# Rapid analysis of major components and potential authentication of South African olive oils by quantitative $^{13}$C nuclear magnetic resonance spectroscopy

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**We have developed a rapid, quantitative $^{13}$C NMR spectroscopic method for the determination of the major fatty acids (oleic, linoleic and saturated acids) contained as triacylglycerides in South African extra-virgin olive oils. With the judicious use of a shiftless NMR relaxation agent, Cr(acetylacetonate)$_3$, it is possible to determine the principal fatty acids in 0.4 g of olive oil within 20 min. This method also allows for the estimation of the distribution of the oleic and linoleic acids in the naturally occurring triacylglycerides in olive oils, expressed as α/β ratios for the triacylglycerides. Moreover, a fake product, marketed in South Africa as an 'extra-virgin olive oil', was also analysed to demonstrate the use of $^{13}$C NMR spectroscopy as a rapid means of establishing the authenticity of the claimed product on the local market. We further examined a selection of extra-virgin olive oils produced in various regions of the Western Cape province in 2002 and 2003, using $^{13}$C NMR spectroscopy, in a preliminary attempt to determine their major fatty acid compositions, and to establish whether any regional and cultivar-based differences in these products can thereby be detected.**

## Introduction

Olive oil is widely recognized to confer health benefits, which are not generally associated with other oils. The traditional 'Mediterranean diet' in which olive oil is the principal source of fat is considered to be a model for healthy eating as it is associated with low rates of heart disease, certain cancers and other diet-related diseases.[1] The benefits of olive oil are thought to be due not only to the high mono-unsaturated (oleic) fatty acid content but also to the presence of minor components, for example, the phenolic compounds hydroxytyrosol and tyrosol, which are present especially in higher quality (virgin and extra-virgin) olive oils and have been shown to protect against oxidative cellular damage in humans.[2,3] The pleasant taste and aroma of olive oil combined with its health benefits have resulted in extra-virgin olive oil becoming a high-value product. Unfortunately, this provides an incentive for the marketing of inferior quality products as extra-virgin olive oil at best, or at worst for the promotion of fake vegetable oils treated with colourants to resemble olive oil. This problem is of global extent, so that bodies such as the European Union (EU) have published strict guidelines for the analysis of olive oils in an effort to detect and prevent adulteration.[4] Unfortunately, South Africa has not avoided such transgressions. Moreover, since current legislation regarding the purity of olive oil is not as well developed as in Europe or the United States, recent newspaper headlines, such as 'Sunflower oil containing poisonous colourant sold to the SA public as olive oil', are cause for serious concern,[5] and underscore the need for a rapid technique for authenticating olive oil and quality control in this country. The expanding production of extra-virgin olive oil in South Africa provides scope for potential fraud, particularly in the absence of a regulatory framework.

In light of these developments, we sought to develop a rapid spectroscopic method for the authentication and analysis of major components of olive oils. The EU regulations for the analysis of these oils[4] stipulate a number of relatively time-consuming methods for determining their chemical composition, such as the quantity of free fatty acids, the peroxide value and the iodine value, among other quality criteria. Some of these methods assess the quality of an olive oil but will not necessarily establish the authenticity of an extra-virgin olive oil, so that in our view obtaining a rapid, unambiguous 'fingerprint' to distinguish a genuine olive oil from fake oil is clearly desirable. The most widely used and officially accredited technique to determine quantitatively the major triacylglyceride fatty acids contained in vegetable oils is gas chromatography (GC).[4] Since the relative quantities of these fatty acids are generally known to vary in different vegetable oils within defined limits, this form of analysis can differentiate between certain vegetable oils. While GC has many advantages, it can be relatively time-consuming and relies on having to manipulate and derivatize the fatty acids chemically prior to analysis.

We have explored the use of high-resolution $^{1}$H and $^{13}$C nuclear magnetic resonance (NMR) spectroscopy as a potential rapid 'fingerprinting' method for extra-virgin olive oils as well as for the quantitative determination of the major fatty acids they contain. Shoolery demonstrated in 1977 that quantitative $^{13}$C NMR spectroscopy in particular can readily be used to determine major fatty acid components of triacylglyceride mixtures found in vegetable oils.[6] Recent advances in high magnetic field Fourier transform NMR (FT-NMR) spectrometers have significantly improved the sensitivity and resolution of the $^{13}$C NMR spectra attainable, facilitating the unique identification and quantification of all carbon atoms that make up the major fatty acids in an olive oil. The advantage of the FT-NMR technique is that it is non-destructive and samples do not require any special chemical pre-treatment, unlike for gas chromatography, so that it is potentially less time-consuming, and bias free. To date, $^{1}$H and $^{13}$C NMR spectroscopy have not yet been accredited for the quantitative determination of the major fatty acids of olive or other vegetable oils. Nevertheless, recent papers have demonstrated how much molecular and compositional information about olive oils (besides the major fatty acid composition) can be obtained using $^{1}$H and $^{13}$C NMR spectroscopy.[7–10] Such studies help to promote the acceptance of $^{1}$H and $^{13}$C NMR spectroscopy as a tool for studying olive oil composition.[8] Moreover, FT-NMR studies have investigated regional and cultivar-based differences in the composition of olive oils produced in Greece[11] and Italy.[12]

In this paper, we show that quantitative $^{13}$C NMR spectroscopy can lead rapidly (in less than 20 min) to the quantitative determination of the major fatty acids contained as triacylglycerides in extra-virgin olive oils (oleic, linoleic and saturated fatty acids) as well as give the distribution α/β ratios of the oleic and linoleic fatty acids in the triacylglycerides (Fig. 1). Moreover, a fake product, marketed in South Africa as an 'extra-virgin olive oil', was also analysed to demonstrate the use of $^{13}$C NMR spectroscopy as a rapid means of establishing the authenticity of these oils. We

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further present a preliminary study of the major fatty acid compositions of extra-virgin olive oils produced in various regions of the Western Cape in 2002 and 2003.

