Supplementary material of the manuscript published in Vitis **60**, 69–75 (2021):

Comparison of two sample preparation methods for ^{1}H-NMR wine profiling: Direct analysis and solid-phase extraction

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Supplementary Fig. S1-S3: ^{1}H-NMR expansion spectra (700 MHz, D_2O, 25 °C) of eight wine samples by DA-NMR.

Supplementary Fig. S4-S6: ^{1}H-NMR expansion spectra (700 MHz, MeOD-d_4, 25 °C) of eight wine samples by SPE-NMR.

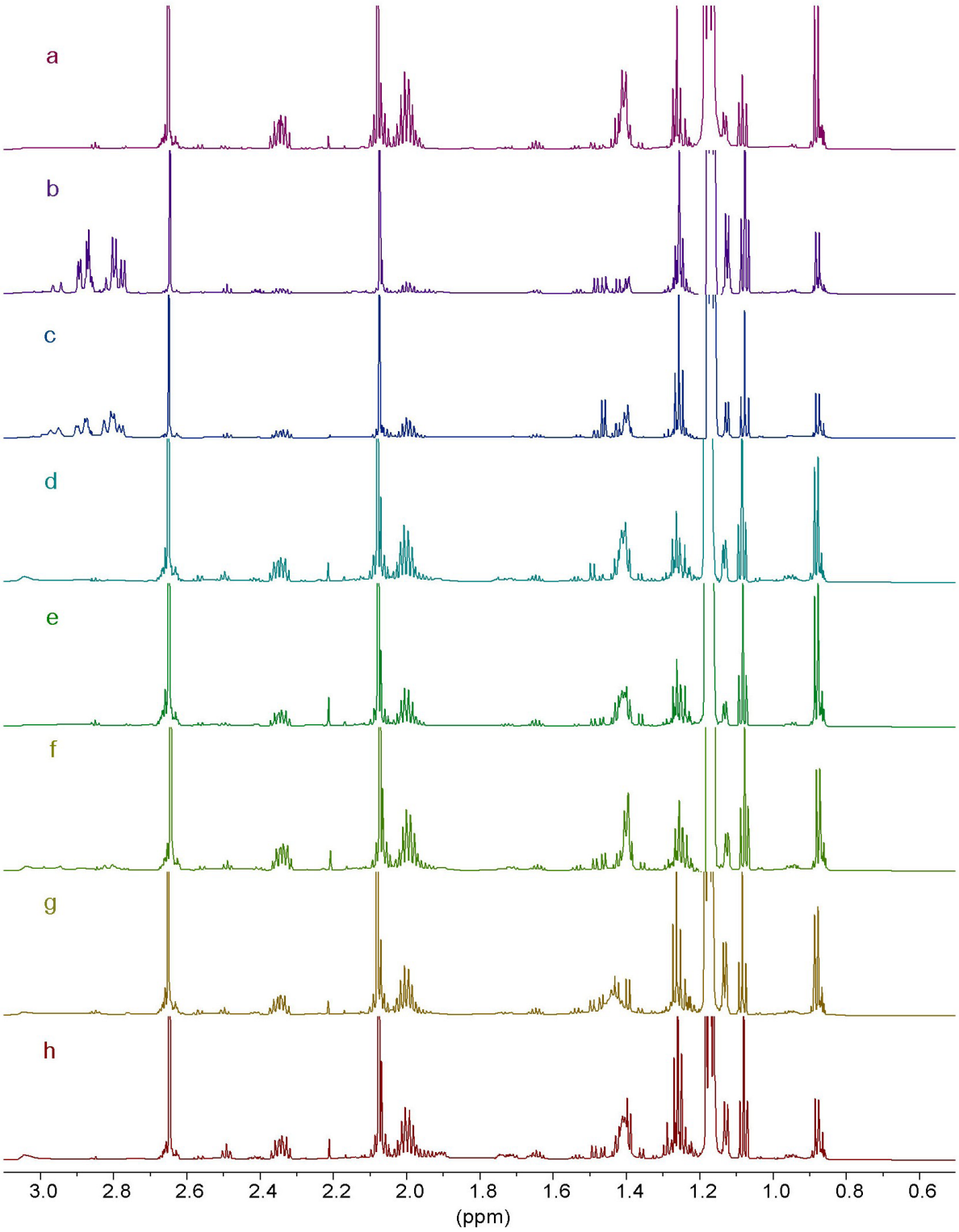
Supplementary Fig. S7: ^{1}H-RMN spectra (700 MHz, MeOD-d_4, 25 °C) of triplicate methanol extracts of a 'Cabernet Sauvignon' wine sample performed to evaluate SPE-NMR repeatability.

