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A novel approach to the rapid assignment of ^{13}C NMR spectra of major components of vegetable oils such as avocado, mango kernel and macadamia nut oils

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Assignment of ^{13}C nuclear magnetic resonance (NMR) spectra of major fatty acid components of South African produced vegetable oils was attempted using a method in which the vegetable oil was spiked with a standard triacylglycerol. This proved to be inadequate and therefore a new rapid and potentially generic graphical linear correlation method is proposed for assignment of the ^{13}C NMR spectra of major fatty acid components of apricot kernel, avocado pear, grapeseed, macadamia nut, mango kernel and marula vegetable oils. In this graphical correlation method, chemical shifts of fatty acids present in a known standard triacylglycerol is plotted against the corresponding chemical shifts of fatty acids present in the vegetable oils. This new approach (under carefully defined conditions and concentrations) was found especially useful for spectrally crowded regions where significant peak overlap occurs and was validated with the well-known ^{13}C NMR spectrum of olive oil which has been extensively reported in the literature. In this way, a full assignment of the ^{13}C{1H} NMR spectra of the vegetable oils, as well as tripalmitolein was readily achieved and the resonances belonging to the palmitoleic acid component of the triacylglycerols in the case of macadamia nut and avocado pear oil resonances were also assigned for the first time in the ^{13}C NMR spectra of these oils.

Supporting information may be found in the online version of this article.

Keywords: NMR; ^{13}C; olive oil; apricot kernel oil; avocado pear oil; grapeseed oil; macadamia nut oil; mango kernel oil; marula oil; tripalmitolein

Introduction

Nuclear magnetic resonance (NMR) spectroscopy has found application in the identification and quantitative determination of the major fatty acid components of vegetable oils, in particular olive oil.[1–5] One of the significant advantages of NMR spectroscopy is that, unlike other analytical techniques, it does not in general require extraction, separation or chemical modification of the vegetable oil to be analyzed. On the other hand the relatively low sensitivity of NMR spectroscopy limits its use to major and minor components of vegetable oils, rendering it generally unsuitable for analyzing trace components. Of the numerous vegetable oils available, olive oil has been the most widely studied by NMR spectroscopy, presumably in view of its high-value and wide consumption. This has resulted in the need for methods of authentication and the detection of adulteration of olive oil with other vegetable oils such as hazelnut and sunflower oils.[6–8] ^{1}H NMR spectroscopy has been used in the analysis of olive oil for measuring diglyceride content, determinations of squalene, cyclo-arthenol and Mg-depleted chlorophyll, and analysis of sterols as well as other components responsible for the taste and aroma of olive oil such as acetic acid, *trans*-2-hexenal and formaldehyde.[1,2] Although GC (gas chromatography) is the most commonly used technique for the qualitative and quantitative determination of fatty acid residues in vegetable oils, ^{13}C NMR spectroscopy can also be used for analyzing these major components.[1–5] The distinct advantage of using ^{13}C NMR spectroscopy in this context is that no chemical derivatization of the sample is required whereas for GC the fatty acid methyl esters must be prepared from the triacylglycerols before analysis is undertaken. ^{13}C NMR spectroscopy has been successfully used to determine quantitatively the major fatty acid residues in olive oils[5,7] and moreover can give direct information about the positional distribution of the fatty acids on the glycerol backbone. Much success has been found in detecting adulteration of olive oil by other oils using ^{13}C NMR spectroscopy. In conjunction with chemometric methods ^{13}C NMR spectroscopy can also be used to distinguish between geographic and cultivar-based differences of olive oils.[1–3,9]

We have recently become interested in the analysis of other high-value vegetable oils, including apricot kernel, avocado pear, grapeseed, macadamia nut, mango kernel and marula oils. To our knowledge, the ^{13}C NMR spectra of these oils have not been examined or fully assigned to date. The conventionally used method for the full assignment of ^{13}C NMR spectra of vegetable

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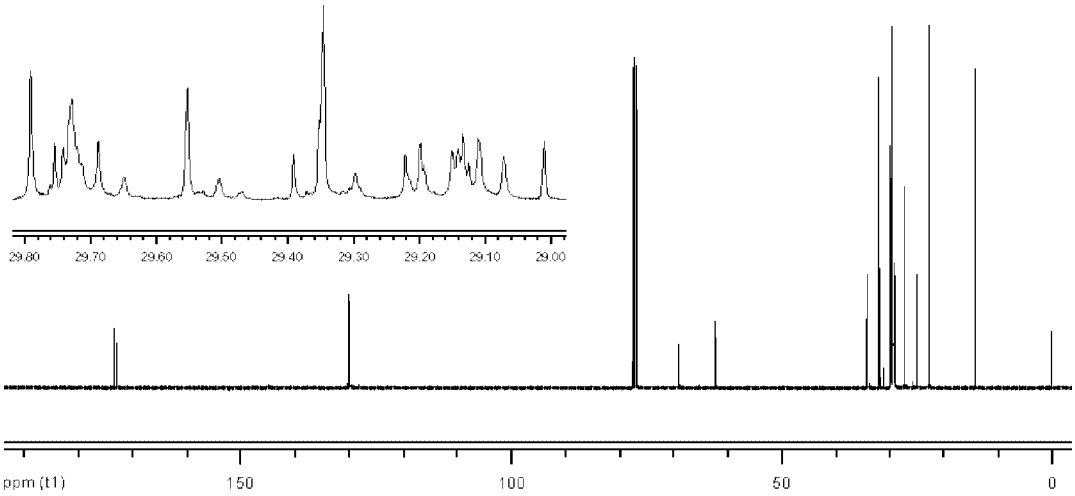

Figure 1. ^{13}C NMR spectrum of macadamia nut oil in $CDCl_3$ with an expansion of the crowded 29 ppm region.

