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Polyphenols Fingerprinting in Olive Oils Through Maximum-Quantum NMR Spectroscopy

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Abstract An NMR protocol based on multiple-quantum spectroscopy is presented for an analytical screening of polyphenols in olive oils. Three Italian olive oils with different total polyphenols content were used as study case. A total of 24 compounds were identified as follows: 1 polyphenol in the 5Q–1Q, 15 more in the 4Q–1Q, and 8 components in the 3Q–1Q spectra, consisting of organic phenols, secoiridoids, lignans, and flavonols. In the three Italian olive oils investigated here, the polyphenols profile turned out to be significantly different, with specific characteristics going beyond simple considerations based on the total polyphenol content. The approach presented here can be easily extended for rapid qualitative and semi-quantitative screening of the polyphenol composition in many food products.

Keywords Olive oil · Polyphenols · NMR spectroscopy · Multiple-quantum · MaxQ

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Introduction

Plant-derived natural products have potential applications in medicinal chemistry, by means of chemotherapeutic, chemopreventive, antioxidant properties, protection against cancer, resistance towards coronary heart diseases, and various other biological functions (Newman and Cragg 2007; Yang et al. 2007). Therefore, the analytical description of plant extracts rich in polyphenols is of considerable interest for their classification and for understanding structure-activity relationships, which can guide the synthesis of analogue compounds with improved bio-availability, stability, nutritional quality, potency, and specificity (Quideau et al. 2011; Duthie et al. 2000; Ferguson 2001). Among foodstuff, dietary polyphenols from olive oil have been a large part of discussions, notably in the Mediterranean realm and its vast diversity of locally grown olives (Yang et al. 2007; Gutfinger 1981; Tsimidou et al. 1992; Aparicio and Luna 2002; Vinha et al. 2005; Haddada et al. 2007; Ocakoglu et al. 2009). Several classes of phenolic molecules (e.g., simple phenols, secoiridoids, lignans, and flavonoids) impart sensory and nutritional quality of olive oils, while providing multiple pharmacological properties (Bendini et al. 2007; Franco et al. 2014; Visioli et al. 1998). The total polyphenol content and the polyphenol profiling of an olive oil depend on many factors such as olive cultivar, geographical origin, the production, processing and storage techniques, and the olive oil age. While the total polyphenol content provides a quality gauge, identification of individual phenols can be useful as a fingerprint to characterize typical extra virgin olive oils. The most commonly used chemical and analytical methods applied to this purpose have been reviewed recently (Ignat et al. 2011).

As a technique of choice, NMR has been applied either on its own or as a chromatographic detector (LC/NMR) for the characterization of olive oils (Sacchi et al. 1998;

Christophoridou and Dais 2009; Christophoridou et al. 2005; Mannina et al. 2001, 2002, 2010; Alonso-Salces et al. 2010; Fuentes De Mendoza et al. 2013; Bastoni et al. 2001; Longobardi et al. 2012; Camin et al. 2016; Mannina and Sobolev 2011). Direct NMR approaches, although technically easier to setup and perform than hyphenated techniques, can encounter bottlenecks in the study of complex mixtures, mainly stemming from spectral overlaps. By increasing the spectral resolution, multidimensional correlation NMR experiments significantly aid the spectral analyses of mixtures (Lin and Shapiro 1997). This has been demonstrated recently in the case of polyphenols (Charisiadis et al. 2017). For instance, NMR-based diffusion experiments (Johnson 1999; Nilsson et al. 2004; Reddy et al. 2013), alone or in combination with other NMR techniques, can greatly enhance the spectral simplification (Barjat et al. 1998; Viel and Caldarelli 2008). More recently, multiple-quantum (MQ) NMR, an experiment originally introduced at the beginning of 2D NMR (Munowitz and Pines 1986; Ernst and Bodenhausen 1990), has found new life for the structural analysis (Baishya et al. 2008; Manjunatha Reddy et al. 2009a, b) and the characterization of mixtures of small molecules (Dalvit and Bohlen 1997; Martineau et al. 2011, 2012) (Reddy et al. 2013, Guennec et al. 2015, Reddy and Caldarelli 2010, 2011, 2012; Piotto et al. 2011; Manjunatha Reddy and Caldarelli 2013; Manjunatha Reddy et al. 2016). In this latter case, the highest possible excitable MQ coherence (also referred to as maximum-quantum; MaxQ) order has shown intriguing potential for the speciation of mixtures, particularly of aromatic molecules. With respect to other popular 2D NMR methods, such as HSQC or TOCSY, the MaxQ NMR protocol is particularly apt to speciate multicomponent mixtures since it identifies in succession molecular fragments as a function of their number of protons. MaxQ NMR has been successfully applied for the characterization of aromatic region of the ^{1}H NMR spectra in a mixture of mono- and polyaromatic hydrocarbons (Reddy and Caldarelli 2010), phenolic compounds in both a model mixture and in the polar part of an olive oil extract (Reddy and Caldarelli 2011) and environmentally relevant molecules (Reddy and Caldarelli 2012). The enhancement in spectral resolution of the MaxQ experiment stems from a dramatic reduction in the number of signals, which is due to two factors. Firstly, only molecular fragments of mutually coupled protons can produce MQ signals, thus providing a first filter (such as the commonly employed double-quantum filter for removing singlets). Then, the higher the observed MQ order, the fewer are the number of signals, with the limit case of a simple singlet for the MaxQ order. For example, in a network of five-coupled spins, there are a maximum of nearly 200 NMR signals that can be compressed into a singlet by observing the five-quantum order, a two-order magnitude reduction in the spectral complexity. This facilitates the identification of the molecules in the analyzed mixture. Since molecular fragments do not possess the same number of coupled protons, several orders must be recorded in principle to obtain a full analysis. For polyphenolics, for instance, MQ can vary between 1 and 5. MaxQ NMR can be employed, in principle, to any case where a detectable MaxQ coherence order can be excited. Even higher resolution could be achieved by including the MaxQ approach in 3D NMR experiments, and the advantage/cost ratio for going to these more complex techniques should be evaluated on a case-by-case basis (Manjunatha Reddy et al. 2016). Quantitative protocols were also established within the context of MaxQ, which requires the use of calibration curves, but their implementation was outside the scope of this paper (Reddy and Caldarelli 2012). Indeed, here we present a MaxQ NMR analysis for profiling polyphenols in extra virgin olive oils as a complement to total polyphenol content assessment. Although the polyphenols content and speciation may depend on the geographical origin, extraction methods, and processing and storage techniques, it was suggested that commonly encountered polyphenols such as lignans, ligstroside, and tyrosol derivatives are expected to be in relatively higher concentrations (Christophoridou et al. 2005) and thus dominate the MQ spectra as well. Since the NMR spectral parameters for these molecules exist in the literature, the MaxQ-NMR signals can be annotated accordingly, along the lines of a previously proof-of-principle work (Christophoridou and Dais 2009).

