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NMR ANALYSIS OF ALDEHYDES IN SICILIAN EXTRA-VIRGIN OLIVE OILS BY DPFGSE TECHNIQUES

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ABSTRACT. The DPFGSE NMR sequences open new perspectives in the volatile compounds analysis of food matrices. Many fresh extra-virgin Sicilian olive oils, analyzed by this technique, show two main resonances in the aldehydic spectral region ($9 - 10$ ppm), at 9.18 and 9.58 ppm. The former was never reported so far, the latter was sometime highlighted as a minor aldehydic component signal of spectra showing stronger resonances at 9.45 and 9.70 ppm. Thermal treatment at 220°C of extra virgin olive oil samples lead to the complete transformation of the resonances at 9.18 and 9.58 ppm into those at 9.45 and 9.70 ppm in 50 minutes. Analogous transformation takes place place in $CDCl_3$ at rt in several weeks. These results suggest the transformation of relatively unstable compounds into thermodynamically more stable products whose resonances are commonly reported in the literature. Even though these chemical changes involve minimal amount of product, they are of crucial importance to define: i) organoleptic extra virgin olive oil properties; ii) fraudulent chemical or thermal treatment detection; iii) extra virgin oil ageing.

1. Introduction

Because of the remarkable success in last two decades, NMR (Nuclear Magnetic Resonance) spectroscopy can be considered part food science history [1]. Indeed despite the low sensitivity of NMR, which apparently limits detection of minor components in complex mixtures, the quick and wide data-acquisition can discriminate product features, quality and frauds [2]. This is demonstrated also by the constantly growing rate of publications in this area [3]. Experiments are acquired on both water soluble food derivates such as fruit juices [4], and on hydrophobic media, for instance, to characterize vegetable fat compositions [5]. Among the NMR possible applications in food science, the analysis of simple high-resolution spectra (in solution) is now flanked by relaxation times measurements of water in food [6], and by solid-state analyses [7]. In general, NMR applications are still limited by low sensitivity. Nonetheless they provide: a) non-variable experimental error (high reproducibility); b) simultaneous quantitative information on a broad group of chemical species; c) minimal sample pre-treatment [8]. These features encouraged us to apply this technique to analysis of olive oil. Olive oil is the hydrophobic phase obtained by squeezing the Olea Europea drupes. Because of its high quality among the vegetable

oils and its main role in the Mediterranean diet, it is probably the most studied product in Italy. The presence of interesting natural products, of antioxidant species, and the appropriate relative concentration of the main fatty acids, need suitable methods able to certify genuine origin, good quality, unmasking possible frauds. ^{1}H and ^{13}C NMR techniques provide different information: the ^{1}H NMR spectrum allows the measurement of minor components of olive oils such as β-sitosterol, hexanal, 2-(E)-hexenal, formaldehyde, squalene, cycloartenol and linolenic acid [9]; the ^{13}C NMR spectrum detects major components such as glycerol tri-esters of olive oils, defining also acyl composition and positional distribution on the glycerol moiety [10]. The main sensitivity concern about ^{1}H NMR analysis is the dynamic range [11], which makes the detection of signals below the threshold imposed by the ADC (analogue to digital converter) impossible. Therefore, in a standard extra virgin oil spectrum, trace component detection is limited to signals more intense than 10^{-5} times the intensity of the fatty acid CH_2 signals. Weak signals above the dynamic range threshold are in any case affected by severe baseline distortion. Selective pulses coupled to gradient spin-echo refocusing such as DPFGSE [12], in NMR modern instruments, allow these limitations to be circumvented, paving the way for the easy detection of minor components, especially those presenting resonances away from other interfering resonances. The power of these new sequences could be demonstrated by the detection of aldehydes, carotenoids or other specific minor components whose resonances fall in a relatively free region. Here, we present a specific DPFGSE analysis on the aldehydic components of some extra virgin olive oils, mostly responsible of the sensorial properties of extra virgin olive oil. Comparison with standard ^{1}HNMR spectra show tremendous improvement of quantitative and qualitative information with drastic instrumental time reduction.