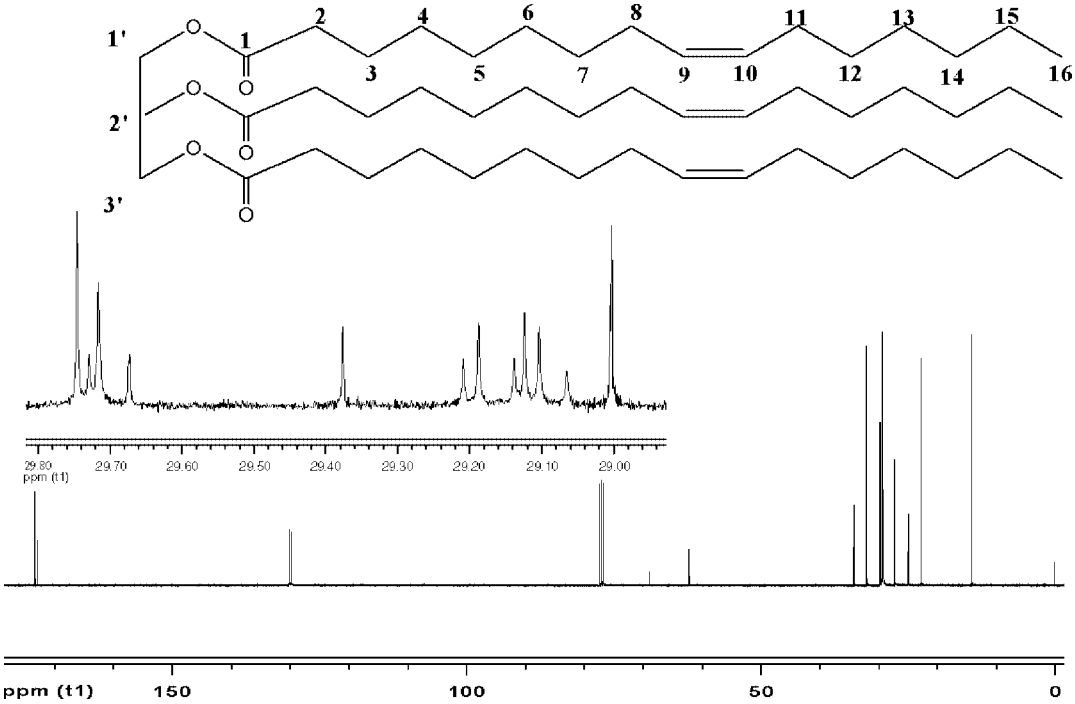

Figure 2. ^{13}C NMR spectrum of tripalmitolein in $CDCl_3$ with an expansion of the crowded 29 ppm region and numbered structure.

oils is by means of standard addition (spiking) of the vegetable oil with a standard triacylglycerol.[10] Comparison of the resulting ^{13}C NMR spectra of the unspiked and spiked vegetable oil leads to the assignment of the ^{13}C resonances in the NMR spectrum of the different fatty acid components present in the vegetable oil. Besides this method having some practical disadvantages, we found that it was not possible to achieve a full, unambiguous assignment of the ^{13}C NMR spectra of the desired oils using this technique. Desirous of developing a rapid method for the accurate assignment of the ^{13}C resonances of the various major components in a vegetable oil, we developed and tested a graphical linear correlation method for the assignment of the ^{13}C resonances of a vegetable oil. The technique was tested and validated with extra-virgin olive oil, for which the ^{13}C NMR spectrum has been well characterized in the literature. Using this approach one could easily achieve the full assignment of the ^{13}C NMR spectra of six locally produced South African vegetable oils in $CDCl_3$ solution.

Experimental

Materials and sample preparation

Standard triacylglycerols, tripalmitin, tripalmitolein, tristearin, triolein and trilinolein were purchased from Sigma-Aldrich and used without further purification (≥99% purity). Samples of olive oil were provided by Brenn-o-Kem (Wolseley, South Africa). Apricot kernel, avocado pear, grapeseed, macadamia nut, mango kernel and marula oils were supplied by Specialized Oils (Paardeneiland, Cape Town, South Africa). All oils were filtered before use. For storage, the oils were flushed with nitrogen gas.

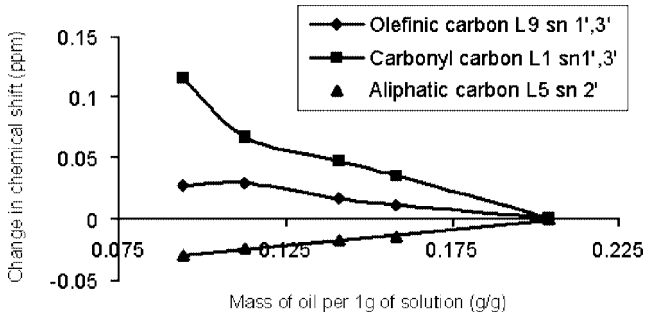

Figure 3. Change in chemical shift for different types of carbon atoms plotted against the mass of oil per 1 g of solution.

^{13}C NMR data collection and processing

Approximately 100 µl of each oil in 700 µl of $CDCl_3$ with TMS as reference was used for NMR analysis at 25 °C. ^{13}C NMR spectra were run on a 400 MHz Varian *Unity* Inova NMR spectrometer operating at 100 MHz for ^{13}C. Acquisition parameters similar to those recommended by Mannina *et al*.[10] were used for collecting the ^{13}C NMR spectra: number of points 256 K; spectral width 195 ppm; relaxation delay: 7 s; acquisition time 4.5 s. When processing, a line-broadening of −0.092 and Gaussian enhancement of 0.7 was used to optimize the resolution of the spectra. The precision of the procedure is estimated by the average half height of the resonances in the crowded region which is at most 0.7 Hz (0.007 ppm). In fact if we do the procedure with the graphical linear correlation method we get even better results, and a precision of ±0.004 ppm.

GC analysis of vegetable oils

Methyl esterification of vegetable oils for GC analysis[11] was carried out as follows: Sodium (0.5 g) was dissolved in 100 ml methanol. The sodium methoxide solution (0.3 g) together with 2 g of the specific vegetable oil was placed in a vial and heat sealed. The heat sealed vial was left for 2 h at 85–90 °C in an oil bath, with occasionally shaking. GC analysis of the vegetable oil samples was carried out to determine their fatty acid content in order to compare with the ^{13}C NMR spectroscopy data obtained. 20 µl of each sample was diluted with 1 ml of dichloromethane and 1 µl portions were injected into a HP 5890 Series 2 GC equipped with a fused silica capillary (30 m × 0.25 mm i.d., 0.2 mm film thickness) coated with a 100% cyanopropylpolysiloxane non-bonded phase. A temperature programmed elution from 40 to 240 °C at a rate of 4 °C/min was used.