Supplementary Fig. S8: Control chart of the first component PC1 that explains 94 % of the data variability. Cliff plot showing the explained variance of the PCA model with a principal component.

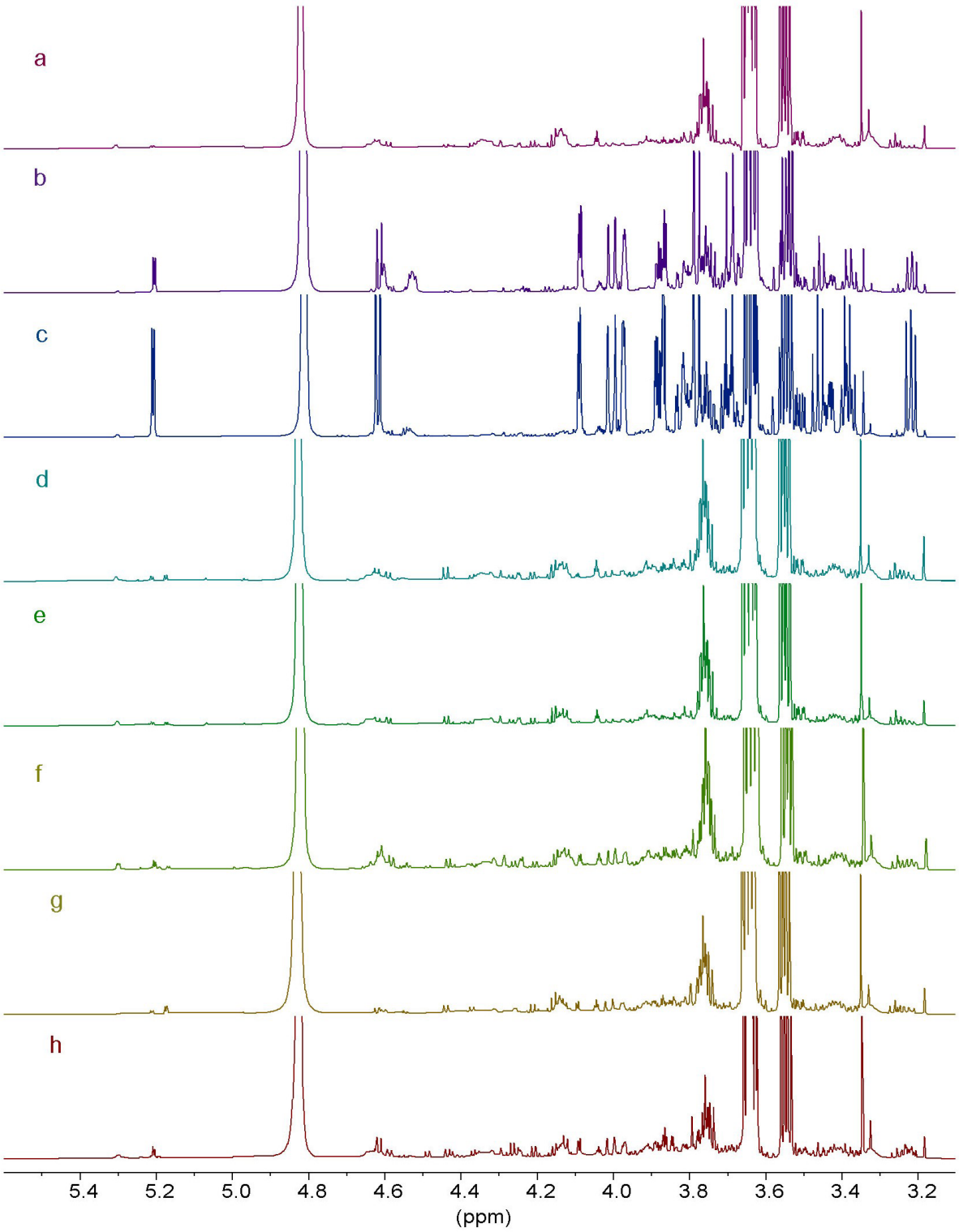
Supplementary Fig. S9-S10: Overlapped ^{1}H-NMR spectra of phenethyl and isoamyl alcohol signals in the eight wine samples by DA-NMR and SPE-NMR.

