Electronic Supplementary Information

Maximum-Quantum (MaxQ) NMR for the speciation of mixtures of phenolic molecules

G N Manjunatha Reddy and Stefano Caldarelli*

Université Paul Cézanne (Aix-Marseille III), Institut des Sciences Moléculaires de Marseille ISM2-UMR CNRS 6263, Faculté des Sciences et Techniques, Service 512 13397 Marseille cedex 20 France.

Extraction of the polar fraction of the oil, sample preparation and experimental section:

Samples

Two mixtures were used in the present study. In the first one, the following molecules (as supplied from Sigma Aldrich): gallic acid, syringic acid, caffeic acid, ferulic acid, vanillic acid, pyrocatechol, *o*- and *p*-coumaric acid, 4-hydroxybenzoic acid, *t*-cinamic acid and benzoic acid were dissolved in a 3:2:1 DMSO:D_2O:$CDCl_3$ mixture. The concentration of each compound was of about 10mM.

The second sample was an extract of a commercially available extra virgin olive oil (PUGET Classic, Lesieur Products), obtained with the following protocol.[27]First, 8 grams of oil was dissolved in 8 ml of methanol/water, 80/20, v/v. The mixture was then stirred in a vortex for 15 mins (3 times) and centrifuged at 5000 rpm for about 10 mins, at 298 K, (3 times) and the methanol fraction collected. Methanol was evaporated under vacuum at 60^0 C and the residue was washed with 8 ml of hexane (3 times). Hexane was then evaporated at 30^0 C. The residue was finally dissolved in 500µl of CD_3OD and subjected to NMR experiments. The total polyphenols content in this sample was of at least 250 mg/kg, according to the nutritional composition information provided by the supplier.

NMR spectroscopy

Experiments were performed on a Bruker AV-500 NMR spectrometer equipped with a TXI triple resonance cryo probe, able to generate pulsed field gradients up to 55 G/cm. The excitation-reconversion of ^{1}H multiple-quantum coherences was empirically optimized on a one dimensional version of the basic pulse sequence $(90)_x$-τ/2-$(180)_x$-τ/2– $(90)_\varphi$-t_1- $(90)_\varphi$-t_2, with a fixed t_1 value of 3 microseconds. The delay, τ, was varied to obtain the best intensity along with the a uniform as possible excitation of the coherence order of choice, *p*. Desired coherences were effectively selected using a pair of half-sine shaped pulsed field gradients,[26] G_1 and G_2, placed before and after the final 90^0 pulse, respectively. The ratio of the gradient pulse was selected to fulfill $G_2 = p*G_1$, with G_2 being kept fixed at 24.75 G/cm and G_1 is respectively varied, for both mixtures.

For the standard mixture 400 increments of 4 scans each were acquired for the indirect dimension for the coherence orders (*p*=3,4 and 5, with τ values of 166.6 ms, 62.5 ms, 100 ms respectively), corresponding to a resolution in the MQ dimension of 1.22 Hz/pt and 3.42 Hz/pt, respectively along F2 and F_1 axes, for approximately 1.50 hrs acquisition time on each order. For the extra virgin olive oil extract all MQ experiments were performed (*p*=3, 4 with τ values 250 ms, 62.5 ms respectively) with512 t_1 increments of 16 scans each. The digital resolution in this latter case was of 1.01 Hz/pt and 1.95 Hz/pt respectively at F_2 and F_1 axes with about 7.19 hrs acquisition time for 3Q, the analogue values for 4Q experiment are 1.01 Hz/pt and 1.46 Hz/pt at 6.45 hrs recording time.The 2D spectra were processed with sine bell window functionsmultiplication to finally produce a 1024 by 1024 matrix and are displayed in magnitude mode.

An additional MaxQ NMR experiment, over a reduced spectral span was performed when further resolution was needed (4Q expanded, top left Fig 3). Resolution along the MQ axis was about 0.17 Hz/pt, for the acquision of 1.7 hrs with 256 increments and 4 scans each. with a fixed t_1 value of 3 microseconds, varying the delay τ to obtain the best intensity along with a uniform as possible excitation of the coherence order of choice, *p*.

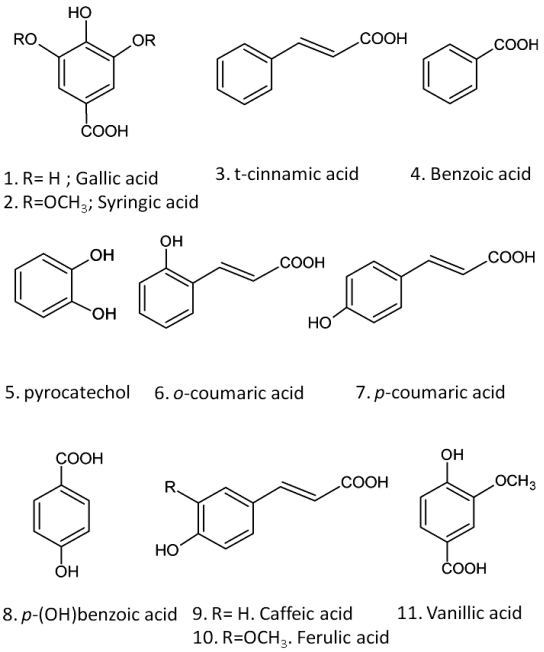

Figure S1: Chemical structures of the components of the standard set of simple phenols; the numbering follows the spectral assignment in the MaxQ spectra, Fig 2.