### Brief overview of high-resolution Fourier transform NMR spectroscopy

For readers unfamiliar with modern $^{1}$H and $^{13}$C FT-NMR spectroscopy, a brief overview might be helpful. The power of $^{1}$H and $^{13}$C NMR spectroscopy for structure determination of molecules in solution is well known and undisputed.[13] The development of superconducting magnets in the last three decades has led to the general availability of moderate (4.9 T) to high magnetic field (>9.8 T) FT-NMR spectrometers. This has greatly enhanced both the sensitivity and resolution of this technique, making it possible to identify and assign uniquely virtually every resonance in a $^{1}$H and $^{13}$C NMR spectrum to an individual H or C atom in typical small (100–600 Da) to moderately large (>600–2000 Da) organic molecules.

The origin of an NMR spectrum lies in the fact that certain nuclei, with nuclear spin-quantum number $I = ½$, possess magnetic moments, so that in a strong external magnetic field these nuclei tend to align themselves either with or against this magnetic field. In the simplest cases of $^{1}$H or $^{13}$C, the nuclei distribute themselves between only two energy states (both nuclei have $I = ½$). These nuclei can then be induced to undergo transitions between these energy states by absorption of low-energy radio-frequency radiation of specific frequencies. A plot of resonance frequency against intensity of either $^{1}$H or $^{13}$C nuclei in different chemical environments gives rise to the high-resolution NMR spectrum. Since organic molecules are usually composed of at least H and C atoms, $^{1}$H and $^{13}$C NMR spectroscopy is the method of choice for structure elucidation. In NMR spectroscopy, $^{1}$H nuclei (protons) are the most readily observed nuclei in view of their high natural abundance (abundance 99.98%, relative NMR sensitivity = 1), whereas $^{13}$C NMR spectroscopy tends to be time-consuming as a result of the low natural abundance of $^{13}$C (1.11% natural abundance, relative NMR sensitivity = $1.59 \times 10^{-2}$). In a $^{1}$H NMR spectrum, quantitative information can readily be obtained by integration of the signals, which gives values for the relative number of equivalent protons in the molecule, as well as a measure of the relative concentration of the molecules in question (given an internal standard). Unfortunately, the spectral range in $^{1}$H NMR spectra is fairly narrow (typically 0–15 ppm), which together with inter-nuclear $^{1}$H –$^{1}$H scalar J coupling, can result in significant overlap of $^{1}$H resonance peaks, particularly for larger molecules with many similar protons (e.g. long aliphatic chains), thus limiting quantitative analysis of $^{1}$H NMR spectra to some extent. This problem is made worse for *mixtures* of different substances, requiring sophisticated methods of assignment. In such situations, the use of $^{13}$C NMR can be very useful, since even though $^{13}$C nuclei have a natural abundance of only 1.11% (the rest of the C atoms being NMR-inactive $^{12}$C and $^{14}$C), the spectral range of $^{13}$C signals is often about 15–20 times wider than that for the $^{1}$H peaks. Thus, the proton-decoupled $^{13}$C NMR spectrum of, say, an olive oil gives a $^{13}$C NMR spectrum in which *all* chemically unique C atoms in the molecules of the major components can be seen as separate peaks and can be readily assigned, provided that spectral resolution is high enough.

A major limitation of $^{13}$C NMR spectroscopy is its low sensitivity and the need to take specific precautions for obtaining integrals of $^{13}$C peaks, which are proportional to the concentration of the molecules. The problem of low sensitivity has been overcome by the use of high magnetic fields (>9.8 T) as well as the use of pulsed-excitation FT-NMR spectroscopy, which acquires multiple digital spectra per experiment, resulting in an improved signal-to-noise ratio over time. In proton-decoupled $^{13}$C spectroscopy, however, during spectral acquisition, the intensities of the resultant $^{13}$C peaks in the spectrum can be significantly distorted by (i) insufficient time to ensure complete relaxation of specific $^{13}$C nuclei in a molecule due to long $T_1$ relaxation times (see below), and (ii) due to the complex phenomenon of the nuclear Overhauser effect (nOe), which operates as a result of proton decoupling in the standard $^{13}$C NMR experiment. In his seminal paper, Shoolery[6] demonstrated that it is possible to obtain accurate concentration-related information from $^{13}$C NMR spectra of mixtures by $^{13}$C NMR spectroscopy, provided suitable precautions during acquisition of the $^{13}$C NMR spectrum are taken.