Supplementary work flow S11: Structural elucidation of tyrosol using 1D and 2D NMR spectra in 'Merlot' wine.

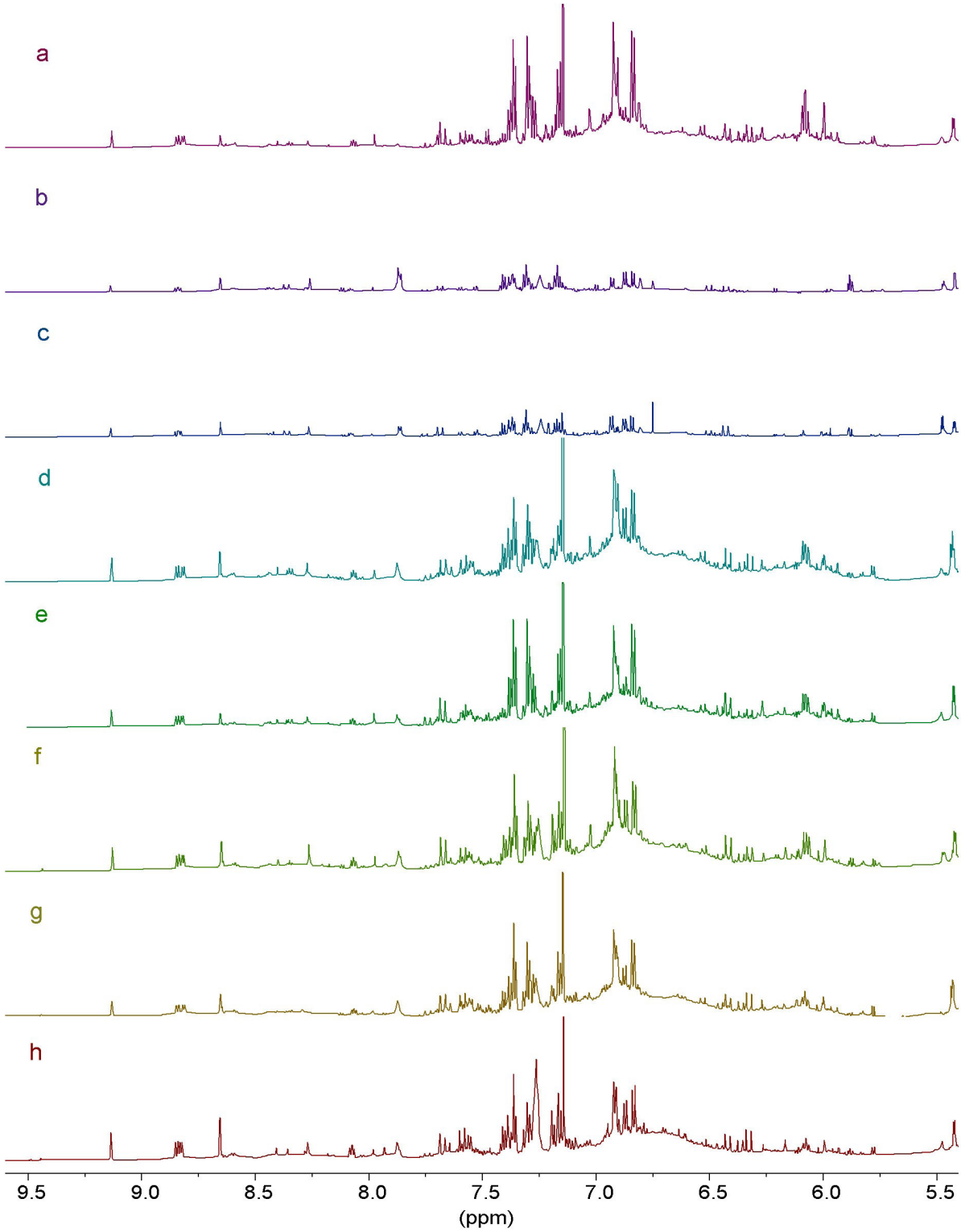
Supplementary Fig. S12-S16: ^{1}H, ^{13}C, COSY, ed-HSQC and HMBC spectra (700 MHz, MeOD-d_4, 25 °C) of 'Merlot' wine extracted by SPE-NMR. Signal assignments of tyrosol.