Materials and Methods

Samples

Three Italian extra virgin olive oils (batches I, II, and III) with different polyphenols content were provided by UNAPROL (Italian Consortium of Olive Oils). The polyphenol content as determined by UNAPROL according to a standard methodology (Montedoro et al. 1992) was of 595, 109, and 237 mg/kg of oil for batches I, II, and III, respectively.

The three olive oils were subjected to a liquid/liquid extraction procedure (Montedoro et al. 1993) using a binary solvent mixture, water/methanol, 20/80 *v*/*v* (Lesage-Meessen et al. 2001) 16 g of olive oil, in each case, was mixed with 16 mL of solvent and stirred in a vortex for about 20 min (three times) and centrifuged at 5000 rpm for about 10 min (three times), and the methanol fraction was collected. Methanol was evaporated under vacuum at 60 °C, and the residue was washed with 16 mL of hexane (three times). Hexane was then evaporated at 30 °C, and a solid residue was obtained. Three individual samples were prepared by dissolving 24.8, 15.0, and 14.6 mg of batches I, II, and III samples respectively in 500 μL of methanol-*d*.

NMR Spectroscopy

For all the extracts, the ^{1}H (500 MHz) one-pulse and two-dimensional MaxQ–1Q correlation NMR experiments were performed using a Bruker Avance III 500 MHz NMR spectrometer equipped with a TXI cryogenic probe. For one-pulse experiments, 32 co-added transients were acquired using a relaxation delay of 4 s to an overall experimental time of 3 min. For MaxQ experiments, a three-pulse sequence, $(\pi/2)$-$(\tau/2)$-$(\pi/2)$-$(\tau/2)$-$(\pi/2)$, was used to excite multiple-quantum transitions where τ was allowed to vary for optimal signal uniformity and sensitivity. Optimal τ values were found to be 220 ms for 3Q and 110 ms for 4Q excitation. No search for the optimal MQ excitation efficiency was performed (Köcher et al. 2016), for economy of time. Two-dimensional MaxQ spectra were acquired with 512 FIDs, each with 16 co-added transients, corresponding to a FID resolution of 9.76 × 0.625 Hz (p = 4) and 11.72 × 0.67 (p = 3), respectively along MaxQ and 1Q axes. Coherence selection was obtained through two half-sine-shaped pulsed field gradients placed before and after the reconversion pulse in a ratio of 1:3 and 1:4, respectively, for 3Q and 4Q coherences. For each sample batch, the total experimental time was about 16 h (8 h for each MaxQ-order). The MaxQ spectra were zero-filled to a 1024 × 2048 (1Q × MaxQ) data matrix and weighed with sine bell window functions. All spectra are displayed in magnitude mode. The MaxQ dimension was scaled by the p-quantum order in order to provide a homogeneous display along the MQ series. Spectral assignments were carried out based on the 1Q chemical shifts and on the multiplet structure.

Results and Discussion

Figure 1 illustrates one-pulse ^{1}H spectra of three batches of olive oil extracts in methanol-d_4 plotted on top of each other for a comparison of aliphatic, aromatic, and aldehydes signals. Inspection of the aliphatic signals proves that, while the extracts are dramatically enriched in polyphenols, a few other molecules were present in low (and variable) content. Notice the spectral complexity in the aromatic spectral region, whereby signals from several hundreds of protons resonate in a spectral span of about 1 ppm, which makes it hard to distinguish the nature of polyphenols between the sample batches.

Figures 2, 3, and 4 illustrate the full and expanded views of MQ (p = 3–5) spectra recorded for the three olive oils, along with the numbering for the identified spin systems. The indirect dimension was plotted with a scale in which the axis is reduced by the p-quantum order, for numerical homogeneity and the sake of an easier comparison along the pQ series (Reddy and Caldarelli 2010). In this scale, the singlet corresponding to the maximum-quantum order appears at the weighed average of signals of the corresponding 1Q trace (i.e., the sum of the chemical shifts of the resonance times their integral, divided by the total integral). This is best exemplified by the peak at 6.99 ppm in the 5Q spectra of extracts of batches I and III. The numbering corresponds to the annotation as reported in Table 1, and the chemical structures (simple phenols, lignans, flavonols, and secoiridoids) are shown in Fig. 5. A visual comparison of the spectra in Fig. 2 suggests, to a first approximation, some qualitative variation in the speciation of the polyphenols content, which will be discussed more in details below. Note that increased noise level in the indirect dimension in the 4Q spectrum of the batch I sample can be linked to solid microparticles suspended in the sample that could not be filtered out.

5Q–1Q NMR Correlation Spectra

Signals in these spectra arise only from monosubstituted benzene rings. Figure 2 shows that only one 5Q signal (compound 1) was observed, which could not be assigned to a known phenolic molecule on the basis of existing databases. This molecule was detected in batches I and III, but not in batch II. Qualitatively, the evolution of the intensity of this peak reflects the observed total concentration in polyphenolics of the three batches.