### Experimental

Olive oil samples were collected from various producers in the Western Cape province. Specimens were kept in cool, dark conditions and analysed as soon after collection as possible to avoid any oxidative and light-induced degradation of the oil. All FT-NMR spectra were recorded, at 40°C, on a Varian Inova 600 MHz (14.09 T) spectrometer operating at 600 MHz for $^{1}$H and 150 MHz for $^{13}$C. $^{1}$H spectra were collected under standard conditions. Samples contained 40% oil (w/w, weight ratio) in $CDCl_3$ (approx. 0.4 g oil, accurately weighed, per 0.6 g $CDCl_3$). Pure pyrazine (*c.* 15 mg, accurately weighed) was used as internal standard as recommended by Mavromoustakos *et al.*[11] The $^{13}$C $T_1$ relaxation times of a typical olive oil sample were measured by the standard inversion-recovery method, and all were found to be <4.5 s for the peaks of interest. After collection of the $^{13}$C data, Gaussian multiplication was applied to the FID (free induction decay) using a line-broadening of –0.092 and Gaussian enhancement of 1.088, to optimize resolution. Prior to Fourier transformation of the FID, zero-filling to 524k was applied. Integration of the peaks could then be carried out using deconvolution analysis to obtain the most accurate peak integrals.

In this study, we examined five experimental $^{13}$C NMR acquisition methods in an attempt to find the shortest possible means of quantitatively determining the major triacyglycerides without loss in accuracy. Mavromoustakos *et al.* showed an excellent correlation between the GC and $^{13}$C NMR data obtained for the analysis of the triacylglyceride composition of a range of Greek olive oils.[11] We chose this procedure as 'control' Method 1, against which we evaluated our modifications.

Method 1: the most important features of this published $^{13}$C NMR procedure are the use of 300 90°-pulses (acquisition time 5.44 s) at 75 MHz $^{13}$C resonance frequency (7.05 T magnet) together with a long pulse delay of 45 s (>5 × longest $T_1$ for our samples), with inverse-gated proton-decoupling to suppress any nOe. Mavromoustakos *et al.* used a narrowed spectral window to cover only the region of interest from 195 to 105 ppm. The spectral window, however, excludes other peaks present in the $^{13}$C spectrum. The total data collection time for one sample using this method was 4 h 12 min. In this work, we used an identical procedure with a 14.09-T spectrometer operating at 150 MHz for $^{13}$C. In the higher magnetic field one can expect roughly twice the overall sensitivity.

Method 2: This was virtually identical to Method 1, the only difference being that a full spectral window (200 to –5 ppm) was used, allowing all the $^{13}$C resonances of the olive oil to be recorded and avoiding the artefact of 'spectral folding' of peaks, so reducing unnecessary noise in the spectrum. Presumably, Mavromoustakos *et al.* chose to use a narrowed spectral window to ensure equal excitation of the $^{13}$C nuclei over this spectral region.[11] With our spectrometer this was achieved over the entire spectrum (see below). The total acquisition time was 4 h 12 min.

Method 3: identical to Method 2, but with the total number of pulses set to 77 (yielding an adequate signal-to-noise ratio), so reducing the total acquisition time for one sample to 65 min.

Method 4: pulse sequence identical to Method 3 but with the pulse delay reduced further to 17.1 s. This meant the total delay between pulses (pulse delay plus acquisition time) was set to ~ 5 × the longest $T_1$ measured for the peaks of interest at <4.5 s.

The total acquisition time was 29 min.

Method 5: oil samples were made up in chloroform as above but with addition of the relaxation agent chromium(III) acetylacetonate, $Cr(acac)_3$, to a maximum of 0.6 mg $Cr(acac)_3$ per g of sample (oil + $CDCl_3$). More $Cr(acac)_3$ resulted in some loss of resolution due to line broadening and is not recommended. An identical pulse sequence as for Method 4 was used, but addition of the relaxation agent allowed for the pulse delay to be further reduced to 9.6 s, resulting in a total acquisition time of 20 min.

## Results and discussion

Olive oils consist mostly of triacylglycerides of 18-carbon atom fatty acids of varying degrees of unsaturation. The position at which the fatty acids are attached to the glycerol backbone (α or β) is characteristic of the oil, and the distribution of the different fatty acids present in the triacylglycerides may be used to define the composition of the oil. Figure 1 shows schematically the structure of a typical triacylglyceride as found in a typical olive oil. These triacylglycerides consist mostly of 18-C oleyl and linoleyl unsaturated fatty acid groups; oleic acid has one double bond (abbreviated as 18:1) and linoleic acid has two double bonds (18:2). In addition to these unsaturated fatty acids, olive oil also contains some saturated palmitic (16:0) and stearic (18:0) acids. The observed α/β ratio for olive oils varies somewhat for naturally occurring triacylglycerides, and this ratio might be a useful parameter in defining the origin and variety of the oils (see below). Natural triacylglycerides generally do not have the same type of fatty acid attached to all three positions of the glycerol backbone, but often two or three different types, reflected in the α/β ratio. An exception is found for olive oils, in which a triacylglyceride derived from only oleic acid, triolein, is commonly found.[14] The relative amounts of fatty acids found in the triacylglycerides of olive oils are typically in the range 8.0–23.5% saturated, 56.0–83.0% oleic, and 3.5–20.0% linoleic fatty acids, with other fatty acids also being present in trace amounts. Variations in these percentages may be used to fingerprint different olive oils to some extent.