Results and Discussion

The striking superficial resemblance of the $^{13}C\{^1H\}$ NMR spectrum of a typical macadamia nut oil in $CDCl_3$ shown in Fig. 1 to that of a $^{13}C\{^1H\}$ NMR spectrum of olive oil, suggests that similar major triacylglycerols are present in all these oils. In order to aid the rapid but unambiguous identification of the individual major triacylglycerols present in all six vegetable oils from their $^{13}C\{^1H\}$ NMR spectra we examined a series of oils and developed a simple method with which to assign all $^{13}C\{^1H\}$ resonances of the major triacylglycerols in such oils. GC analysis was first used to determine the fatty acid components of the triacylglycerols. Although GC analysis is the accepted analytical technique for fatty acid compound analysis and quantification in vegetable oils, it has the main disadvantage of being a destructive and time-consuming technique since chemical modification (esterification) of the triacylglycerols is required before analysis, unlike $^{13}C\{^1H\}$ NMR spectroscopy. GC analysis of the oils indicated that apricot kernel, avocado pear, grapeseed, macadamia nut, mango kernel and marula oils contained the same major fatty acid components, namely oleic acid, palmitic acid, linoleic acid and stearic acid found in olive oil, although palmitoleic acid not detected in any olive oil samples was observed to be present in macadamia nut and avocado pear oil in significant amounts. With the exception of tripalmitolein, the ^{13}C NMR spectra of the other triacylglycerols present in the six vegetable oils have previously been assigned by Mannina *et al*.[10] The full assignment of the ^{13}C NMR spectrum of a sample of pure tripalmitolein (Fig. 2) was carried out by methodologies given by Mannina *et al*.[10] for other triacylglycerols, and comparison of chemical shift trends observed for mainly triolein (Table 1).

Table 1. Assignment of ^{13}C NMR spectrum of tripalmitolein

Carbon	Position on glycerol backbone	Chemical shift (ppm)
C1	sn 1′, 3′	173.205
	sn 2′	172.795
C2	sn 1′, 3′	34.021
	sn 2′	34.185
C3	sn 1′, 3′	24.879
	sn 2′	24.879
C4	sn 1′, 3′	29.082
	sn 2′	29.044
C5	sn 1′, 3′	29.167
	sn 2′	29.188
C6	sn 1′, 3′	29.100
	sn 2′	29.115
C7	sn 1′, 3′	29.695
	sn 2′	29.709
C8	sn 1′, 3′	27.162
	sn 2′	27.162
C9	sn 1′, 3′	129.691
	sn 2′	129.665
C10	sn 1′, 3′	129.984
	sn 2′	129.995
C11	sn 1′, 3′	27.220
	sn 2′	27.224
C12	sn 1′, 3′	29.729
	sn 2′	29.729
C13	sn 1′, 3′	29.984
	sn 2′	29.986
C14	sn 1′, 3′; sn 2′	31.783
C15	sn 1′, 3′; sn 2′	22.655
C16	sn 1′, 3′; sn 2′	14.094
CHO		68.877
CH_2O		62.086

With the aim of developing a rapid method with which to determine the major triacylglycerols present in apricot kernel, avocado pear, grapeseed, macadamia nut, mango kernel and marula oils using $^{13}C\{^1H\}$ NMR spectroscopy we initially investigated the use of the procedure developed for mainly olive oils[10] which involves the addition of standard triacylglycerols

Table 2. Assignment of ^{13}C NMR resonances of the major fatty acid residues in olive oil

Section	Assignment	Position on glycerol backbone	Chemical shift (ppm) measured	Chemical shift (ppm) Literature assignments[3,4,13]		
				Vlahov *et al.*[3]	Sacchi *et al.*[4]	Shaw *et al.*[13]
A	P1	sn 1′, 3′	173.236		173.27	173.113
	V/E		173.223			173.101
	O1	sn 1′, 3′	173.204		173.2	173.084
	L1	sn 1′, 3′	173.194		173.17	173.075
	O1	sn 2′	172.837		172.83	172.688
	L1	sn 2′	172.787		172.77	172.679
B	L13	sn 2′	130.198	130.15	130.22	130.105
	L13	sn 1′, 3′	130.191	130.15	130.22	130.097
	O10	sn 2′	130.016	129.96	130.04	129.945
	O10	sn 1′, 3′	130.001	129.94	130.02	129.93
	L9	sn 1′, 3′	129.984	129.91	129.98	129.9
	L9	sn 2′	129.958	129.89	130.01	129.874
	V/E		129.918			
	V/E		129.822			
	O9	sn 1′, 3′	129.702	129.65	129.69	129.64
	O9	sn 2′	129.676	129.63	129.72	129.614
	L10	sn 2′	128.095	128.07	128.12	128.053
	L10	sn 1′, 3′	128.077	128.05	128.11	128.035
	L12	sn 1′, 3′	127.911	127.89	127.94	127.874
	L12	sn 2′	127.899	127.88	127.93	127.862
C	CHO		68.911	68.90		68.8852
	CH2O		62.108	62.06		62.0478
D	O2/L2	sn 2′	34.206	34.16		34.1408
	P2	sn 1′, 3′	34.064	34.01		33.9972
	UK		34.054			
	O2/L2	sn 1′, 3′	34.041	33.990		33.9758
E	P14		31.959	31.940	34.26	31.9283
	O16		31.938	31.920	34.2	31.9074
	UK		31.818	31.80 (UK)		
	L16		31.555	31.53	34.15	31.5167
F	O12		29.796	29.770		29.7586
	O7	sn 2′	29.745			
	P10		29.735	29.720 (UK)		29.6637
	O7/P12	sn 1′, 3′	29.731			
	P11		29.718	29.68 (UK)		
	P8		29.693	29.64 (UK)		
	P7	sn 1′, 3′	29.653			29.6637
	L7	sn 2′	29.650			29.6203
	L7	sn 1′, 3′	29.635			
	O14	sn 2′	29.560	29.54		29.5289
	O14	sn 1′, 3′	29.558	29.54		29.5289
	P5		29.508	29.49		29.4748
	P13		29.397	29.38 (S15)*		29.3688 (S15)*
	L15		29.377	29.36		29.3419
	O13		29.356	29.34		29.3156
	O15		29.353	29.34		29.3156
	P6		29.302	29.28		29.2686
	O5/L5	sn 2′	29.225	29.20		29.1869
	O5/L5	sn 1′, 3′	29.203	29.18		29.1656
	O6/L6	sn 2′	29.153	29.10		
	P4		29.145	29.10		
	O6/L6	sn 1′, 3′	29.136	29.10		
	O4	sn 1′, 3′	29.114	29.10		29.0717
	L4	sn 1′, 3′	29.108	29.10		29.0717
	O4/L4	sn 2′	29.075	29.05		29.0324

Table 2. (*Continued*)

Section	Assignment	Position on glycerol backbone	Chemical shift (ppm) measured	Chemical shift (ppm) Literature assignments[3,4,13] Vlahov *et al.*[3]	Sacchi *et al.*[4]	Shaw *et al.*[13]
G	O11	sn 2′	27.249	27.22	27.31	27.1477
	O11	sn 1′, 3′	27.245	27.22	27.31	27.1477
	L14		27.226	27.20	27.22	27.1657
	L8		27.214	27.18		27.1815
	O8		27.194	27.16	27.31	27.2009
H	L11		25.653	25.62	25.67	25.6084
	O3	sn 2′	24.908	24.88		24.8605
	L3	sn 2′	24.898	24.88		24.8605
	P3		24.890	24.86	24.84	24.8466
	O3	sn 1′, 3′	24.869	24.84	24.8	24.8239
	L3	sn 1′, 3′	24.861	24.84	24.7	24.8239
I	P15		22.720	22.70 (S17)*	22.82	22.6841 (S17)*
	O17		22.711	22.70	22.8	22.6743
	L17		22.602	22.58	22.61	22.5656
J	P16		14.133	14.09 (S18)*	14.13	14.0824 (S18)*
	O18		14.127	14.09	14.13	14.0769
	UK		14.119			
	L18		14.081	14.05	14.09	14.037

O, olein; P, palmitin; L, linolein; S, saturated; UK, unknown; V/E, vaccenin or eicosenoin.
* The chemical shifts of the omega 1, 2 and 3 carbons (P14, 15 and 16) have been correlated with the corresponding omega carbons in the literature.