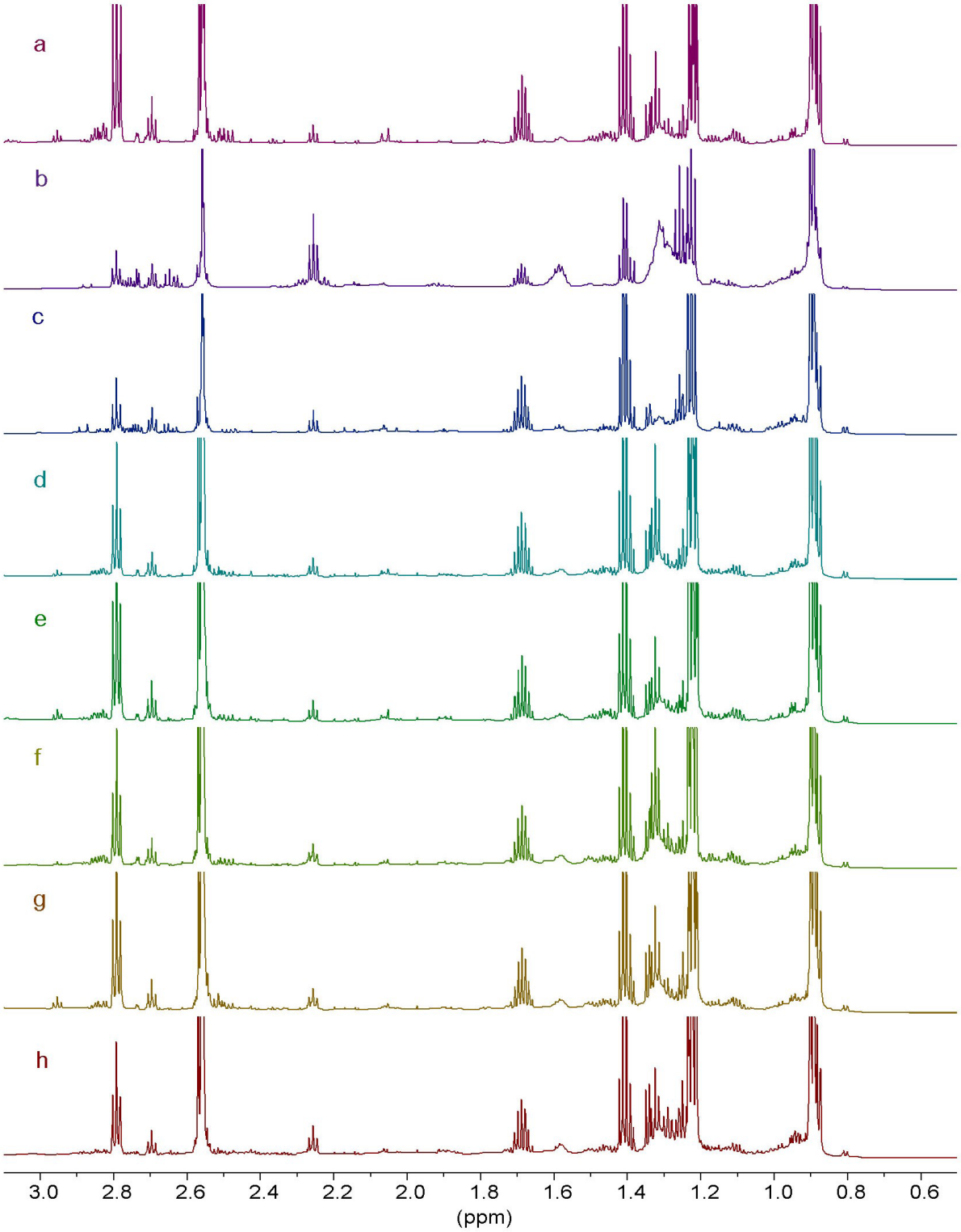
Supplementary Fig. S1: ^{1}H-NMR expansion spectra (700 MHz, D_2O, 25 °C) from 0.5 to 3.1 ppm of eight wine samples by DA-NMR. Wines: **a**) 'Cabernet Sauvignon', **b**) mixture of 'Chenin Blanc' and 'Colombard', **c**) 'White Zinfandel', **d**) 'Petite Sirah', **e**) 'Merlot', **f**) 'Zinfandel', **g**) 'Nebbiolo' and **h**) 'Barbera'.