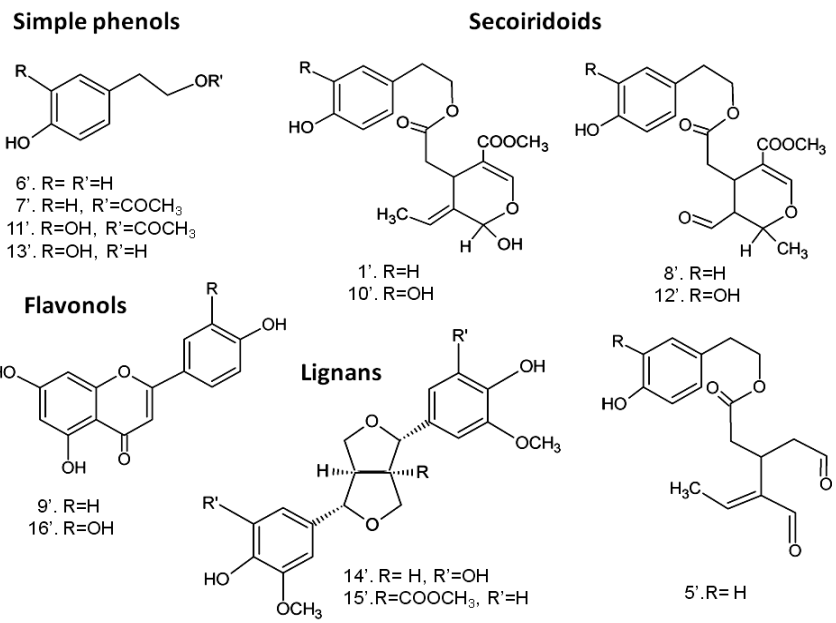

Figure S2: Chemical structures of the molecules identified in the olive oil extract, Numbering follows the one of MaxQ Spectra in Fig. 3. Simple phenols (6': tyrosol and 7': tyrosol acetate, 11': hydroxyltyrosol acetate, and 13': hydroxyltyrosol), secoiridoids (1': ligstroside aglycon, 5': dialdehydic form of ligstroside lacking carboxymethyl group, 8': dialdehydic form of ligstroside, 10': oleuropein aglycon, and 12': aldehydic form of oleuropein), flavonols (9': apigenin, 16': luteolin), and lignans (14': (+)- pinoresinol, 15': (+)-1-acetoxypinoresinol).

Table S1: Summary of the NMR MaxQ chemical shift of the phenolic molecular fragments identified for the two mixtures analyzed in this work.

Nº	Mixture of standard phenols		Nº	Phenolic fraction of an extra virgin olive oil	
	Molecule (MaxQ)	δ_{MaxQ} (ppm)		Molecule (MaxQ)	δ_{MaxQ} (ppm)
1	Gallic acid (1Q)	6.96	1’	Ligstroside aglycon (4Q)	6.860
2	Syringic acid (1Q)	7.19	2’	---	6.863
3	t-cinnamic acid (5Q)	7.46	3’	---	6.865
4	Benzoic acid (5Q)	7.67	4’	---	6.867
5	Pyrocatechol (4Q)	6.69	5’	Dialdehydic form of ligstroside lacking the carboxymethyl group (4Q)	6.870
6	o-coumaric acid (4Q)	7.09	6’	Tyrosol (4Q)	6.885
7	p-coumaric acid (4Q)	7.11	7’	Tyrosol acetate (4Q)	6.894
8	4-hydroxybenzoic acid (4Q)	7.31	8’	Aldehydic form of ligstroside (4Q)	6.897
9	Caffeic acid (3Q)	6.93	9’	Apigenin (4Q)	7.42
10	Ferulic acid (3Q)	7.07	10’	Oleuropein aglycon (3Q)	6.63
11	Vanillic acid (3Q)	7.25	11’	Hydroxytyrosol (3Q)	6.64
			12’	Aldehydic form of oleuropein (3Q)	6.65
			13’	Hydroxytyrosol acetate(3Q)	6.68
			14’	Pinoresinol(3Q)	6.84
			15’	Acetoxy pinoresinol(3Q)	6.88
			16’	Luteolin(3Q)	7.27

Additional References

26. Keeler, J.; Clowes, R. T.; Davis, A. L.; Laue, E. D., Academic Press Inc,: San Diego, 1994; Vol. 239, p 145.
27. Montedoro, G.; Servili, M.; Baldioli, M.; Selvaggini, R.; Maniati, E. *J. Agri. Food Chem.,* **1992,** 40, 1571-1576.