4Q–1Q NMR Correlation Spectra

Line shapes in these spectra are of two kinds, both possessing at least four coupled protons: singlets (MaxQ signals) in the indirect, MQ dimension for disubstituted benzene rings, and multiplets (non-MaxQ signals) for monosubstituted rings. In general, but not in all cases, non-MaxQ signals do not carry new information on the sample composition, as the associated molecule could be more easily identified in MQ spectra of a higher order. Expanded regions of the 4Q–1Q spectra of three different sample batches are given in Fig. 3. The right-hand side panels are zoomed out to a factor 16 for a better visual representation of spectral resolution. Fourteen molecular components were identified in the 4Q–1Q correlation spectrum (see Fig. 5 for chemical structures). Due to the presence of magnetically equivalent protons, 1, 4-disubstituted six-membered aromatic molecules have a relatively better signal to noise ratio in their 4Q spectra as compared to 1,2 and 1,3 disubstituted analogues (Manjunatha Reddy and Caldarelli 2013). It has to be noted that the relative signal intensities are also sensitive to the choice of the 4Q excitation duration in the pulse sequence (Manjunatha Reddy and Caldarelli 2013). The spectral resolution obtained for the 4Q spectra allows to distinguish seven MaxQ signals in less than 0.04 ppm (25 Hz) in the 4Q dimension and about half a ppm in the 1Q dimension. In other words, about 28 protons

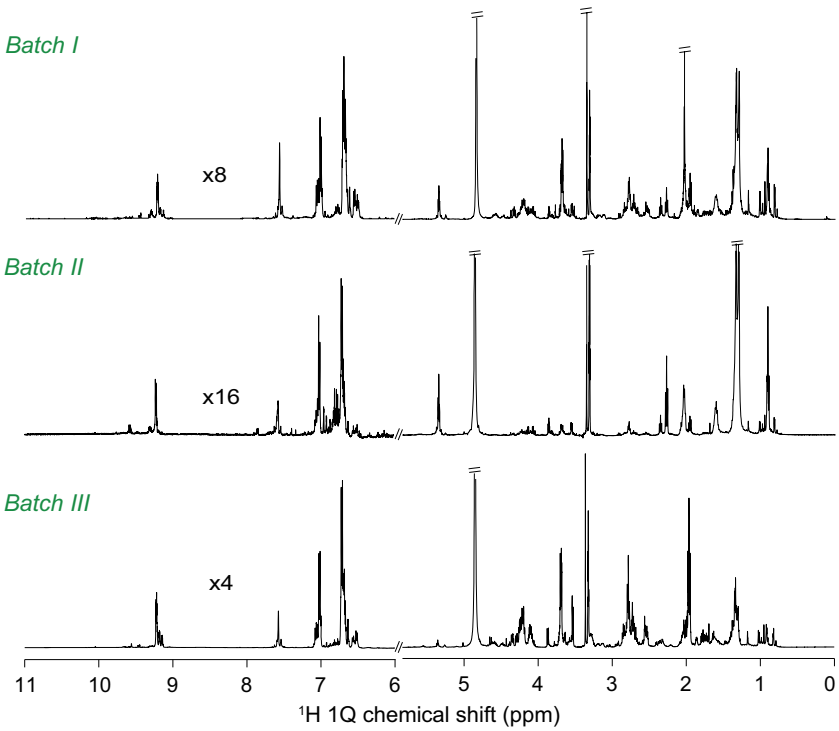

Fig. 1 ^{1}H (500 MHz) one-pulse NMR spectra of three different olive oil extracts in methanol-*d*. Thirty-two co-added transients were acquired in each case to an overall experimental time of 3 min. The aromatic and aldehydes spectral regions are magnified for a better visual appreciation