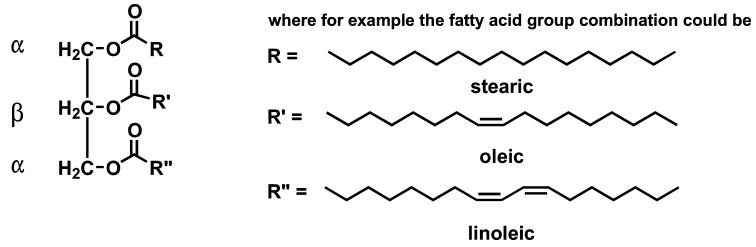


**Fig. 1**. General structure of a triacylglycerol as found in olive oils. The outer carbon positions of the glycerol backbone are labelled α, the inner carbon position β.

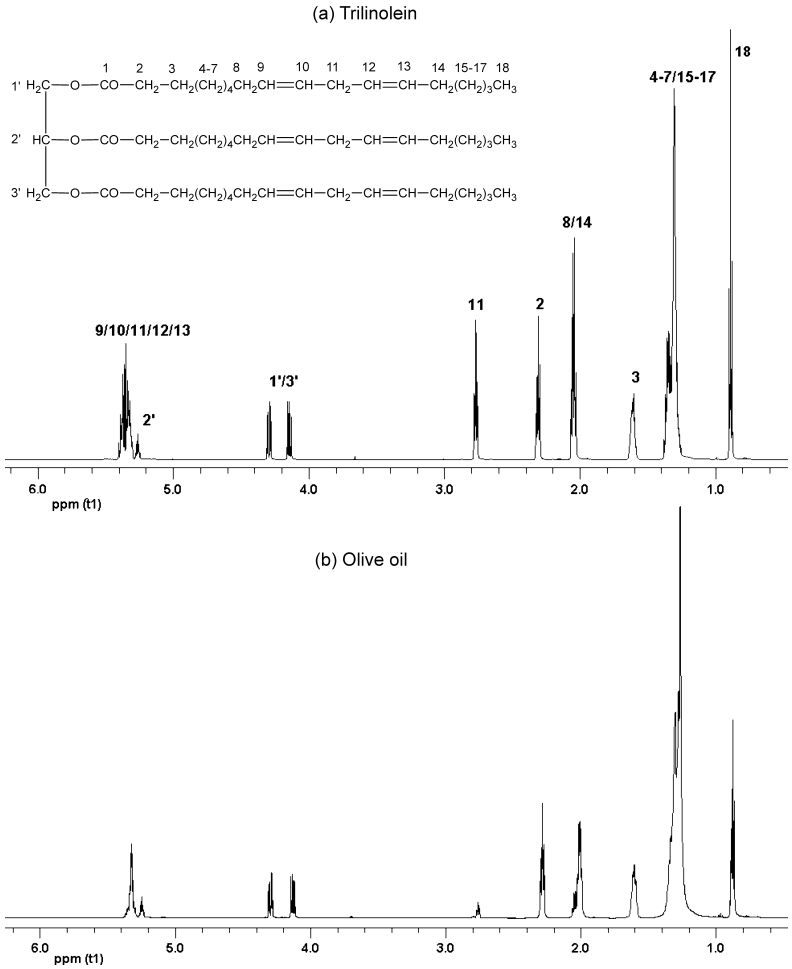


**Fig. 2**. (**a**) A 600-MHz $^1H$ spectrum of commercially available trilinolein (structure shown) with peak assignments as indicated. (**b**) $^1H$ spectrum of an extra-virgin olive oil in $CDCl_3$.

A fully assigned $^1H$ NMR spectrum of the commercially available trilinolein is shown in Fig. 2(a). Unfortunately, due to the narrow spectral range of the $^1H$ NMR spectrum, many $^1H$ peaks overlap significantly for the various fatty acids present in these oils, compromising the advantages of the speed and sensitivity of $^1H$ NMR spectroscopy. Figure 2 compares a $^1H$ NMR spectrum of trilinolein with that of an extra-virgin olive oil [Fig. 2(b)]; these spectra are remarkably similar, the only noticeable difference being the relative peak intensities. The intensity differences are due to the overlapping presence of other fatty acids in the oil, the $^1H$ peaks of which occur at similar chemical shifts to the trilinolein peaks. This illustrates the weakness of $^1H$ NMR spectroscopy for olive oil analysis, as the peaks of the various similar fatty acid components overlap markedly in the spectrum.

By contrast, in a $^{13}C$ NMR spectrum of an extra-virgin olive oil, overlapping of $^{13}C$ peaks is substantially reduced, since they are dispersed over a much wider spectral range of approximately 200 ppm for $^{13}C$ compared to the 10–15 ppm for a $^1H$ spectrum. Figure 3 shows a proton-decoupled $^{13}C$ spectrum of an extra-virgin olive oil obtained by Method 1; the main aliphatic region ($CH_3$s and $CH_2$s), the glycerol backbone peaks, the olefinic region (double bonds), and the carbonyl (–OC=O) region in the spectrum have been labelled. The full assignment of the $^{13}C$ spectrum of all the major triacylglycerides found in olive oils has only recently been achieved with the aid of a series of 1D and 2D INADEQUATE NMR experiments.[15] Thus, it is now possible to assign uniquely all $^{13}C$ signals in an olive oil spectrum to individual carbon atoms of the component oleic, linoleic and saturated fatty acids. Mannina *et al.*[16] reported that the $^{13}C$ NMR shifts of the triacylglycerides are slightly concentration dependent, something that should be kept in mind when analysing olive oils by $^{13}C$ NMR.