(triolein, tristearin, etc.) to the sample of vegetable oil and using deconvolution of the $^{13}C\{^1H\}$ NMR spectrum collected to detect enhancement of particular resonances.[7,8,10] Although this approach has been successfully applied to olive and other oils in the literature, its limitations include the time-consuming nature (requiring repeated ^{13}C NMR spectra to be acquired for each standard triacylglycerol added) and the use of expensive but potentially unavailable pure triacylglycerol standards. In our hands moreover, a major limitation with this standard addition approach was that the complete assignment of the spectra of the six oils of interest was not always possible. Although a large number of $^{13}C\{^1H\}$ resonances in the spectrum could be assigned by inspection and comparison with data in the literature, the method of standard additions could not be applied to all regions in the $^{13}C\{^1H\}$ spectrum, notably in the spectrally crowded 29 ± 1 ppm region in which several CH_2 carbon resonances are found resulting in significant spectral overlap (Fig. 1). Spiking the oil with a standard in this crowded region leads to the expected intensity increases of specific resonances, but unfortunately can also result in significant loss of resolution due to spectral overlap in this region making clear assignments of individual resonances very difficult if not impossible. This is probably the reason why this region of the $^{13}C\{^1H\}$ spectrum of olive oil has not been fully assigned in the literature to our knowledge. A more invidious problem with the standard additions method is small but detectable concentration dependence of $^{13}C\{^1H\}$ chemical shifts of triacylglycerols and thus the possibility of this leading to peak overlap on addition of the standard; in the case of olive oils a limited concentration dependence of $^{13}C\{^1H\}$ has been reported by Mannina *et al.*[12] It is obvious that such concentration dependence of $^{13}C\{^1H\}$ chemical shifts may lead to ambiguity when using the standard addition method for the assignment of the ^{13}C spectra of vegetable oils. In this context we propose a new approach to aid in the rapid and reliable assignment of ^{13}C NMR spectra in vegetable oils.

Assignment of ^{13}C NMR spectra using the graphical linear correlation method

The proposed method is based on the reasonable expectation that the $^{13}C\{^1H\}$ chemical shifts of a fatty acid residue of a particular triacylglycerol in a given solvent at a specified concentration should all be affected, to a first approximation, in a similar manner by the factors responsible for the observed concentration dependence. Moreover it may be expected that saturated sp^3 carbon atoms might be differently affected to unsaturated sp^2 carbon atoms for a given fatty acid residue (*vide infra*). On this basis it would be reasonable to expect that $^{13}C\{^1H\}$ shifts of all carbon resonances of a fatty acid of a pure, standard triacylglycerol (e.g. triolein, tripalmitin, etc.) would be linearly correlated to the corresponding fatty acid residues of the triacylglycerols in a vegetable oil mixture in a given solvent at a specified concentration range. To test this expectation the dependence of the $^{13}C\{^1H\}$ chemical shifts of concentration changes has been determined for olive oil in $CDCl_3$ and we found that for sp^3 carbon atoms the method is satisfactorily independent on concentration of the vegetable oil within the concentration range of approximately 0.10–0.20 g oil/g of $CDCl_3$ solution. Similar trends were found for the other vegetable oils. For each concentration of vegetable oil, the $^{13}C\{^1H\}$ shifts of selected carbon atoms were found to result in linear trends with identical gradients but slightly differing intercepts at 'zero' concentration, which indicates that at a practical concentration range of approximately 0.10–0.20 g oil/g of $CDCl_3$ solution, all aliphatic $^{13}C\{^1H\}$ shifts are affected to the same degree, and importantly in a linear fashion as a result of small concentration changes.

This can be clearly seen in Fig. 3 where the chemical shifts of an aliphatic sp^3 carbon L5 of trilinolein is shown to change linearly with a change in concentration from 0.095 to 0.20 g oil/g solution.

Interestingly the concentration dependence of resonances of sp^2-type carbon atoms (e.g. olefinic and carbonyl carbon atoms) shows a rather more complex non-linear trend (Fig. 3). This suggests that linear concentration dependences for such $^{13}C\{^1H\}$ shifts and thus correlations cannot generally be expected for such carbon atoms. As these resonances are in non-crowded spectral regions they are generally easily assigned by inspection in most vegetable oils making this effect not too serious a limitation. Nevertheless we have found that within a limited concentration range (approximately 0.10–0.20 g oil/1 g of $CDCl_3$ solution) the proposed graphical linear correlation method can, as a first approximation, also be used for assignments of sp^2-type carbon resonances. Comparing the graphs of the sp^2 and sp^3 (Fig. 3)

Table 3. Assignment of ^{13}C NMR resonances of the major fatty acid residues in avocado pear and macadamia nut oil