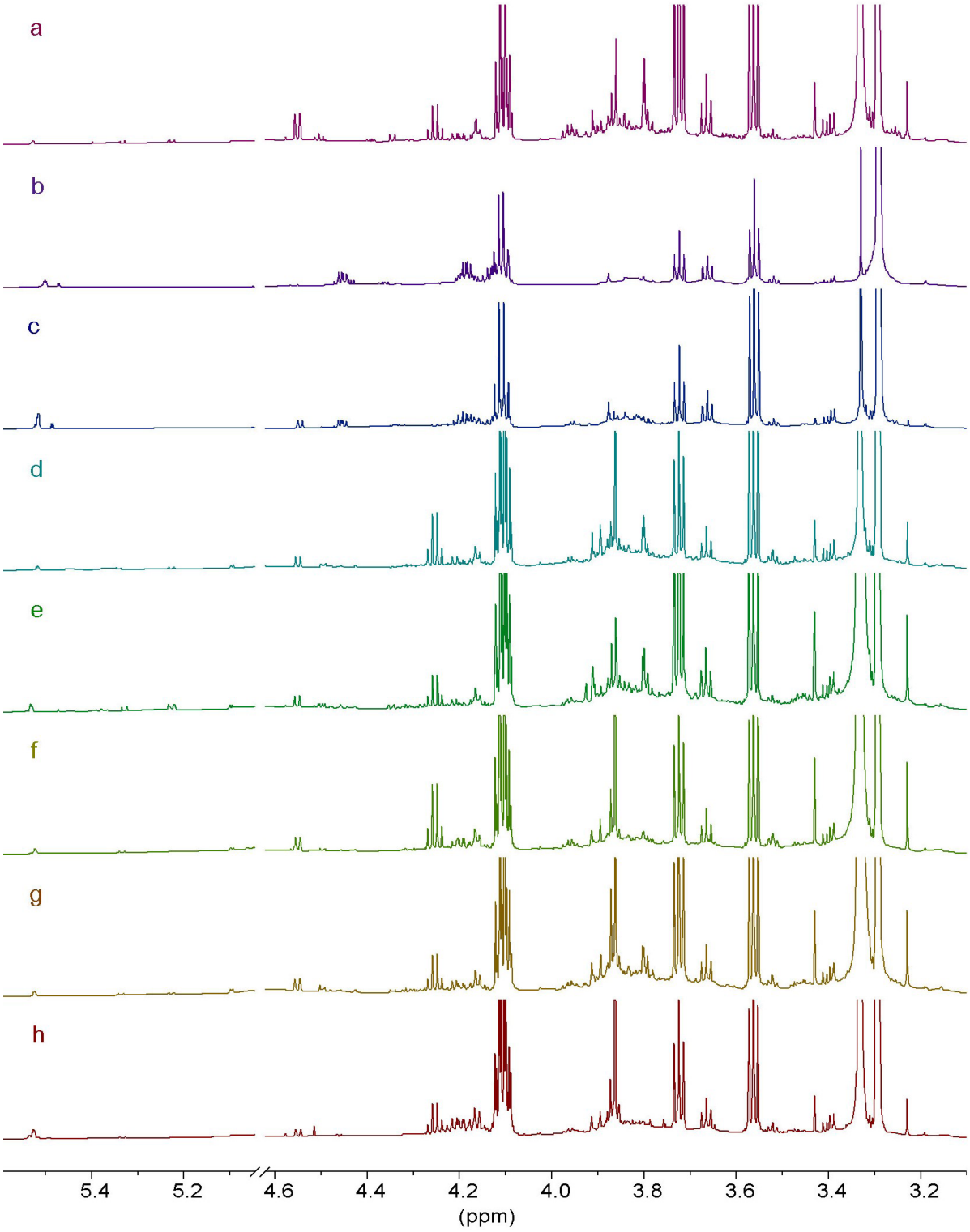
Supplementary Fig. S2: ^{1}H-NMR expansion spectra (700 MHz, D_2O, 25 °C) from 3.1 to 5.6 ppm of eight wine samples by DA-NMR. Wines: **a**) 'Cabernet Sauvignon', **b**) mixture of 'Chenin Blanc' and 'Colombard', **c**) 'White Zinfandel', **d**) 'Petite Sirah', **e**) 'Merlot', **f**) 'Zinfandel', **g**) 'Nebbiolo' and **h**) 'Barbera'.