Inspection of the carbonyl [Fig. 3(b)] and olefinic [Fig. 3(c)] regions of the 150-MHz $^{13}C$ NMR spectrum of olive oil shows that peaks of the different triacylglyceride fatty acid components can

readily be identified, in contrast to that in the corresponding $^{1}$H NMR spectrum. The carbonyl region of the $^{13}$C NMR spectrum (170–180 ppm) shows all the peaks belonging to oleic, linoleic, and saturated fatty acids, while the olefinic region (125–130 ppm) shows peaks due to oleic and linoleic acids. Unfortunately, the different saturated fatty acids (e.g. palmitic and stearic) cannot be distinguished in the $^{13}$C spectrum. Figures 3(b) and (c) also show that the oleic and linoleic fatty acids attached to the 'outer' α carbons of the glycerol backbone give rise to signals distinct from those attached to the 'inner' β glycerol carbon (Fig. 1), allowing for the α/β ratios for the for differentiating between olive oils and is a good parameter to identify adulterated oils, since in olive oil unsaturated fatty acids show a preference for the β position in the triacylglycerides and it is rare for a saturated fatty acid to be found in the β position.

According to EU regulations, an olive oil should have less than 1.5% saturated fatty acids at the β position.[4] If there were no preference for the α or β positions, the expected α/β ratio for a triacylglyceride would be 2:1; a ratio greater or less than 2:1 implies favouring the α or β positions, respectively. Thus, in olive oils we expect an α/β ratio <2:1 for the oleic and linoleic fatty acids, indicating a preference of an unsaturated fatty acid for the β position of the glycerol backbone.

Figure 4 compares a $^{13}$C NMR spectrum of a genuine extra-virgin olive oil and that of a fake olive oil marketed in South Africa as having been produced in Italy*. It is readily apparent that the fake oil is not an olive oil, as the relative intensities of the $^{13}$C peaks clearly differ in the two spectra. These findings concur with recently published results, which also used $^{13}$C NMR spectroscopy to identify adulteration of virgin olive oils with other vegetable oils.[17] It was shown that adulterations as low as 5 mol% could be detected by this technique.

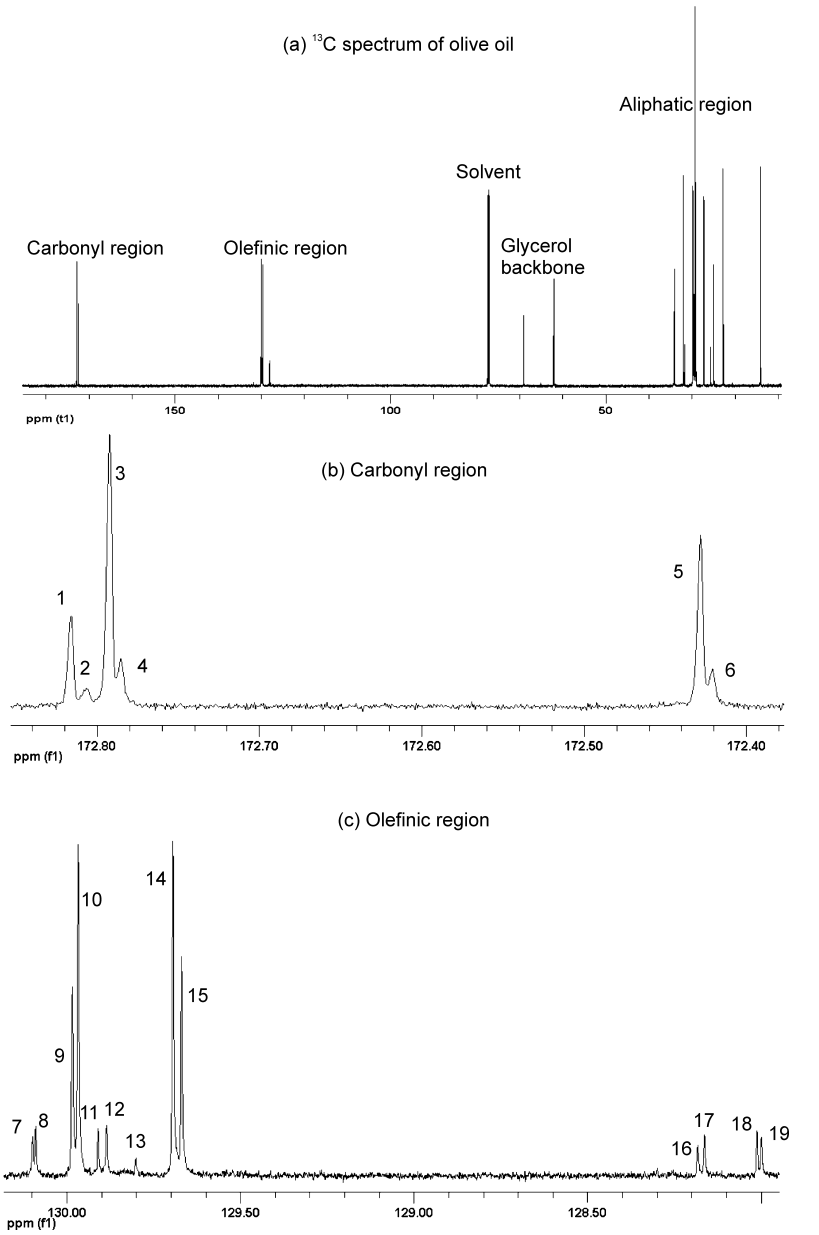


**Fig. 3**. (**a**) $^{13}$C spectrum of a typical extra-virgin olive oil. (**b**) Carbonyl region of an olive oil spectrum. (**c**) Olefinic region of an olive oil spectrum. Peaks correspond to the following carbons (oleic = O, linoleic = L): 1, C1 saturated ; 2, C1 *cis*-11 monoenoic; 3, O1α; 4, L1α; 5, O1β; 6, L1β; 7, L13β; 8, L13α; 9, O10β; 10, O10α; 11, L9α; 12, L9β; 13, C11 cis-11 monoenoic; 14, O9α; 15, O9β; 16, L10β; 17, L10α; 18, L12α; 19, L12β.