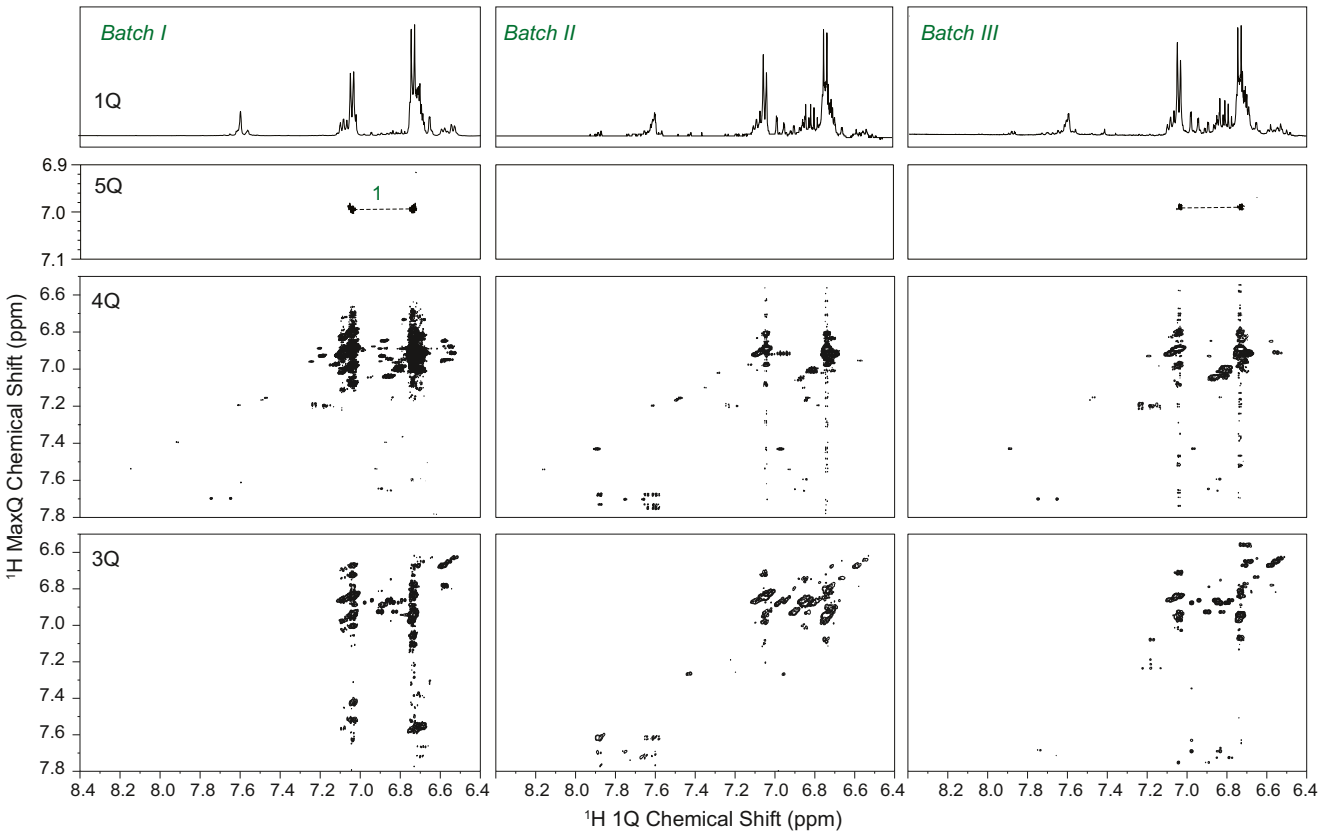

Fig. 2 ^{1}H (500 MHz) one pulse NMR spectra followed by two-dimensional 5Q–1Q, 4Q–1Q, and 3Q–1Q correlation NMR spectra. For multiple-quantum experiments, 16 transients were co-added for each of 512 t_1 increments corresponding to a total experimental time of 16 h using a relaxation delay of 2 s. The spectra are displayed in magnitude mode, and the MaxQ dimension is plotted with the reduced scale described in the text

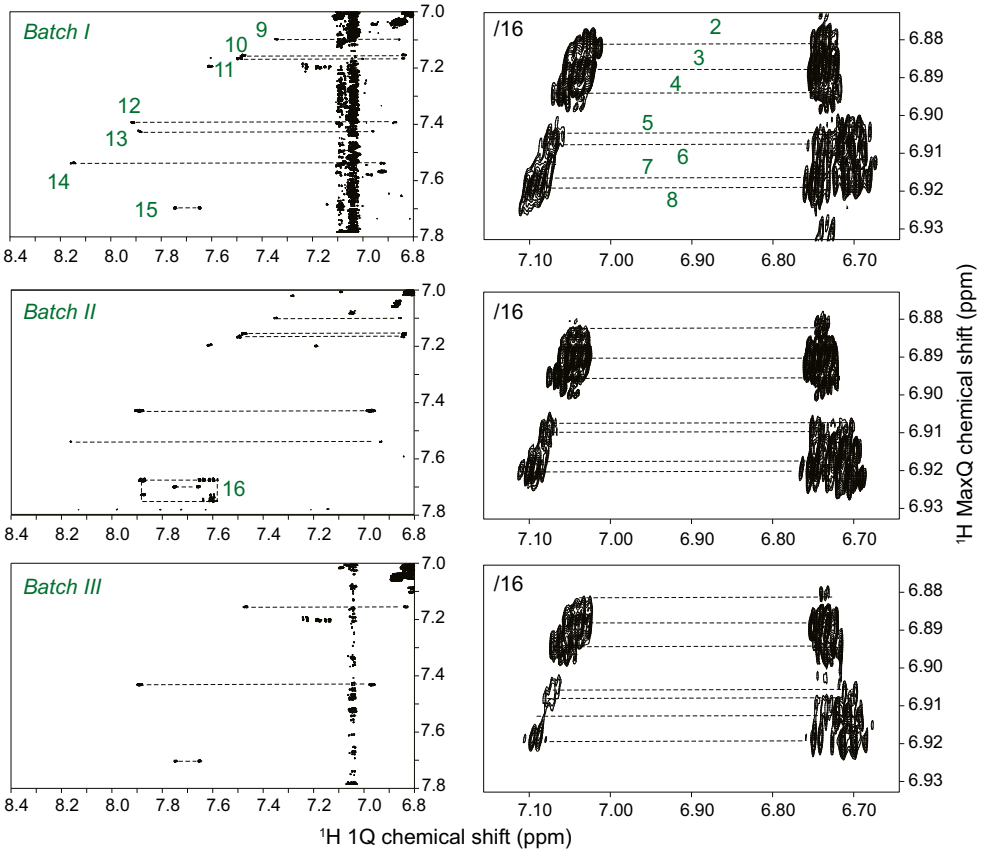

Fig. 3 Expanded regions of ^{1}H 4Q–1Q correlation NMR spectra showing the annotation of some of the molecular components. Relatively low intense peaks are spread up to 2 ppm (left) and the signals with higher intensities are resonating in a narrow spectral region in less than half a ppm (right-hand side, spectral intensity is downscaled to a factor 16). All spectra are displayed in magnitude mode, and the MaxQ dimension is plotted with the reduced scale described in the text. See Table 1 for the details of the annotation and Fig. 5 for the corresponding molecular structures

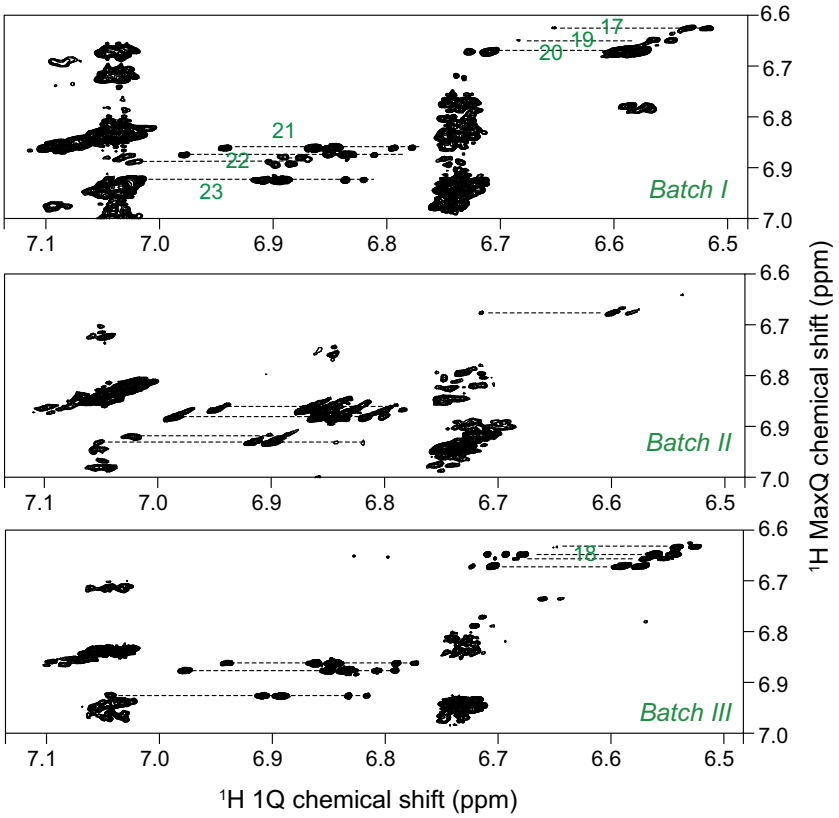

Fig. 4 Expanded regions of ^{1}H 3Q–1Q correlation NMR spectra, depicting the identification of MaxQ signals of trisubstituted polyphenols. All spectra are displayed in magnitude mode, and the MaxQ dimension is plotted with a reduced scale. See Table 1 for spectral annotation and Fig. 5 for molecular structures