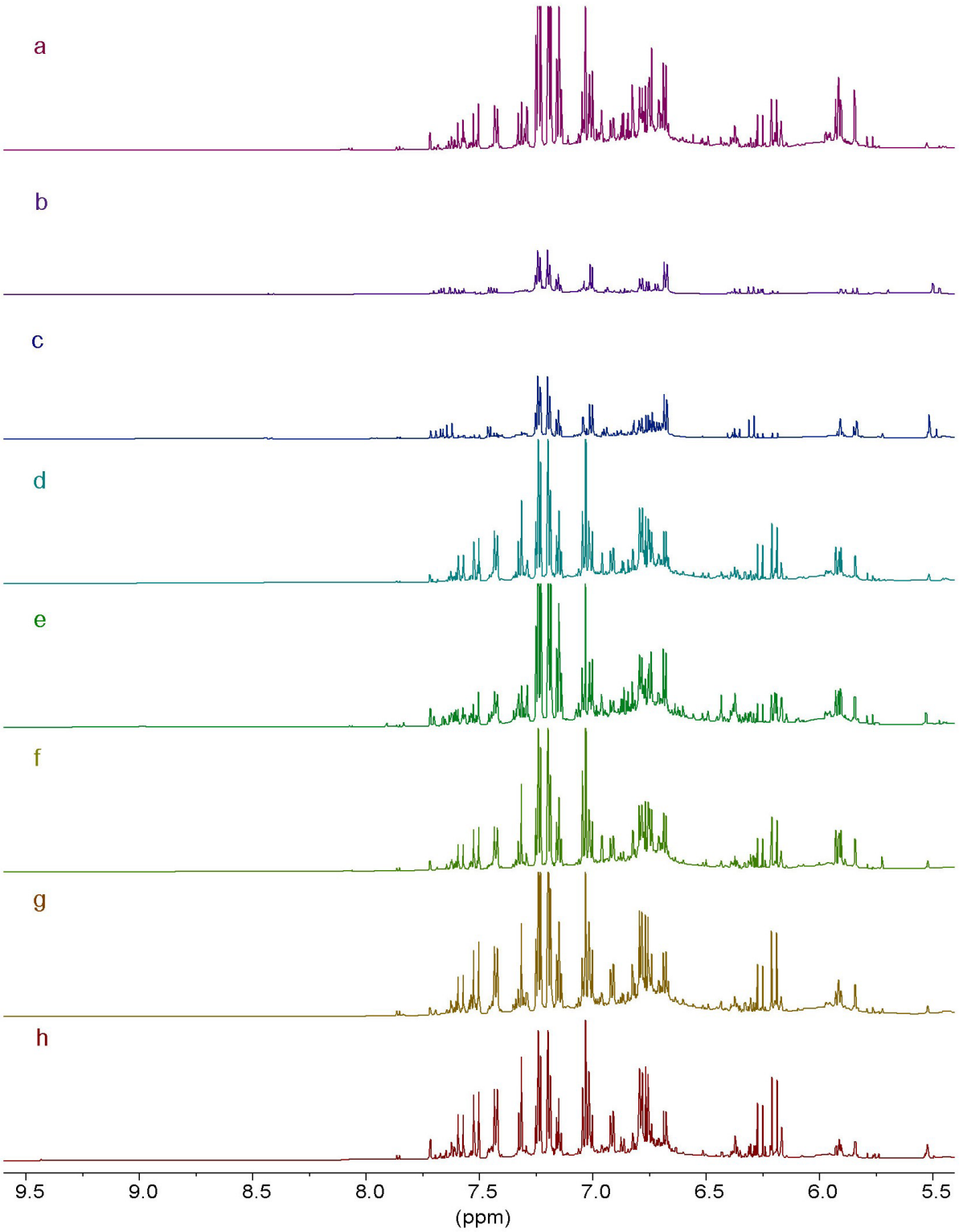
Supplementary Fig. S3: ^{1}H-NMR expansion spectra (700 MHz, D_2O, 25 °C) from 5.4 to 9.6 ppm of eight wine samples by DA-NMR. The aromatic region was vertical multiplied by factor of thirty. Wines: **a**) 'Cabernet Sauvignon', **b**) mixture of 'Chenin Blanc' and 'Colombard', **c**) 'White Zinfandel', **d**) 'Petite Sirah', **e**) 'Merlot', **f**) 'Zinfandel', **g**) 'Nebbiolo' and **h**) 'Barbera'.