Carbon	Position	Avocado pear oil (ppm)	Carbon	Position	Macadamia nut oil (ppm)
P1	sn 1′,3′	173.266	P1	sn 1′, 3′	173.256
Pa1	sn 1′,3′	173.253	Pa1	sn 1′, 3′	173.243
O1	sn 1′,3′	173.235	O1/L1	sn 1′, 3′	173.226
L1	sn 1′,3′	173.226	O1/Pa1/L1	sn 2′	172.818
O1/Pa1	sn 2′	172.825	L13		130.201
L1	sn 2′	172.815	O10/Pa10	sn 2′	130.017
L13	sn 1′,3′	130.202	O10/Pa10/L9	sn 1′, 3′	130.003
L13	sn 2′	130.195	L9	sn 2′	129.923
O10/Pa10	sn 2′	130.017	V/E		129.825
O10/Pa10	sn 1′,3′	130.003	V/E		129.822
L9	sn 2′	129.989	O9/Pa9	sn 1′, 3′	129.705
L9	sn 1′,3′	129.962	O9/Pa9	sn 2′	129.679
V/E		129.92	L10	sn 2′	128.093
V/E		129.824	L10	sn 1′, 3′	128.074
Pa9		129.709	L12	sn 1′, 3′	127.909
O9	sn 1′,3′	129.704	L12	sn 2′	127.896
O9/Pa9	sn 2′	129.678	CHO		68.907
L10	sn 2′	128.092	CH2O		62.11
L10	sn 1′, 3′	128.075	O2/Pa2/L2	sn 2′	34.208
L12	sn 1′, 3′	127.91	P2	sn 1′, 3′	34.065
L12	sn 2′	127.898	Pa2	sn 1′, 3′	34.056
CHO		68.913	O2/L2	sn 1′, 3′	34.043
CH2O		62.114	P14		31.955
O,L,Pa2	sn 2′	34.208	O16		31.933
P2	sn 1′,3′	34.065	Pa14		31.811
Pa2	sn 1′,3′	34.056	L16		31.55
O2/L2	sn 1′,3′	34.042	UK		30.897
P14		31.955	O12		29.792
O16		31.934	Pa12	sn 2′	29.763
Pa14		31.812	Pa12	sn 1′, 3′	29.755
L16		31.551	O8/Pa7	sn 2′	29.743
O12		29.793	P10	sn1,3	29.731
Pa12	sn 2′	29.763	O8/P12	sn 1′, 3′	29.729
Pa12	sn 1′,3′	29.756	Pa7/P11	sn 1′, 3′	29.721
O7	sn 2′	29.743	P9		29.714
P10/Pa7	sn 2′	29.732	P8		29.689
O7/P12/Pa7	sn 1′,3′	29.728	L7/P7		29.65
P11		29.715	O14		29.554
P8		29.689	P5	sn 1′, 3′	29.505
P7/L7	sn 1′,3′/sn2(L)	29.65	P13		29.392
L7	sn 1′,3′	29.633	L15		29.372
O14		29.555	O13		29.353
P5		29.506	O15		29.347
P13		29.393	P6	sn 1′, 3′	29.298
L15		29.372	O5/L5	sn 2′	29.222
O13/P6		29.353	Pa5	sn 2′	29.216
O15		29.349	O5/L5	sn 1′, 3′	29.2

Table 3. (*Continued*)

Carbon	Position	Avocado pear oil (ppm)	Carbon	Position	Macadamia nut oil (ppm)
P6	sn 1′,3′	29.299	Pa5	sn 1′, 3′	29.194
O5/L5	sn 2′	29.222	O6/L6	sn 2′	29.151
Pa5	sn 2′	29.217	Pa6/L6/P4	sn 2′/sn1,3	29.142
O5/L5	sn 1′,3′	29.201	O6	sn 1′, 3′	29.135
Pa5	sn 1′,3′	29.194	Pa6	sn 1′, 3′	29.126
O6/L6	sn 2′	29.152	O4/Pa4/L4/P4	sn 1′, 3′	29.111
L6/P4/Pa6	sn 1′,3′	29.145	O4/Pa4/L4	sn 2′	29.072
O6	sn 1′,3′	29.135	Pa13		29.011
Pa6	sn 2′	29.126	O11/Pa8	sn 2′	27.246
O4/L4/Pa4	sn 1,3	29.111	O11	sn 1′, 3′	27.243
O4/L4/Pa4	sn 2′	29.073	L14		27.223
Pa13		29.011	L8		27.212
O11/Pa8	sn 2′	27.247	O8		27.193
O11	sn 1′,3′	27.243	Pa11		27.187
L14		27.224	L11		25.651
L8		27.212	O3/Pa3/L3	sn 2′	24.905
O8		27.193	P3		24.888
Pa11		27.188	O3/Pa3/L3/P3	sn 1′, 3′	24.865
L11		25.651	P17		22.717
O3/Pa3	sn 2′	24.906	O17		22.707
L3	sn 2′	24.896	Pa15		22.681
P3	sn 1′,3′	24.888	L17		22.598
O3/L3/Pa3	sn 1′,3′	24.866	P16		14.131
P15		22.717	O18		14.125
O17		22.707	Pa16		14.117
Pa15		22.682	L18		14.085
L17		22.599			
P16		14.131			
O18		14.125			
Pa16		14.116			
L18		14.084			

O, olein; P, palmitin; Pa, palmitolein; L, linolein; UK, unknown; V/E, vaccenin or eicosenoin; FFA, free fatty acid.

carbon atoms, it can be seen that as the concentration increases the chemical shifts of the sp^2 carbon atoms move upfield, while the sp^3 carbon atoms chemical shifts move downfield. It is therefore evident that the sp^2 and sp^3 carbon atoms are affected very differently with a change in concentration, and thus they should clearly not be plotted on the same graph.

On the basis of these observations therefore, we have tested a new method for the possible assignment of $^{13}C\{^1H\}$ resonances of triacylglycerols in vegetable oils by establishing a correlation of the observed $\delta^{13}C\{^1H\}$ of the various peaks of the major components with those of the pure component obtained under similar conditions at similar concentrations. The graphical correlation method therefore consists of correlating the chemical shift values of defined fatty acid carbon atoms in the standard triacylglycerols (triolein, tripalmitin, etc.) with the corresponding peaks present in the $^{13}C\{^1H\}$ NMR spectrum of the vegetable oil. Essentially we employ the following modus operandi: Firstly, the $^{13}C\{^1H\}$ NMR spectrum of the vegetable oil is divided into sections each representing different carbon atoms of the same type present in the triacylglycerols. Section A covers the ^{13}C resonances due to the carbonyl sp^2-carbon resonances, Section B those of the olefinic sp^2-carbons, while Sections D–J shows the crowded spectral region due to aliphatic sp^3-carbon ^{13}C signals (Table 2).

Certain of these regions are easily assigned by inspection using the relative percentages of each fatty acid residue present in the oil as determined by GC analysis and can be approximately related (If necessary quantitative $^{13}C\{^1H\}$ NMR spectra can be recorded to confirm this (see ref 5).) to resonance intensities in the NMR spectrum, which further assists in assigning such additional resonances by inspection. In addition it is known from the work of Vlahov *et al*.[3] that carbons from a saturated fatty acid chain is generally observed somewhat further upfield than the equivalent carbon in an unsaturated fatty acid chain. These concepts can be applied in order to assign Sections A, B, E, I and J. Subsequently by plotting the $^{13}C\{^1H\}$ chemical shifts of each of all unambiguously assigned $^{13}C\{^1H\}$ resonances of a particular fatty acid residue in the vegetable oil on the *y*-axis against the $^{13}C\{^1H\}$ shift of that resonance in the standard triacylglycerol along the x-axis, one obtains a remarkably linear correlation as shown for instance for trilinolein in macadamia nut oil in Fig. 4.