2. Experimental

Six extra-virgin olive oil samples (*Arbequina 2009, Arbosana 2009, Mandanicese 2009, Triolo 2009, Cerasuola 2009* and *Cerasuola 2008*) coming from monovarietal sicilian cultivars were analyzed by NMR to assess features and differences. Samples were directly provided by producers within the province of Messina, together with the relative production cards where the percentage (100%) for each mentioned cultivar is certified. All the samples were milled in the 2009 oil campaign, except for the *Cerasuola 2008* sample (produced by the same trees of *Cerasuola 2009*), which was included among the analyzed oils to check both, the aging effect in optimal conditions, and the possible inter-year differences concerning the minor components (specifically aldehydes). According to most of the previous studies [5], we prepared samples by adding 700 μl of $CDCl_3$ and 20 μl of DMSO-d_6 to 20 μl of olive oil. However, provided there were not such remarkable differences, we also prepared 150 μl olive oil samples in 450 μl of $CDCl_3$ in order to enhance the sensitivity and simplify the analyzed system. Spectra were run on a Varian 300 and a Varian 500 spectrometers equipped with the gradient device. Beyond standard ^{1}H spectra, the DPFGSE (double pulsed field gradient spin echoes) together with relative 1D-NOESY and 1D-TOCSY versions were run on several olive oil samples. Major signal free results were analysed with Mestrec (version 4.9.9.6 for PC). Standard 1H spectra were run at 500 MHz with 4000 transients and 2 s as relaxation time, leading to 4 hours of experimental time; the ^{1}H-DPFGSE of the region $7.5 - 10.5$ ppm shown in Fig. 3 and Fig. 4 were run

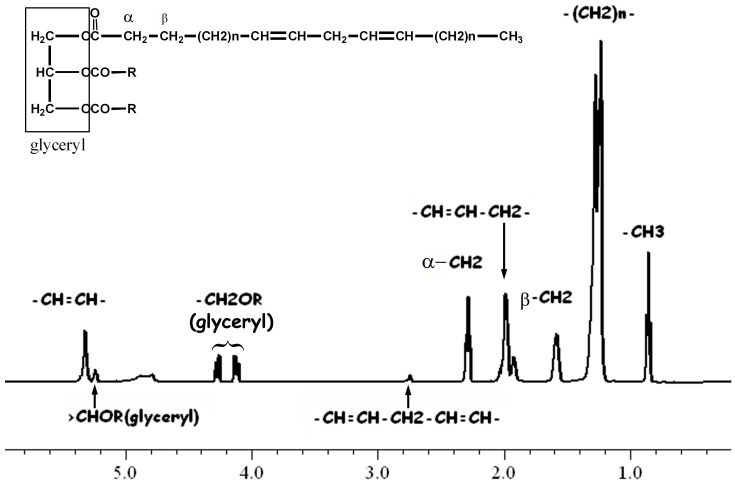

Figure 1. Main signal assignment in a ^{1}H spectrum of the *Arbequina 2009* olive oil.

with 128 transients and 2 s of relaxation delay in about 10 minutes. The 1D-TOCSY [13] experiments for any of the aldehydic species were run at 500 MHz in about 22 minutes with 256 transients, 2.5 s of acquisition time and 2.5 s of relaxation delay.