Table 1 MaxQ NMR chemical shifts together with 1Q chemical shifts for a direct comparison of the polyphenols content in three Italian olive oils (batches I, II, III) are listed

No.	Compound	δ_{1Q} ppm	MQ spectrum	$\delta_{Max\text{-}Q}$ ppm		
				Batch I	Batch II	Batch III
1	Unknown	7.02, 6.95	5Q	6.99	–	6.99
2	Tyrosol	7.02, 6.71	4Q	6.87	6.87	6.87
3	Hemiacetal of the dialdehidic form of ligstroside lacking carboxymethyl group	7.03, 6.71	4Q	6.87	6.87	6.87
4	Dialdehidic form of ligstroside lacking carboxymethyl group	7.03, 6.72	4Q	6.88	6.88	6.88
5	4-Hydroxyphenylacetic acid	7.04, 6.73	4Q	6.88	6.88	6.88
6	Tyrosol acetate	7.05, 6.72	4Q	6.89	6.89	6.89
7	Aldehydic form of ligstroside	7.06, 6.72	4Q	6.89	6.89	6.89
8	Ligstroside aglycon	7.01, 6.71	4Q	6.90	6.90	6.90
9	Unknown	6.86, 7.34	4Q	7.10	7.10	–
10	*p*-Coumaric acid	6.84, 7.48	4Q	7.16	7.16	7.16
11	Unknown	6.85, 7.49	4Q	7.17	7.17	–
12	Apigenin	6.86, 7.92	4Q	7.39	7.39	7.39
13	4-Hydroxybenzoic acid	6.97, 7.88	4Q	7.43	–	–
14	Unknown	6.92, 8.16	4Q	7.54	7.54	–
15	Unknown	7.75, 7.65	4Q	7.70	7.70	7.70
16	*t*-Cinnamic acid[a]	7.43, 7.58, 7.9	4Q	–	7.65–7.75[a]	–
17	Oleuropine aglycon	6.65, 6.70, 6.53	3Q	6.63	–	6.63
18	Hydroxytyrosol	6.68, 6.70, 6.56	3Q	–	–	6.64
19	Aldehydic form of oleuropein	6.69, 6.72, 6.58	3Q	6.65	6.65	6.65
20	Hydroxytyrosol acetate	6.69, 6.71, 6.58	3Q	6.68	–	6.68
21	Pinoresinol	6.94, 6.77, 6.81	3Q	6.84	6.84	6.84
22	Acetoxy pinorecinol	6.81, 6.82, 6.87 6.91, 7.00, 6.75	3Q 3Q	6.83 6.88	6.83 6.88	6.83 6.88
23	Caffeic acid	7.07, 6.99, 6.80	3Q	6.95	6.95	–
24	Luteolin	6.95 (1), 7.43 (2)	3Q	–	7.27	–

[a] Obtained from non-MaxQ signals in the 4Q and 3Q spectra

resonating in two distinct multiplets were identified. Neglecting the effect of intermolecular interactions on chemical shifts in the polyphenolic mixtures studied here, we assigned NMR resonances in this crowded region to tyrosol (2 and 6) and ligstroside (3, 4, 7, and 8) derivatives based on their 1Q chemical shifts in NMR databases obtained in previous studies (Christophoridou and Dais 2009; Christophoridou et al. 2005). Indeed, these commonly observed polyphenols have very similar chemical structures (Fig. 5), so that their NMR signals resonate in a narrow spectral region (6.7 to 7.1 ppm). 4-Hydroxyphenylacetic acid (5) was the last molecule identified in this spectral region. Seven more molecular components were found at relatively weaker intensities (Fig. 3, left column), three of which could be assigned to *p*-coumaric acid (10), apigenin (12), and 4-hydroxybenzoic acid (13). The remaining four molecular components could not be determined on the basis of the literature. Finally, a non-MaxQ signal (i.e., a multiplet in the *p*Q dimension) was observed in the 4Q spectrum of batch II, not related to any resonance measured in the 5Q spectrum. Nonetheless, it was possible to identify this compound on the basis of its 1Q signals to *t*-cinnamic acid, a monosubstituted phenolic molecule. The absence of its signal in the 5Q spectrum is most likely due to a non-optimal choice of the sequence parameters.

3Q–1Q Correlation NMR Spectra

In analogy to what was explained for the previous spectra, these spectra encompass singlets, MaxQ signals from trisubstituted benzenes and multiplets from di- and monosubstituted benzenes. Expansions of the 3Q–1Q correlation spectra are depicted in Fig. 4. Here, together with the 3Q signals, correlations appear from molecular fragments, with four and five spin networks already detected in the form of non-MaxQ signals, and can be easily recognized. Seven more molecular fragments were identified