This highly linear correlation thus facilitates the assignment of the remaining $^{13}C\{^1H\}$ resonances, which were not directly assignable by inspection particularly in a spectrally crowded region encompassing the aliphatic carbon resonances. It is thus possible by using a previously assigned $^{13}C\{^1H\}$ shift of the pure triacylglycerol (obtained from the literature or preferably from a

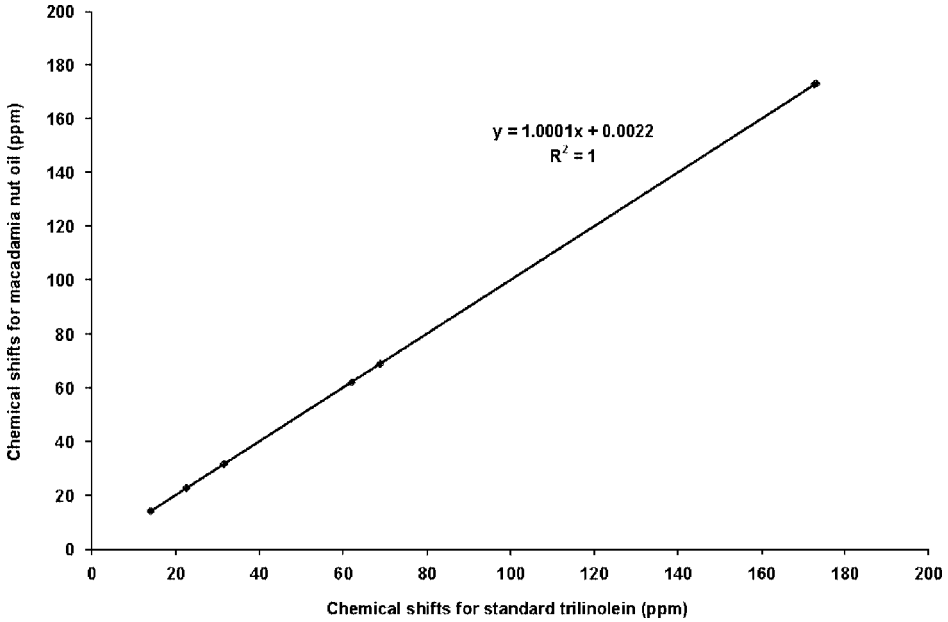

Figure 4. Linear correlation obtained from plotting the chemical shifts of the sp^3 carbon resonances belonging to standard trilinolein against those in macadamia nut oil.

$^{13}C\{^1H\}$ spectrum of a sample of pure triacylglycerol recorded in the same sample and approximate concentration plotted as ordinate) to predict the corresponding chemical shift value of the corresponding $^{13}C\{^1H\}$ shift of that component in the oil as abscissa (Fig. 4).

In this way all the relevant ^{13}C resonances can be assigned to the appropriate carbon of the fatty acid residues, particularly in the crowded spectral region of the $^{13}C\{^1H\}$ NMR spectrum. This method is most suitable for application in regions D, F, G and H where significant spectral overlap occurs and for the same sp^3 carbon type. Moreover the degree of linearity obtained by regression analysis (as measured by the regression coefficient r^2) is sensitive to the correct assignment since miss-assignments result in the rapid deviation of the r^2 from close to 1, typically correct assignments lead to r^2 values of 0.999. This linear method is only used for the sp^3 hybridized carbons as the carbonyl and olefinic sp^2 carbon can easily be assigned by inspection. Indeed the sp^2 and sp^3 carbons should not be used on the same linear graph as a result of their non-linear concentration dependence shown previously (Fig. 3), although for very similar concentrations of standard triacylglycerol and vegetable oil in the same solvent remarkable linear correlations of the $^{13}C\{^1H\}$ shifts for all carbon types also result. The limitation due to the variable concentration dependencies of the $^{13}C\{^1H\}$ shifts of sp^2 and carbonyl type carbon atoms is not a serious limitation in this context, since resonances resulting from such carbon atoms are generally easily assigned by inspection of other methods.

Validation and assignment of $^{13}C\{^1H\}$ of vegetable oils

Assessment and validation of the linear correlation method described here was first carried out by application to the well-known $^{13}C\{^1H\}$ spectrum of olive oil, previously assigned and reported extensively in the literature[3,4,13] as shown in Table 2. A comparison of the assignment obtained using the graphical linear correlation method with those from the literature confirms that these $^{13}C\{^1H\}$ assignments agree well with those found by other researchers for all carbon atoms of each fatty acid residue of the major triacylglycerols in olive oil. The various $^{13}C\{^1H\}$ chemical shift values for spectral regions A, B, C, E, I and J correspond well with published data[3,4,13] with some exceptions in other regions: C-2 of all fatty acid residues present in section D corresponds reasonably well with the literature,[4] however the resonances of the C-2 atoms of triolein and trilinolein in the α positions of the glycerol backbone were clearly separated in our $^{13}C\{^1H\}$ spectrum and could therefore be separately assigned; Shaw *et al*. found only one resonance representing both these carbon atoms, presumably due to fortuitous overlap. Although most assignments in spectral section G correlate with published data,[3] we also observed two resolved resonances for C-8 of the olein residue, indicating separate peaks for C-8 in the α and β positions, whereas Shaw *et al*. report only a single resonance for this carbon atom. The same pertains for C-3 of the olein and linolein residues in spectral region H, for which separate resonances could be observed and assigned. Upon first glance, region F appears more difficult to assign unambiguously which probably accounts for why several of these resonances have not previously been assigned in the literature to our knowledge. Nevertheless using our proposed correlation method, it becomes relatively straight forward to assign some of these resonances, some of the unassigned resonances in this section could also be due to the vaccenin or eicosenoin residues or possibly some other saturated residues not previously reported or detected by $^{13}C\{^1H\}$ NMR (due to their low concentrations) which are likely to mostly overlap with the palmitin $^{13}C\{^1H\}$ resonances. Since palmitin is the major saturated component present in olive oil, the assignment of the saturated resonances was carried out using the tripalmitin chemical shifts as a reference. As expected for olive oil, saturated fatty acid residues, for instance for palmitin, are known not to be present in the β position of a triacylglycerol.[1] This is thought to be due to the observation that in natural olive oils saturated fatty acid residues such as palmitin are found at the β position of the triacylglycerols in amounts of less than 2%. Indeed, the presence of substantial amounts of palmitin in the β position of the glycerol