3. Discussion

Nowadays the typical main pattern of an extra-virgin olive oil ^{1}H-1D spectrum is well known as well as its differences from other vegetable oils [14]. Beyond the ready assignment of proton resonances belonging to major components (Fig. 1), it is possible to amplify the intensity scale to assign and detect resonances belonging to minor components (Fig. 2). To obtain reasonable results, NMR spectra have to be run with a high number of scans (the signal to noise ratio is proportional to the root square of this parameter) and careful baseline correction must be applied (this is fundamental for integration of smaller peaks). Alternatively, the DPFGSE sequence provides the chance to reduce experimental time improving spectral quality. Spectra of fresh Sicilian oil (*Mandanicese 2009*), run at 300 and 500 MHz in 10' of experimental time are reported in Fig. 3. Instead of the resonances previously reported, these spectra show two other bands at 9.18 and 9.58 ppm (Fig. 3). The former, to our knowledge, has never been reported so far. The latter appears in some papers as a relatively weak signal attributed to branched alkanals [15]. On the basis of this unexpected result, we extended our study over other Sicilian extra virgin olive oils. As shown in Fig. 4, the two resonances at 9.18 and 9.58 ppm are always present as important aldehydic components of the samples studied, sometimes flanked by the known 9.45 and 9.70 ppm signals. The sample comparison defines *Triolo 2009* as the poorest and *Mandanicese 2009* as the richest sample in aldehydic fraction. This is the reason why *Triolo 2009* shows the worst signal to noise ratio, whereas *Mandanicese 2009* (showing a well defined spectrum) was chosen as the representative sample used for further studies. The

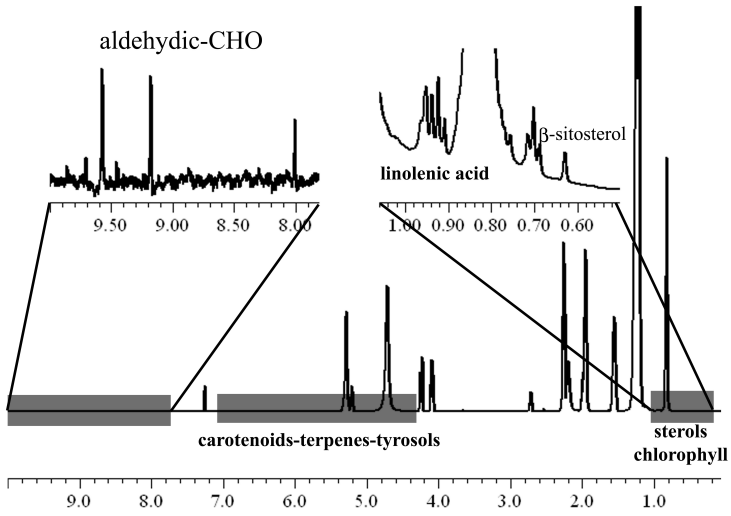

Figure 2. Selected expanded regions highlighting the presence of quantifiable species even just in traces. Reliable integration of the aldehydic region of a normal ^{1}H NMR spectrum requires about 4000 transients and about 4 hours of experimental time.

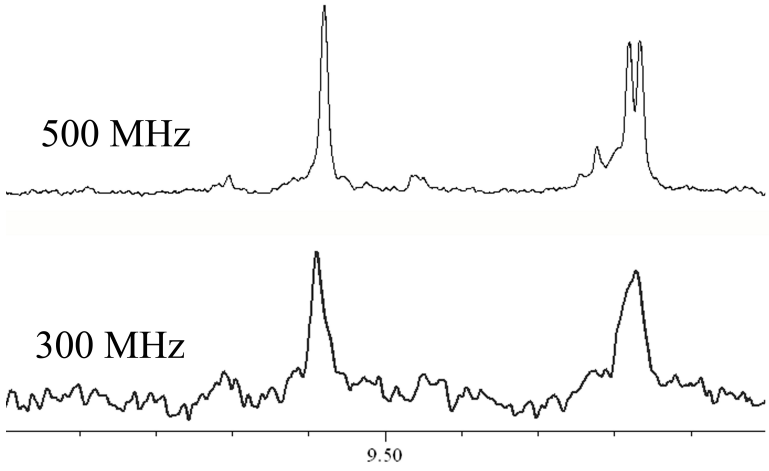

Figure 3. Expansion of the $9-10$ ppm region of the DPFGSE-^{1}H NMR spectra run respectively at 300 and 500 MHz. Spectra were acquired with 64 transients in about 10 minutes.