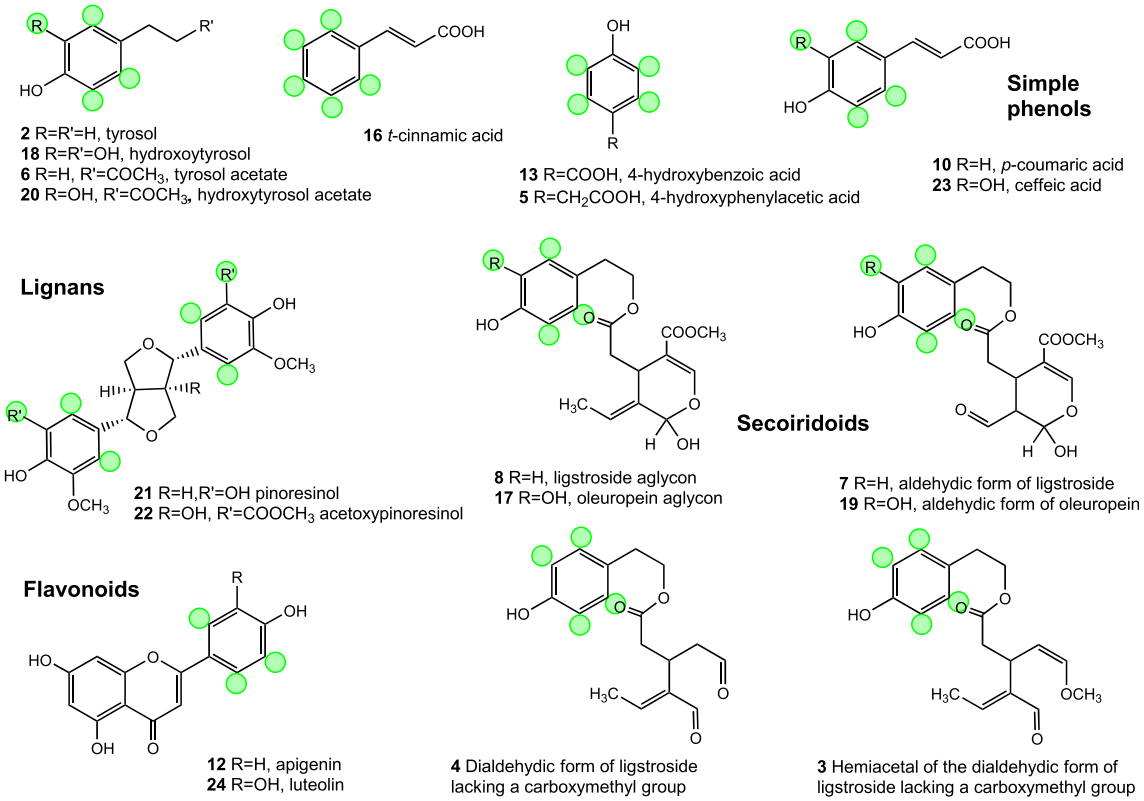

Fig. 5 Chemical structures of polyphenols identified in olive oil extracts; simple phenols (2, 5, 6, 10, 13, 16, 18, 20, and 23), lignans (21 and 22), flavonols (12 and 24), and secoiridoids (3, 4, 7, 8, 17, and 19). Green-color circles represent the ^{1}H coupled-spin networks.

in the 3Q–1Q correlation spectra, namely, oleuropein aglycon (17), hydroxytyrosol (18), aldehydic form of oleuropein (19), hydroxytyrosol acetate (20), (+)-pinoresinol (21), (+)-1-acetoxypinoresinol (22), caffeic acid (23), and luteolin (24). This corresponds to an assignment of 24 proton chemical shifts in eight different molecules. Table 1 summarizes the qualitative polyphenols profiles of the olive oils studied here. As previously mentioned, the overall polyphenols content but even more its profile in terms of actual molecular species can depend on many different factors. In the case of the investigated olive oils, in batch I, which contains the highest total polyphenols content, 21 compounds were observed whereas there were 19 in batch II and 17 in batch III which has the lowest total phenol content. More specifically, 13 compounds were common to all three samples (2–8, 10, 12, 15, 21, and 22). Indeed, as described in previous studies, *p*-coumaric acid, apigenin, lignans, tyrosol, and ligstroside derivatives are major components in fresh olive oils. Concerning the compounds more specific to each batch, 4-hydroxybenzoic acid is present in batch I only whereas oleuropein aglycon, hydroxytyrosol acetate and an unknown structure (1) are present in batches I and II. In addition, signals of *t*-cinnamic acid and luteolin were found in batch II only. Specifically absent from the batch II sample were caffeic acid and three unknown structures (9, 11 and 14).

It can be concluded that MaxQ NMR analyses provide a fine characterization of the polyphenol content in extra virgin olive oils (EVOOs), as exemplified in three sample olive oils from Italy. Focusing on the signal of the six-membered aromatic rings, a series of spectra ranging from 5Q to 3Q allowed similarities and differences in polyphenol content to be determined.

Conclusions

To summarize, MaxQ NMR is capable of providing information on the qualitative and semi-quantitative polyphenol profile for olive oils. This approach is easy to implement, reasonably fast, and sensitive. Most importantly, it does not require a physical separation of the polyphenols. It can be anticipated that the information obtained by this technique could be applied to the study of olive oil aging or adulteration, by monitoring temporal charges of specific polyphenols as a function of chemical and biological processes. MaxQ NMR databases could be easily built for the further expansion of applicability of this method to probe polyphenols in various other mixtures of natural origin. Thus, application to the analysis of the polyphenol content in other foodstuff is a natural extension of the method.

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Conflict of Interest G. N. Manjunatha Reddy declares that he has no conflict of interest. Luisa Mannina declares that she has no conflict of interest. Anatoly P. Sobolev declares that he has no conflict of interest. Stefano Caldarelli declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

References

Alonso-Salces RM, Moreno-Rojas JM, Holland MV, Reniero F, Guillou C, Kr H (2010) Virgin olive oil authentication by multivariate analyses of 1H NMR fingerprints and δ13C and δ2H data. J Agric Food Chem 58:5586–5596. https://doi.org/10.1021/jf903989b

Aparicio R, Luna G (2002) Characterisation of monovarietal virgin olive oils. Eur J Lipid Sci Technol 104:614–627. https://doi.org/10.1002/1438-9312(200210)104:9/10%3C614::AID-EJLT614%3E3.0.CO;2-L

Baishya B, Reddy GNM, Prabhu UR, Row TNG, Suryaprakash N (2008) Simplifying the complex 1H NMR spectra of fluorine-substituted benzamides by spin system filtering and spin-state selection: multiple-quantum−single-quantum correlation. J Phys Chem A 112:10526–10532. https://doi.org/10.1021/jp8055174