## Rapid quantitative determination of major fatty acid composition of olive oils by $^{13}$C NMR

In order to use $^{13}$C NMR for the quantitative determination of major fatty acids in olive oils, several factors alluded to in the introduction should be taken into consideration. First, all the $^{13}$C nuclei over the entire spectral range should be equally excited by the radio-frequency pulse; second, account must be taken of the different $T_1$ relaxation times for carbon nuclei, so as to allow a sufficient time between excitation pulses for nuclear relaxation to occur, thus avoiding peak intensity distortions resulting from too high a pulse repetition rate; third, the proton-decoupling must be gated 'off' during the (relatively long) relaxation delay in order to suppress any nuclear Overhauser enhancement of $^{13}$C nuclei which have protons attached, leading to intensity distortions for these peaks relative to others.[6] The excellent separation of $^{13}$C peaks of the oleic, linoleic and saturated fatty acids observed in the 150-MHz $^{13}$C NMR spectrum of genuine extra-virgin olive oil, shown in Fig. 3, suggests that their quantitative determination should easily be possible. We thus investigated the use of appropriate $^{13}$C NMR methods to develop a rapid means for obtaining accurate results in the shortest possible time. Examination of the published quantitative $^{13}$C NMR method for olive oils[11] shows this to be relatively lengthy. Hence, we compared four potentially shorter methods (2–5 described in the experimental section) with Method 1.

Methods 1 and 2 use 300 pulses for the data collection, as the original experiments of Mavromoustakos *et al.* were carried out on a 300-MHz instrument (7.05 T magnet), requiring a time of 4 h 12 min per sample. Since we had available a 600-MHz NMR instrument (with a 14.09 T magnet, resulting in nominally twice the absolute sensitivity), the simplest way to decrease the overall acquisition time significantly was to reduce the number of pulses. We found that the number of pulse repetitions could be reduced to 77 (resulting in a total acquisition time of 65 min), while still achieving a comparable signal-to-noise ratio for the $^{13}$C spectra with that of the published method; we refer to this as Method 3. Given the relatively long pulse delay of 45 s in Methods 1–3, one might expected a further reduction in the spectral acquisition time by optimizing the pulse delay time to match the generally recommended 3–5 × the *longest* $T_1$ in the sample.[6] By means of the inversion recovery experiment, we

*Sample kindly supplied by Vesuvio Estate, Paarl.

estimated the longest $T_1$ in our samples of extra-virgin olive oil in $CDCl_3$ to be no longer than 4.5 s for any $^{13}C$ peak of interest. Thus, the optimum pulse repetition rate should be at most *c.* 22.5 s ($5 \times T_1$), which resulted, taking into account the data (FID) acquisition time of 5.4 s, in a maximum necessary pulse delay time of 17.1 s. Using this pulse sequence with 77 pulse repetitions reduced the total acquisition time to only 29 min (Method 4).

To reduce the total experimental acquisition time still further, we explored the use of the shiftless relaxation agent, $Cr(acac)_3$, as originally advocated by Shoolery.[6] Nuclear relaxation is generally understood by two related, but separate, relaxation phenomena, these being characterized by the spin-lattice relaxation time $T_1$ and the spin-spin relaxation time $T_2$, respectively. In general for nuclei in non-viscous solutions, it is found that $T_1 \geq T_2$, so pulse delays are determined by the longest $T_1$ times for given $^{13}C$ nuclei, rather than by the $T_2$ times. Moreover, the $T_2$ relaxation time of a particular nucleus is inversely proportional to the peak width at half height, so that for very small $T_2$ values, the $^{13}C$ peak widths become unacceptably broad. Hence, addition of too much of a relaxation agent not only reduces $T_1$ but also results in much smaller $T_2$ values, which are reflected in broader peaks, resulting in a significant and undesirable loss of spectral resolution. By using a judicious amount of relaxation agent, however, the overall $T_1$ times and hence the pulse delays can be reduced, without a significant loss of spectral resolution. This is important for olive oils, since several $^{13}C$ peaks useful for quantification have similar chemical shifts (Fig. 3).