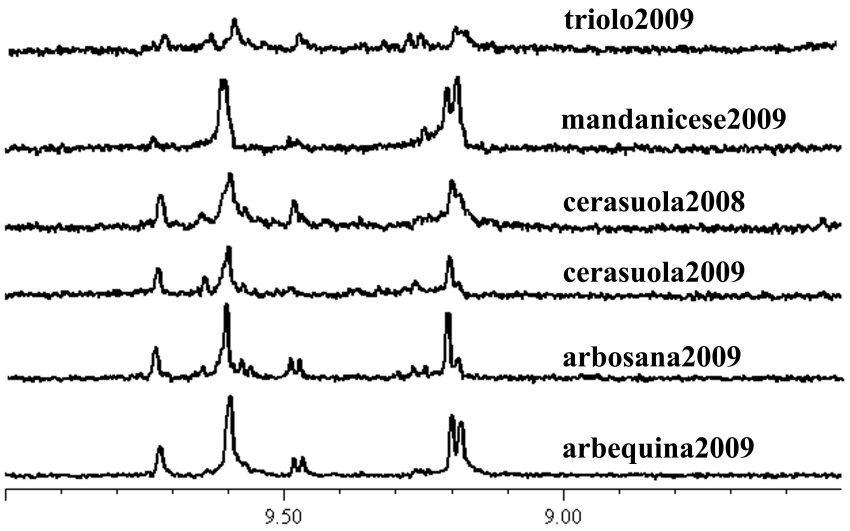

Figure 4. ^{1}H-DPFGSE spectra of six different extra-virgin Sicilian oil samples in $CDCl_3$ at 500 MHz.

comparison between the low- and the high-field spectra, proves that the band at about 9.2 ppm is due to, at list, two separate broad singlets coming from different aldehydes. Time monitoring of *Mandanicese 2009* olive oil in $CDCl_3$ at rt show a very slow decrease of the 9.18 and 9.58 ppm signals in favour of those at 9.45 and 9.70 ppm. Analogous monitoring of the pure olive oil heated at 220°C showed a similar trend leading to the complete disappearance of the former pair of signals in favour of the latter in less than 50 minutes (Fig. 5). These results suggest that the 9.18 and 9.58 ppm signals belongs to relatively unstable species slowly leading to the well known and stable pair of signals previously attributed to hexanal and 2-E-hexenal. The total integration of the aldehydic region shows an overall enhancement, though this is not a definite proof of the labile-to stable aldehyde transformation. Therefore, it is not clear whether the labile aldehydes transform into the others or not; however, some purification studies monitored by NMR are underway to understand if the thermal shock produces aldehydic compounds from the sole saponifiable fraction. To gain information on the nature of the whole set of aldehydic resonances, selective-shaped-pulse 1D-TOCSY were run on the *Mandanicese 2009* olive oil sample before and after thermal treatment. Our spectra undoubtedly show that the doublet at 9.45 ppm belongs to 2-E-hexenal; however, so far, we have not obtained unambiguous results concerning the nature of the other resonances (Fig. 6 and Fig. 7). Even though variations concerning the aldheydic component of an extra-virgin olive oil such as those described in this paper involve minimal change in composition, they play a crucial role in the sensory properties of the oil. Moreover, these chemical transformations might be useful as probe of fraudulent chemical or thermal treatment of olive oil as well as of its ageing-stage.

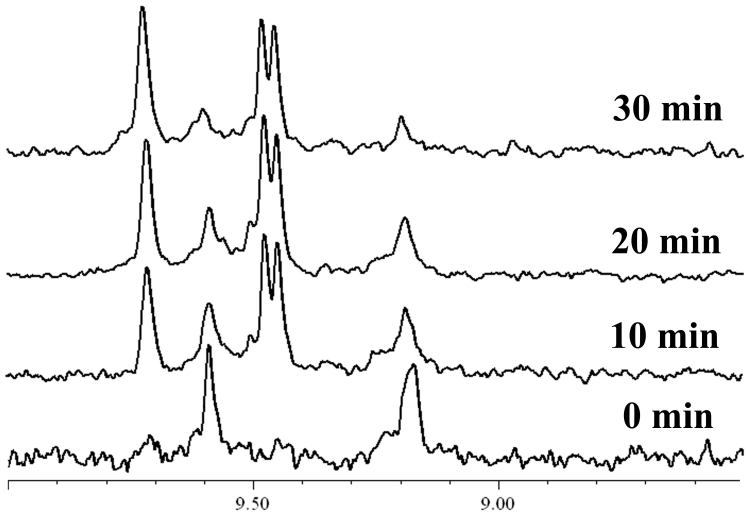

Figure 5. DPFGSE Spectra in $CDCl_3$ at 25°C after several oil heating times at 220°C (indicated).