Barjat H, Morris GA, Swanson AG (1998) A three-dimensional DOSY–HMQC experiment for the high-resolution analysis of complex mixtures. J Magn Reson 131:131–138. https://doi.org/10.1006/jmre.1997.1332

Bastoni L, Bianco A, Piccioni F, Uccella N (2001) Biophenolic profile in olives by nuclear magnetic resonance. Food Chem 73:145–151. https://doi.org/10.1016/S0308-8146(00)00250-8

Bendini A, Cerretani L, Carrasco-Pancorvo A, Gomez-Caravaca A-M, Segura-Carretero A, Fernandez-Gutierrez A et al (2007) Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. Molecules 12:1679–1719

Camin F, Pavone A, Bontempo L, Wehrens R, Paolini M, Faberi A et al (2016) The use of IRMS, 1H NMR and chemical analysis to characterise Italian and imported Tunisian olive oils. Food Chem 196:98–105. https://doi.org/10.1016/j.foodchem.2015.08.132

Charisiadis P, Kontogianni VG, Tsiafoulis CG, Tzakos AG, Gerothanassis IP (2017) Determination of polyphenolic phytochemicals using highly deshielded –OH 1H-NMR signals. Phytochem Anal 28:159–170. https://doi.org/10.1002/pca.2656

Christophoridou S, Dais P (2009) Detection and quantification of phenolic compounds in olive oil by high resolution 1H nuclear magnetic resonance spectroscopy. Anal Chim Acta 633:283–292. https://doi.org/10.1016/j.aca.2008.11.048

Christophoridou S, Dais P, Tseng L-H, Spraul M (2005) Separation and identification of phenolic compounds in olive oil by coupling high-performance liquid chromatography with postcolumn solid-phase extraction to nuclear magnetic resonance spectroscopy (LC-SPE-NMR). J Agric Food Chem 53:4667–4679. https://doi.org/10.1021/jf040466r

Dalvit C, Bohlen JM (1997) Analysis of biofluids and chemical mixtures in non-deuterated solvents with H-1 diffusion-weighted PFG phase-sensitive double-quantum NMR spectroscopy. NMR Biomed 10:285–291. https://doi.org/10.1002/(sici)1099-1492(199709)10:6<285::aid-nbm485>3.0.co;2-1

Duthie GG, Duthie SJ, Kyle JAM (2000) Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. Nutr Res Rev 13:79–106

Ernst RR, Bodenhausen G, Wokaun A (1990) Principles of Nuclear Magnetic Resonance in One and Two Dimensions. Clarendon Press

Ferguson LR (2001) Role of plant polyphenols in genomic stability. Mutat Res, Fundam Mol Mech Mutagen 475:89–111. https://doi.org/10.1016/S0027-5107(01)00073-2

Franco MN, Galeano-Díaz T, López Ó, Fernández-Bolaños JG, Sánchez J, De Miguel C et al (2014) Phenolic compounds and antioxidant capacity of virgin olive oil. Food Chem 163:289–298. https://doi.org/10.1016/j.foodchem.2014.04.091

Fuentes de Mendoza M, De Miguel GC, Marín Expóxito J, Sánchez Casas J, Martínez Cano M, Martín Vertedor D et al (2013) Chemical composition of virgin olive oils according to the ripening in olives. Food Chem 141:2575–2581. https://doi.org/10.1016/j.foodchem.2013.05.074

Guennec AL, Giraudeau P, Caldarelli S, Dumez J-N (2015) Ultrafast double-quantum NMR spectroscopy. Chem Commun (Camb) 51:354–357. https://doi.org/10.1039/c4cc07232d

Gutfinger T (1981) Polyphenols in olive oils. J Am Oil Chem Soc 58:966–968. https://doi.org/10.1007/BF02659771

Haddada FM, Manai H, Daoud D, Fernandez X, Lizzani-Cuvelier L, Zarrouk M (2007) Profiles of volatile compounds from some monovarietal Tunisian virgin olive oils. Comparison with French PDO. Food Chem 103:467–476. https://doi.org/10.1016/j.foodchem.2006.08.023

Ignat I, Volf I, Popa VI (2011) A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. Food Chem 126:1821–1835. https://doi.org/10.1016/j.foodchem.2010.12.026

Johnson CS Jr (1999) Diffusion ordered nuclear magnetic resonance spectroscopy: principles and applications. Prog Nucl Magn Reson Spectrosc 34:203–256. https://doi.org/10.1016/S0079-6565(99)00003-5

Köcher SS, Heydenreich T, Zhang Y, Reddy GNM, Caldarelli S, Yuan H et al (2016) Time-optimal excitation of maximum quantum coherence: physical limits and pulse sequences. J Chem Phys 144:164103. https://doi.org/10.1063/1.4945781

Lesage-Meessen L, Navarro D, Maunier S, Sigoillot JC, Lorquin J, Delattre M et al (2001) Simple phenolic content in olive oil residues as a function of extraction systems. Food Chem 75:501–507. https://doi.org/10.1016/S0308-8146(01)00227-8

Lin M, Shapiro MJ (1997) Mixture analysis by NMR spectroscopy. Anal Chem 69:4731–4733. https://doi.org/10.1021/ac970594x

Longobardi F, Ventrella A, Napoli C, Humpfer E, Schütz B, Schäfer H et al (2012) Classification of olive oils according to geographical origin by using 1H NMR fingerprinting combined with multivariate analysis. Food Chem 130:177–183. https://doi.org/10.1016/j.foodchem.2011.06.045

Manjunatha Reddy GN, Caldarelli S (2013) Improved excitation uniformity in multiple-quantum NMR experiments of mixtures. Magn Reson Chem 51:240–244. https://doi.org/10.1002/mrc.3938

Manjunatha Reddy GN, Guru Row TN, Suryaprakash N (2009a) Discerning the degenerate transitions of scalar coupled 1H NMR spectra: correlation and resolved techniques at higher quantum. J Magn Reson 196:119–126. https://doi.org/10.1016/j.jmr.2008.10.018

Manjunatha Reddy GN, Nayak SK, Guru Row TN, Suryaprakash N (2009b) Proton NMR studies of dihalogenated phenyl benzamides: two-dimensional higher quantum methodologies. Magn Reson Chem 47:684–692. https://doi.org/10.1002/mrc.2449