Table 4. ^{13}C NMR assignments for grapeseed and apricot kernel oil

Carbon	Position	Grapeseed oil (ppm)	Carbon	Position	Apricot kernel oil (ppm)
FFA		173.813	P1		173.244
P1		173.252	UK		173.231
O1	sn 1′,3′	173.221	O1	sn1′, 3′	173.212
L1	sn 1′,3′	173.21	L1	sn1′, 3′	173.202
UK		172.812	L1	sn 2′	172.805
L1/O1	sn 2′	172.801	O1	sn 2′	172.794
UK		145.14	L13	sn1′, 3′	130.203
L13	sn 2′	130.204	L13	sn 2′	130.195
L13	sn 1′,3′	130.196	O10	sn 2′	130.016
O10	sn 2′	130.018	O10	sn1′, 3′	130.002
O10	sn 1′,3′	130.002	L9	sn 2′	129.987
L9	sn 1′,3′	129.988	L9	sn1′, 3′	129.962
L9	sn 2′	129.962	O9	sn1′, 3′	129.703
O9	sn 1′,3′	129.703	O9	sn 2′	129.677
O9	sn 2′	129.676	L10	sn1′, 3′	128.092
L10	sn 2′	128.091	L10	sn 2′	128.074
L10	sn 1′,3′	128.072	L12	sn 2′	127.908
L12	sn 1′,3′	127.906	L12	sn1′, 3′	127.897
L12	sn 2′	127.894	CHO		68.908
CHO		68.908	CH2O		62.106
CH2O		62.109	O2	sn 2′	34.204
L2/O2		34.196	L2	sn 2′	34.199
P2		34.06	P2		34.062
L2/O2	sn 1′,3′	34.032	O2	sn1′, 3′	34.04
P14		31.948	L2	sn1′, 3′	34.034
O16		31.927	P14		31.953
L16		31.545	O16		31.932
UK		30.899	UK		31.811
O12		29.787	L16		31.549
O7	sn 2′	29.737	UK		30.895
O7/P10	sn 1′,3′	29.725	O12		29.791
P12		29.718	UK		29.762
P11		29.708	O7	sn 2′	29.742
P8		29.683	O7/P10,12	sn1′, 3′	29.727
L7	sn 2′	29.641	P11		29.713
L7	sn 1′,3′	29.627	P8	sn 2′	29.687
O14		29.548	L7	sn1′, 3′	29.645
P5		29.499	L7	sn 2′	29.631
P13		29.386	O14		29.553
L15		29.367	UK		29.528
O13		29.347	P5	sn1′, 3′	29.503
O15		29.341	UK		29.47
P6		29.293	P13		29.391
L5/O5	sn 2′	29.215	L15		29.371
L5/O5	sn 1′,3′	29.194	O15,13	sn 1′,3′	29.346
L6/O6	sn 2′	29.147	P6		29.298
L6/O6/P4	sn 1′,3′	29.133	O5/L5	sn 2′	29.22
L4/O4	sn 1′,3′	29.1	O5/L5	sn1′, 3′	29.199
L4/O4	sn 2′	29.062	O6/L6/P4	sn 2′	29.15
O11	sn 2′	27.242	O6/L6	sn1′, 3′	29.133
O11	sn 1′,3′	27.238	O4	sn1′, 3′	29.11
UK		27.231	L4	sn1′, 3′	29.105
L14		27.219	O4	sn 2′	29.071
L8		27.208	L4	sn 2′	29.067
O8		27.188	UK		29.01
L11		25.647	O11		27.242
O3	sn 2′	24.901	L14		27.222
L3	sn 2′	24.892	L8		27.211
P3		24.883	O8		27.191
O3	sn 1′,3′	24.861	L11		25.65
L3	sn 1′,3′	24.854	O3	sn 2′	24.905
P15		22.713	L3	sn 1′, 3′	24.896
O17		22.703	P3		24.887
L17		22.595	O3	sn 1′, 3′	24.865
P16		14.131	L3	sn 2′	24.858
O18		14.125	P15		22.716
L18		14.084	O17		22.706
			UK		22.699
			UK		22.682
			L17		22.598
			UK		14.131
			O18		14.125
			P16		14.118
			L18		14.085

O, olein; P, palmitin; Pa, palmitolein; L, linolein; UK, unknown; V/E, vaccenin or eicosenoin; FFA, free fatty acid.

backbone in olive oil in particular indicates adulteration.[1] The good agreement between our assignments derived from the graphical correlation method and published data for olive oils, acceptably validates the proposed method.

Using the proposed graphical correlation method developed here, we were able to fully assign the ^{13}C NMR spectra of of all six vegetable oils in $CDCl_3$ solution without recourse to the method of standard additions. The maximum relative error determined for the $^{13}C\{^1H\}$ shifts of sp^3 carbon atoms is ± 0.004 ppm and for the sp^2 carbon atoms is ± 0.036 ppm under our conditions. The assignments for macadamia nut and avocado pear oils are shown in Table 3, grapeseed and apricot kernel oils in Table 4 and mango kernel and marula oils in Table 5, and the ease with which these were carried out is an indication of the simplicity of the method presented earlier. In principle, under carefully controlled conditions we believe that this simple correlation method of assignment may compliment the more time-consuming standard additions methodology, as well as more elaborate spectroscpic methods such as $^{13}C-^{13}C$ correlations spectroscopy (e.g. INADEQUATE), and be suitable for the assignment of $^{13}C\{^1H\}$ spectra of similar vegetable oils not previously studied by NMR spectroscopy, something currently underway in our laboratory.

Conclusions

In conclusion, the use of simple linear correlations between ^{13}C NMR shifts of triacylglycerol fatty acid components in vegetable oils against the corresponding chemical shifts of the standard triacylglycerols in the same solvent at concentration ranges of between 0.10 and 0.20 g oil/1 g solution proves to be a simple method to identify and accurately assign the ^{13}C NMR spectra of the major components in such oils, particularly in crowded spectral regions. These major components included