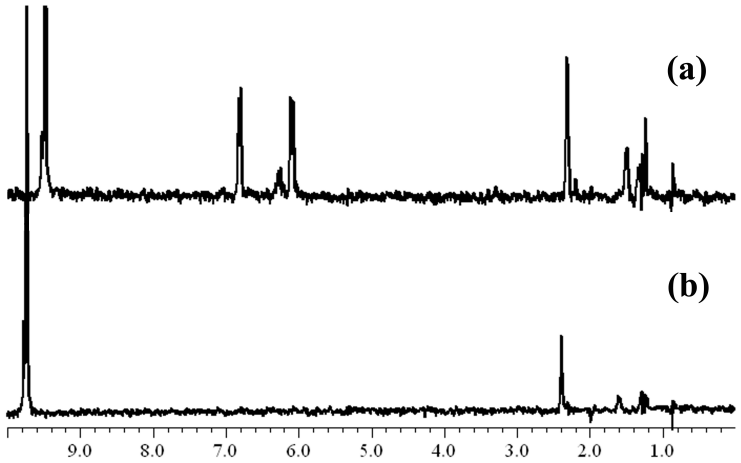

Figure 6. 1D-TOCSY spectra after drastic heating of the *Mandanicese 2009* sample in $CDCl_3$ with the selective excitation of the resonances at 9.45 (a) and 9.71 ppm (b). Comparison with the TOCSY spectra of pure standards shows that these compounds are 2-E-hexenal and hexanal, respectively.

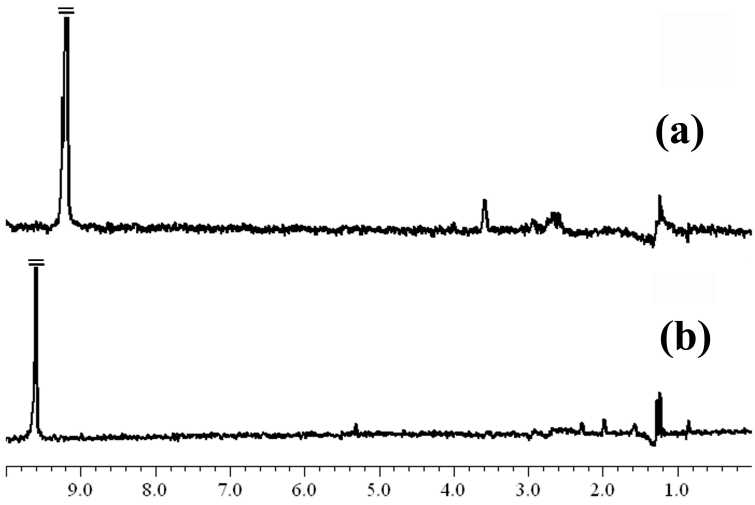

Figure 7. 1D-TOCSY spectra of the fresh *Mandanicese 2009* sample in $CDCl_3$ with the selective excitation of the resonances at 9.18 (a) and 9.58 ppm (b). All the non-excitation signals are very weak and not assigned with certainty so far.

4. Conclusions

The NMR analysis of the aldehydic fraction of Sicilian extra-virgin olive oils reveal, unexpectedly, the presence of new species. Preliminary results suggest that these compounds are not temperature and time stable. Therefore such NMR resonances could be used as probes of oil aging or rectification. Work is still in progress to characterize the new species, which are probably connected with the olive metabolism like the other, already known, aldehydes.

Acknowledgments

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