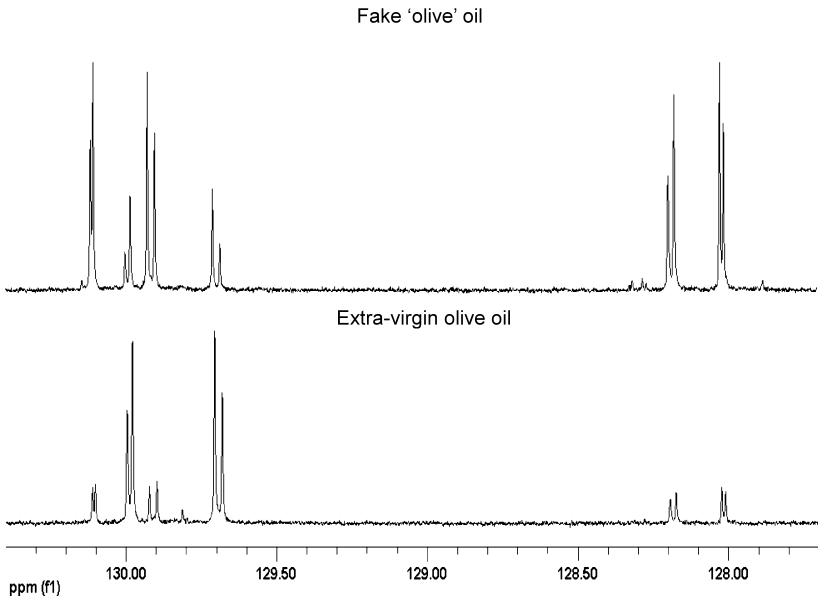


**Fig. 4.** Comparison of the olefinic regions of a sunflower oil (top), that was fraudulently sold on the South African market as an Italian extra-virgin olive oil, and a genuine extra-virgin olive oil (bottom).

**Table 1**. Major fatty acid composition (as mol%) of a South African extra-virgin olive oil as determined by quantitative $^{13}C$ NMR spectroscopy using Methods 1–5 (see text).

| Method | % Oleic[a] | % Linoleic[a] | % Saturates[a] | Oleic α/β ratio ratio | Linoleic α/β ratio |
|---|---|---|---|---|---|
| 1[b] | 68.48 ± 0.44 | 11.04 ± 0.20 | 20.60 ± 0.30 | 1.45 ± 0.02 | 1.20 ± 0.04 |
| 2 | 69.23 ± 0.42 | 11.25 ± 0.11 | 19.52 ± 0.41 | 1.46 ± 0.02 | 1.16 ± 0.02 |
| 3 | 69.24 ± 0.36 | 11.23 ± 0.38 | 19.55 ± 0.41 | 1.46 ± 0.02 | 1.15 ± 0.05 |
| 4 | 69.07 ± 0.47 | 11.16 ± 0.28 | 19.77 ± 0.42 | 1.44 ± 0.03 | 1.22 ± 0.08 |
| 5 | 68.87 ± 0.53 | 11.20 ± 0.24 | 19.94 ± 0.42 | 1.48 ± 0.02 | 1.15 ± 0.09 |

[a]Mol% at 95% confidence interval of mean of six determinations ($t = 2.571$).
[b]After ref. 11.

We found an optimum amount of $Cr(acac)_3$ leading to a reduction of $T_1$ times without significant line broadening to be 0.6 mg $Cr(acac)_3$ per 1 g of sample, or 0.06% (w/w). In the presence of this amount of $Cr(acac)_3$, the longest $T_1$ for any peak of interest was <3 s, so allowing a pulse delay of only 9.6 s, taking into account an acquisition time of 5.4 s. Using this delay with 77 pulse repetitions yielded $^{13}C$ spectra from which accurate peak intensities could be obtained with an acquisition time of only 20 min (Method 5). This is confirmed by data in Table 1, in which are given the mol% of oleic, linoleic and saturated fatty acids for the same extra-virgin olive oil as obtained by 6 determinations with each of the $^{13}C$ NMR Methods 1–5. Clearly, there was no significant difference in the mol% of these fatty acids as found by each of the methods tested at 95% confidence. Thus, by using an appropriate amount of $Cr(acac)_3$ it is possible to determine accurately the mol% of the major fatty acids in olive oil by means of quantitative $^{13}C$ NMR, achieving a time-saving factor of 12.6 over the published method,[11] without loss of accuracy or any special sample preparation.

As part of this study, we were interested in determining the variation (if any) in the major fatty acid composition as a function of variety and region of origin of extra-virgin olive oils produced in the Western Cape in 2002 and 2003. Mannina *et al.*[19] have reported on a cultivar–composition relationship of Sicilian olive oils, while others [19,20] have investigated the use of quantitative $^{13}C$ NMR as a means of characterizing the varietal and geographical origin of these oils. It was found that the relative composition of fatty acid groups may be used to identify oils of the same cultivars, although a link to geographical origin is less well established. To our knowledge, no such study has been carried out on South African olive oils. Table 2 shows the results of our limited study. The mol% saturated acid was obtained from the integration of peak 1 in Fig. 3(b); the mol% oleic acid was obtained from the average integral of peaks 9/10 and 14/15 in Fig. 3(c), while the linoleic value was derived from the average of peak integrals 16/17 and 18/19 in Fig. 3(c). The linoleic peaks 12/13 in Fig. 3(c) were not used as there was some overlap of these with those of minor fatty acid components;[21] the same appeared to be the case for peaks 7/8.

