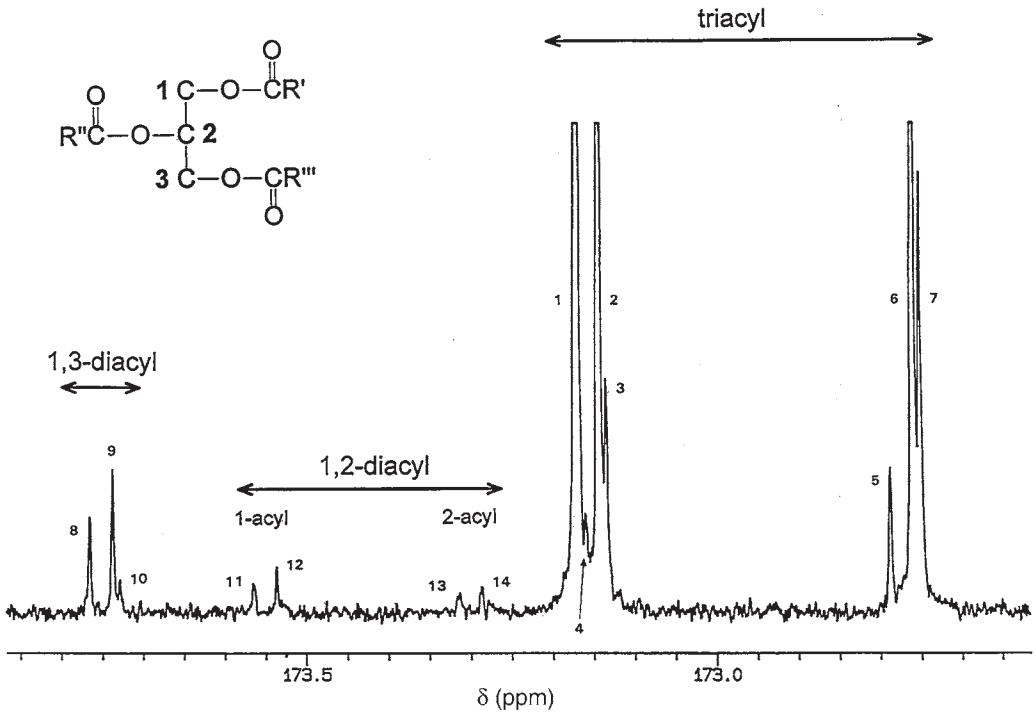

FIG. 3. ^{13}C NMR spectrum (67.94 MHz) of the carbonyl carbons of a sample of palm oil that contains small quantities of diacylglycerols as a result of hydrolysis upon storage; in $CDCl_3$ solution at 55°C (concentration 1:3, vol/vol). Assignments: Peaks 1, 2, 3, and 4 belong to the saturated (palmitic/stearic), oleic, linoleic, and *cis*-vaccenic (10) acyls, respectively, in the 1,3-glycerol positions of the TG; peaks 5, 6, and 7 belong to the saturated, oleic and linoleic acyls, respectively, in the 2-glycerol position in the TG; peaks 8, 9, and 10 belong to the saturated, oleic and linoleic acyls, respectively, in the 1,3-diacylglycerols (1,3-DG); peaks 11 and 12 belong to the saturated and oleic acyls, respectively, in the 1-glycerol position of 1,2-DG; and peaks 13 and 14 belong to the saturated and oleic acyls, respectively, in the 2-glycerol position of 1,2-DG. Peaks 13 and 14 are actually unresolved doublets (separation 0.003 ppm, see Table 3); the small separation may be caused by the nature of the acyl at the 1-position, whether saturated or not.

carbons would not be suitable for quantitative measurements of the composition of the partial acylglycerols by the broadband proton-decoupled mode, and it would be prohibitively long by using the gated-decoupling technique. However, the medium-field spectrum of the carbonyl carbons complements the spectrum of the glycerol carbons, as the chemical shifts in the former differentiate between the various acyl groups, whether saturated, monoene, or diene. Figure 3 shows the high-resolution NMR spectrum of the carbonyl carbons of a sample of palm oil that contains DG as minor components.

Analysis of a known mixture of acylglycerols. The quantitative analysis of the NMR spectrum of the glycerol carbons of the mixture of acylglycerols (see Experimental Procedures section) is shown in Table 4. As the glycerol carbons of all the TG give rise to one peak/band for the α-C and one for the β-C, the area of the peak for a partial acylglycerol is expressed relative to that for the TG, that is, as mole ratio. The analysis can be made with the α-C or with the β-C, although more straightforwardly with the latter. The area ratios from the quantitative NOE-suppressed spectrum are in satisfactory agreement with the mole ratio of the acylglycerols obtained by weighing the samples, within experimental errors. The results from the spectrum at the higher temperature (50°C) appear to be in slightly better agreement, as the spectral lines are narrower.

The results from the broadband proton-decoupled spectrum shown in Table 4 are also in satisfactory agreement with the known mole ratio of the acylglycerols. This confirms that the NOE of the glycerol carbons is full or nearly full and that with small pulse angles (<30°) quantitative NMR spectrum of the glycerol carbons can be obtained efficiently in the broadband proton-decoupled mode.

The ratios of the peak intensity measurements made by electronic integration are given in parentheses in Table 4. The electronic integration values are dependent on the line-shape of the peaks, and the results can differ from the area ratios by more than 10%.

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TABLE 4
Quantitative Analysis of the Glycerol Carbons of a Known Mixture of Acylglycerols[a,b]

	TG	DG	MG[c]
Mole ratio (by weighing)[b]	1.00	0.296	0.301
NOE-suppressed NMR spectrum[b,d,e]			
α-C (28°C)	1.00	0.31	0.34 (G-1)
	(1.00)	(0.309)	(0.349)
			0.33 (G-3)
			(0.314)
β-C (28°C)	1.00	0.31	0.32
	(1.00)	(0.262)	(0.301)
α-C (50°C)	1.00	0.30	0.34 (G-1)
	(1.00)	(0.295)	(0.281)
			0.34 (G-3)
			(0.273)
β-C (50°C)	1.00	0.29	0.33
	(1.00)	(0.266)	(0.332)
Broadband proton-decoupled spectrum[b,d,e]			
α-C (28°C)	1.00	0.30	0.34 (G-1)
	(1.00)	(0.270)	(0.300)
			0.35 (G-3)
			(0.280)
β-C (28°C)	1.00	0.31	0.34
	(1.00)	(0.295)	(0.324)

[a]TG, triacylglycerol; DG, diacyglycerol; MG, monoacylglycerol; NMR, nuclear magnetic resonance; for other abbreviation see Table 3.
[b]See Experimental Procedures section.
[c]Area measurements made for both α-carbons, G-1 and G-3.
[d]Ratio of area of each peak to that of the peak for TG.
[e]Ratio of value of electronic integration of peak intensity to that of the peak for TG given in parentheses.

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