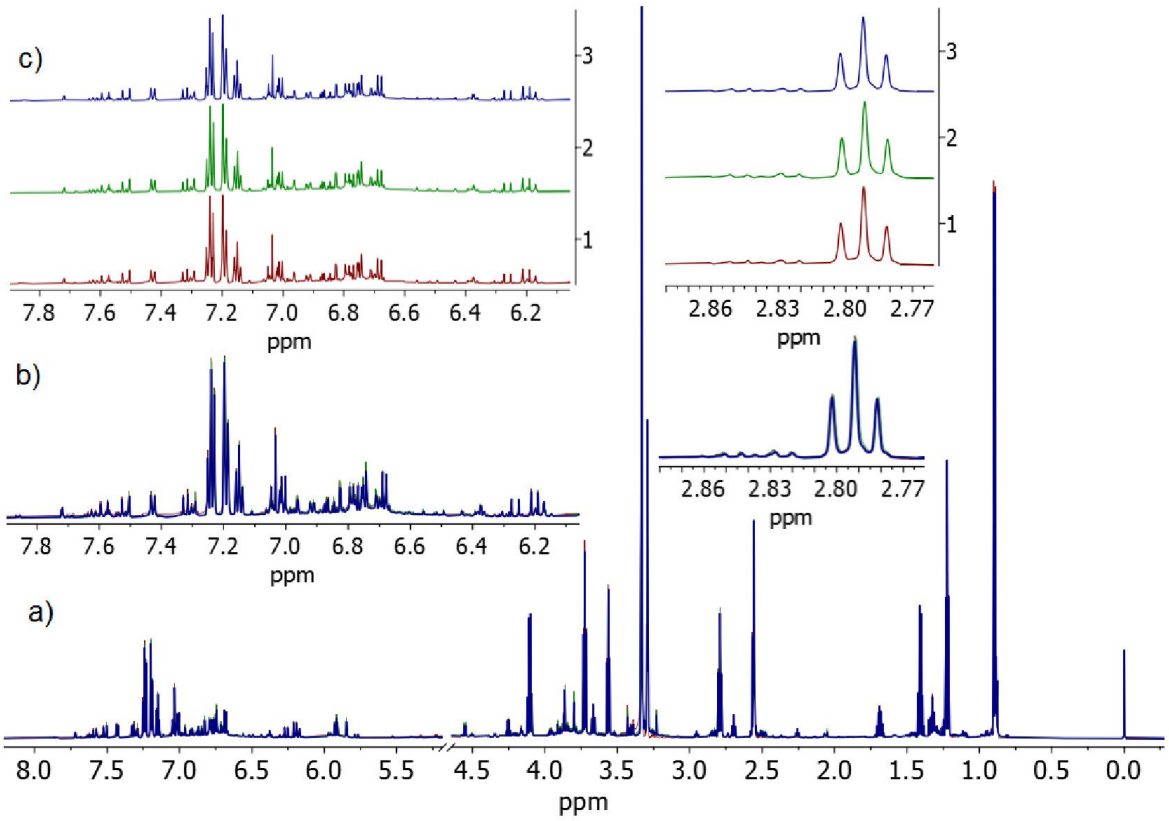
Supplementary Fig. S4: ^{1}H-NMR expansion spectra (700 MHz, MeOD-d_4, 25 °C) from 0.5 to 3.1 ppm of eight wine samples by SPE-NMR. Wines: **a**) 'Cabernet Sauvignon', **b**) mixture of 'Chenin Blanc' and 'Colombard', **c**) 'White Zinfandel', **d**) 'Petite Sirah', **e**) 'Merlot', **f**) 'Zinfandel', **g**) 'Nebbiolo' and **h**) 'Barbera'.