Table 2 shows a marked difference in the mol% fatty acid composition of the fake olive oil compared to the extra-virgin olive oils. The relative mol% of oleic and linoleic fatty acids for the fake oil are clearly out of the range expected for an olive oil, the oleic percentage being about 40% lower and the linoleic proportion being about 40% higher than the average for authentic olive oils. The mol% of fatty acids for the fake olive oil are typical of those expected for sunflower oil[14] and correspond to those measured for a commercially available sunflower oil using $^{13}C$ NMR spectroscopy (25.1% oleic, 61.6% linoleic, 13.3% saturated, 2.1:1 oleic α/β ratio). Some instructive observations on the fatty acid composition of South African olive oils can be made regarding their similarities (Table 2). Samples J and C were both olive oils pressed from the Coratina cultivar, the similarity in mol% fatty acid composition can clearly be seen, even though the two oils were pressed in different years. The same can be said for A and B, although A was a blend containing mostly oil from the Frantoio cultivar, B was a pure Frantoio oil. Sample I was also a Frantoio blend but the fatty acid composition differs from A and B. While the blended samples A and I contain mostly Frantoio, the relative quantities are not known, so that it is not possible to draw conclusions about these samples concerning regional differences. Similar comments pertain to sample K, which contains oil from the Mission cultivar, while G and H are blends, containing mostly oil from the Mission cultivar. Samples K and G are from

**Table 2**. The fatty acid composition (mol% determined by a single quantitative $^{13}C$ NMR measurement) of a selection of extra-virgin olive oils produced in the Western Cape during 2002 and 2003, and also of a fake olive oil sold on the South Africa market.

| Sample | Region | Cultivar | Year | % Oleic | % Linoleic | % Saturates | Oleic α/β ratio | Linoleic α/β ratio |
|---|---|---|---|---|---|---|---|---|
| A | Paarl | Blend (Frantoio) | 2002 | 67.5 | 13.4 | 19.1 | 1.5 | 1.1 |
| B | Somerset West | Frantoio | 2002 | 67.7 | 12.2 | 20.1 | 1.5 | 1.1 |
| C | Somerset West | Coratina | 2002 | 74.2 | 9.6 | 16.2 | 1.6 | 1.2 |
| D | Somerset West | Leccino | 2002 | 72.8 | 7.9 | 19.3 | 1.4 | 1.2 |
| E | Somerset West | Cultivar '177' | 2002 | 73.6 | 10.3 | 16.1 | 1.6 | 1.1 |
| F | Somerset West | Cultivar 'FS17' | 2002 | 69.5 | 11.6 | 18.8 | 1.5 | 1.2 |
| G | Hermanus | Blend (Mission) | 2002 | 74.0 | 10.2 | 15.8 | 1.6 | 1.2 |
| H | Swartland | Blend (Mission) | 2002 | 70.9 | 14.5 | 14.6 | 1.7 | 1.4 |
| I | Paarl | Blend (Frantoio) | 2003 | 70.5 | 10.8 | 18.7 | 1.5 | 1.2 |
| J | Paarl | Coratina | 2003 | 73.1 | 9.8 | 17.0 | 1.5 | 1.4 |
| K | Worcester | Mission | 2003 | 74.8 | 11.7 | 13.5 | 1.7 | 1.0 |
| Fake olive oil | n/a | n/a | n/a | 26.5 | 60.7 | 12.8 | 2.2 | 1.4 |

n/a, not available.

two geographically distinct regions, and from different production years but they had similar fatty acid contents, in contrast to sample H, which was also mainly a Mission oil, but had rather different composition. Although some varietal similarities are evident from Table 2, not much can be said about the differences between oils produced in distinct geographical regions. Moreover, the number of samples we used was probably far too small to show any possible correlations between the fatty acid composition, the geographical origin and possibly seasonal variations in these olive oils, for the same cultivar.

As has been highlighted by other studies,[19] the discrimination of extra-virgin olive oils on the basis of variety and geographical region requires the use of a larger number of samples and multivariate statistical analysis. At present, olive oil production in South Africa is relatively small, which means that quantities of individual cultivars are not large enough to warrant pressing fruit of the different cultivars separately. Some producers grow a range of cultivars, while others, more commonly, grow only one or two cultivars and blend the oils when pressing the fruit.

In general, the data from Table 2 are qualitatively suggestive but more samples need to be collected so a full statistical analysis can be carried out. As the number of olive oil producers in South Africa grows, gathering larger data sets over several seasons should become easier, making a fuller study of any possible correlations of the major component composition of olive oils with geographical, seasonal and cultivar differences a worthwhile undertaking. Such a study in our view will also lead to the development of local know-how and a database of South Africa olive oils, which could be invaluable to the burgeoning number of small producers of this high-value product. Finally, the determination of the numerous minor components in olive oils, which define their aromatic character, should not be ignored in such a survey. It is noteworthy that $^{1}H$ NMR spectroscopy of olives has been used to identify some of the minor components of the oil, such as alkanals and other volatile and steroid-type compounds; these studies established a correlation between composition and geographical origin of Italian olive oils.[12,22]

## Conclusions

We have developed and tested a rapid quantitative $^{13}C$ NMR method for the accurate determination of major fatty acid components of extra-virgin olive oil. A fake 'olive' oil, marketed in South Africa as authentic, could thus easily be shown not to be genuine, but to have a composition consistent with a sunflower oil, which appeared to have been coloured. In our view, $^{13}C$ NMR spectroscopy should be considered for use as a rapid initial method of analysis if a product is suspected of being fraudulently marketed as an olive oil. This preliminary investigation indicated also variations in the fatty acid content of individual cultivars from different regions in the Western Cape. This is supported by studies conducted elsewhere and strengthens the case for further NMR investigations to be carried out on a larger number and range of South African oils, so that proper statistical analysis can be conducted. Such studies are planned.

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