Table 5. ^{13}C NMR assignments for mango kernel and marula oil

Carbon	Position	Mango kernel oil (ppm)	Carbon	Position	Marula oil (ppm)
UK		178.907	FFA		173.873
UK		178.885	FFA		173.842
FFA		173.898	P1	sn 1′, 3′	173.265
FFA		173.866	O1	sn 1′, 3′	173.234
UK		173.723	L1	sn 1′, 3′	173.223
P1		173.287	O1	sn 2′	172.825
O1	sn 1′,3′	173.255	L1	sn 2′	172.812
L1	sn 1′,3′	173.244	L13	sn 2′	130.186
O1	sn 2′	172.844	L13	sn 1′, 3′	130.178
L1	sn 2′	172.834	O10	sn 2′	130.013
UK		145.079	O10	sn 1′, 3′	129.999
L13		130.199	L9	sn 1′, 3′	129.973
O10	sn 1′,3′	130.017	L9	sn 2′	129.948
O10	sn 2′	130.005	UK		129.717
L9	sn 1′,3′	129.989	UK		129.707
L9	sn 2′	129.962	O9	sn 1′, 3′	129.701
UK		129.725	O9	sn 2′	129.675
O9	sn 2′	129.705	L10	sn 2′	128.103
UK		129.689	L10	sn 1′, 3′	128.083
O9	sn 1′,3′	129.679	L12	sn 1′, 3′	127.920
L10		128.094	L12	sn 2′	127.903
L10		128.077	CH2O		68.939
L12		127.912	UK		68.332
L12		127.9	UK		65.041
CHO		68.921	CHO		62.127
DAG		68.349	O2/L2	sn 2′	34.212
DAG		65.051	UK		34.105
CH2O		62.114	P2		34.069
O2/L2	sn 2′	34.215	O2/L2	sn 1′, 3′	34.047
UK		34.122	UK		34.020
UK		34.106	UK		34.005
P2		34.07	P14		31.976
O2/L2	sn 1′,3′	34.047	O16		31.955
UK		33.974	L16		31.568
UK		33.958	O12		29.810
P14		31.963	O7	sn 2′	29.757
O16		31.941	UK		29.754
L16		31.557	UK		29.747
O12		29.798	O7	sn 1′, 3′	29.743
P10,12/O7		29.741	P10		29.736
P11		29.726	P12		29.727
P9		29.711	P11		29.719
P8		29.699	UK		29.711
UK		29.679	P9		29.704
P7/L7	sn 1′,3′	29.661	P8		29.690
L7	sn 2′	29.637	UK		29.670
UK		29.632	L7	sn 2′	29.661
O14	sn 1′,3′	29.565	P7		29.647
O14	sn 2′	29.562	L7	sn 1′, 3′	29.643
P5		29.515	UK		29.578
UK		29.48	UK		29.576
P13		29.402	UK		29.573
L15		29.378	O14		29.571
O13		29.361	UK		29.525
O15		29.357	P5		29.491
P6		29.307	UK		29.415
UK		29.292	P13		29.391
O5/L5	sn 2′	29.229	O13/L15		29.370
O5/L5	sn 1′,3′	29.206	O15		29.363
UK		29.187	UK		29.318
O6/L6	sn 1′,3′	29.158	P6		29.301
P4/L6	sn 1′,3′	29.149	O5/L5	sn 1′, 3′	29.239
O6	sn 2′	29.141	UK		29.226
O4	sn 1′,3′	29.119	O5/L5		29.217
L4	sn 2′	29.117	UK		29.201
UK		29.103	UK		29.196
UK		29.088	O6/L6	sn 1′, 3′	29.164
O4/L4	sn 2′	29.08	UK		29.158
O11	sn 1′,3′	27.252	O6/L6	sn 1′, 3′	29.148
O11	sn 2′	27.248	P4		29.139
UK		27.242	O4		29.126
L14		27.228	L4	sn 2′	29.119
L8		27.217	UK		29.110
UK		27.208	UK		29.093
O8		27.199	O4/L4	sn 1′, 3′	29.088
UK		27.188	O11		27.260
UK		27.183	O11		27.256
L11		25.654	UK		27.249
O3/L3	sn 2′	24.91	L14		27.236
P3		24.892	L8		27.223
UK		24.878	O8		27.204
O3/L3	sn 1′,3′	24.87	UK		27.197
UK		24.753	UK		27.188
UK		24.737	L11	sn 2′	25.662
P15		22.723	O3		24.917
O17		22.713	L3	sn 1′, 3′	24.906
L17		22.604	P3		24.900
P16		14.133	O3/L3		24.878
O18		14.127	UK		24.742
L18		14.087	UK		24.727
			P15		22.734
			O17		22.724
			L17		22.615
			P16		14.137
			O18		14.132
			L18		14.092

O, olein; P, palmitin; Pa, palmitolein; L, linolein; UK, unknown; V/E, vaccenin or eicosenoin; FFA, free fatty acid.

oleic, linoleic and palmitic fatty acids in all six oils and palmitoleic fatty acid in macadamia nut and avocado pear oils. This method has been validated with the well-known ^{13}C NMR spectrum of olive oil, and has been used to fully assign the ^{13}C NMR spectra of the triacylglycerol fatty acid residues of six previously unassigned vegetable oils, apricot kernel, avocado pear, grapeseed, macadamia nut, mango kernel and marula oils. This method is reasonably robust and rapidly leads to accurate ^{13}C NMR assignments in mixtures of triacylglycerols at reasonable concentrations in similar vegetable oils not previously examined, without having to resort to time-consuming additions of pure standards.

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Supporting information

Supporting information may be found in the online version of this article.

References

[1] L. Mannina, A. P. Sobolev, A. Segre, *Spectrosc. Eur.* **2003**, *15*, 6.
[2] L. Mannina, A. Segre, *Grasas y Aceites* **2002**, *53*, 22.
[3] G. Vlahov, C. Schiavone, N. Simone. *Magn. Reson. Chem.* **2001**, *39*, 689.
[4] R. Sacchi, F. Addeo, L. Paolillo, *Magn. Reson. Chem.* **1997**, *35*, S133.
[5] J. M. McKenzie, K. R. Koch, *S. Afr. J. Sci.* **2004**, *100*, 349.
[6] L. Diego, G. Gonzáles, L. Mannina, M. D'Imperio, A. Segre, R. Aparicio, *Eur. Food Res. Technol.* **2004**, *219*, 545.
[7] G. Vlahov, *Magn. Reson. Chem.* **1997**, *35*, S8.
[8] J. Mavromoustakos, M. Zervou, G. Bonas, A. Kolocouris, P. Petrakis, *J. Am. Oil Chem. Soc.* **2000**, *77*(4), 405.
[9] G. Vlahov, A. D. Shaw, D. B. Kell. *J. Am. Oil Chem. Soc.* **1999**, *76*, 1223.
[10] L. Mannina, C. Luchinat, M. C. Emanuele, A. Segre, *Chem. Phys. Lipids* **1999**, *103*, 47.
[11] *Off. J. Eur. Commun.* **1991**, *L248*, 1, Commission Regulation (EEC) No 2568/91.
[12] L. Mannina, C. Luchinat, M. Patumi, M. C. Emanuele, E. Rossi, A. Segre, *Magn. Reson. Chem.* **2000**, *38*, 886.
[13] A. D. Shaw, A. di Camillo, G. Vlahov, A. Jones, G. Bianchi, J. Rowland, D. B. Kell, *Anal. Chim. Acta* **1997**, *348*, 357.