Manjunatha Reddy GN, Yemloul M, Caldarelli S (2016) Combined maximum-quantum and DOSY 3D experiments provide enhanced resolution for small molecules in mixtures. Magn Reson Chem n/a-n/a. https://doi.org/10.1002/mrc.4465

Mannina L, Sobolev AP (2011) High resolution NMR characterization of olive oils in terms of quality, authenticity and geographical origin. Magn Reson Chem 49:S3–S11. https://doi.org/10.1002/mrc.2856

Mannina L, Patumi M, Proietti N, Bassi D, Segre AL (2001) Geographical characterization of Italian extra virgin olive oils using high-field 1H NMR spectroscopy. J Agric Food Chem 49:2687–2696. https://doi.org/10.1021/jf001408i

Mannina L, Dugo G, Salvo F, Cicero L, Ansanelli G, Calcagni C et al (2002) Study of the cultivar−composition relationship in Sicilian olive oils by GC, NMR, and statistical methods. J Agric Food Chem 51:120–127. https://doi.org/10.1021/jf025656l

Mannina L, Marini F, Gobbino M, Sobolev AP, Capitani D (2010) NMR and chemometrics in tracing European olive oils: the case study of Ligurian samples. Talanta 80:2141–2148. https://doi.org/10.1016/j.talanta.2009.11.021

Martineau E, Giraudeau P, Tea I, Akoka S (2011) Fast and precise quantitative analysis of metabolic mixtures by 2D H-1 INADEQUATE NMR. J Pharm Biomed Anal 54:252–257. https://doi.org/10.1016/j.jpba.2010.07.046

Martineau E, Tea I, Akoka S, Giraudeau P (2012) Absolute quantification of metabolites in breast cancer cell extracts by quantitative 2D 1H INADEQUATE NMR. NMR Biomed 25:985–992. https://doi.org/10.1002/nbm.1816

Montedoro G, Servili M, Baldioli M, Selvaggini R, Miniati E (1992) Simple and hydrolysable phenolic compounds in virgin olive oil. J Agric Food Chem 40:1571

Montedoro G, Servili M, Baldioli M, Selvaggini R, Miniati E, Macchioni A (1993) Simple and hydrolyzable compounds in virgin olive oil. 3. Spectroscopic characterizations of the secoiridoid derivatives. J Agric Food Chem 41:2228

Munowitz M, Pines A (1986) Multiple-quantum nuclear magnetic resonance spectroscopy. Science 233:525–531. https://doi.org/10.1126/science.233.4763.525

Newman DJ, Cragg GM (2007) Natural products as sources of new drugs over the last 25 years. J Nat Prod 70:461–477. https://doi.org/10.1021/np068054v

Nilsson M, Duarte IF, Almeida C, Delgadillo I, Goodfellow BJ, Gil AM et al (2004) High-resolution NMR and diffusion-ordered spectroscopy of port wine. J Agric Food Chem 52:3736–3743. https://doi.org/10.1021/jf049797u

Ocakoglu D, Tokatli F, Ozen B, Korel F (2009) Distribution of simple phenols, phenolic acids and flavonoids in Turkish monovarietal extra virgin olive oils for two harvest years. Food Chem 113:401–410. https://doi.org/10.1016/j.foodchem.2008.07.057

Piotto M, Manjunatha Reddy GN, Caldarelli S (2011) Non-uniformly sampled maximum quantum spectroscopy. J Magn Reson 213:107–111. https://doi.org/10.1016/j.jmr.2011.09.016

Quideau S, Deffieux D, Douat-Casassus C, Pouységu L (2011) Plant polyphenols: chemical properties, biological activities, and synthesis. Angew Chem Int Ed 50:586–621. https://doi.org/10.1002/anie.201000044

Reddy GNM, Caldarelli S (2010) Demixing of severely overlapping NMR spectra through multiple-quantum NMR. Anal Chem 82:3266–3269. https://doi.org/10.1021/ac100009y

Reddy GNM, Caldarelli S (2011) Maximum-quantum (MaxQ) NMR for the speciation of mixtures of phenolic molecules. Chem Commun 47:4297–4299. https://doi.org/10.1039/C1CC10226E

Reddy GNM, Caldarelli S (2012) Identification and quantification of EPA 16 priority polycyclic aromatic hydrocarbon pollutants by maximum-quantum NMR. Analyst 137:741–746. https://doi.org/10.1039/C1AN16047H

Reddy GNM, Ballesteros-Garrido R, Lacour J, Caldarelli S (2013) Determination of labile chiral supramolecular ion pairs by chromatographic NMR spectroscopy. Angew Chem Int Ed 52:3255–3258. https://doi.org/10.1002/anie.201209616

Sacchi R, Mannina L, Fiordiponti P, Barone P, Paolillo L, Patumi M et al (1998) Characterization of Italian extra virgin olive oils using 1H-NMR spectroscopy. J Agric Food Chem 46:3947–3951. https://doi.org/10.1021/jf970666l

Tsimidou M, Papadopoulos G, Boskou D (1992) Phenolic compounds and stability of virgin olive oil—part I. Food Chem 45:141–144. https://doi.org/10.1016/0308-8146(92)90025-W

Viel S, Caldarelli S (2008) Improved 3D DOSY-TOCSY experiment for mixture analysis. Chemical Communications 2013-2015. https://doi.org/10.1039/B802789G

Vinha AF, Ferreres F, Silva BM, Valentão P, Gonçalves A, Pereira JA et al (2005) Phenolic profiles of Portuguese olive fruits (Olea europaea L.): influences of cultivar and geographical origin. Food Chem 89:561–568. https://doi.org/10.1016/j.foodchem.2004.03.012

Visioli F, Bellomo G, Galli C (1998) Free radical-scavenging properties of olive oil polyphenols. Biochem Biophys Res Commun 247:60–64. https://doi.org/10.1006/bbrc.1998.8735

Yang D-P, Kong D-X, Zhang H-Y (2007) Multiple pharmacological effects of olive oil phenols. Food Chem 104:1269–1271. https://doi.org/10.1016/j.foodchem.2006.12.058