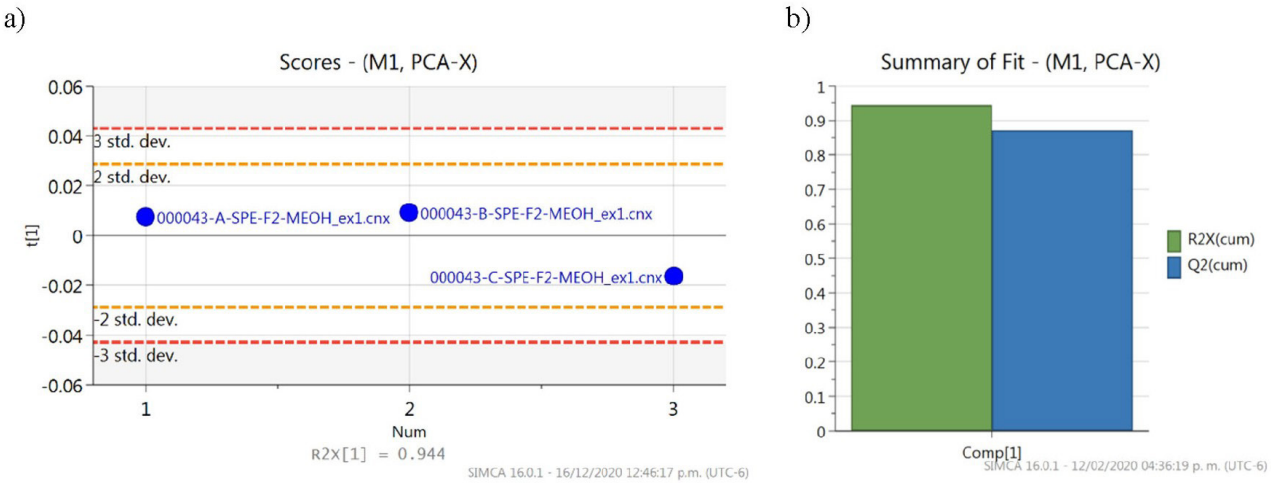
Supplementary Fig. S5: ^{1}H-NMR expansion spectra (700 MHz, MeOD-d_4, 25 °C) from 3.1 to 5.6 ppm of eight wine samples by SPE-NMR. Wines: **a**) 'Cabernet Sauvignon', **b**) mixture of 'Chenin Blanc' and 'Colombard', **c**) 'White Zinfandel', **d**) 'Petite Sirah', **e**) 'Merlot', **f**) 'Zinfandel', **g**) 'Nebbiolo' and **h**) 'Barbera'.



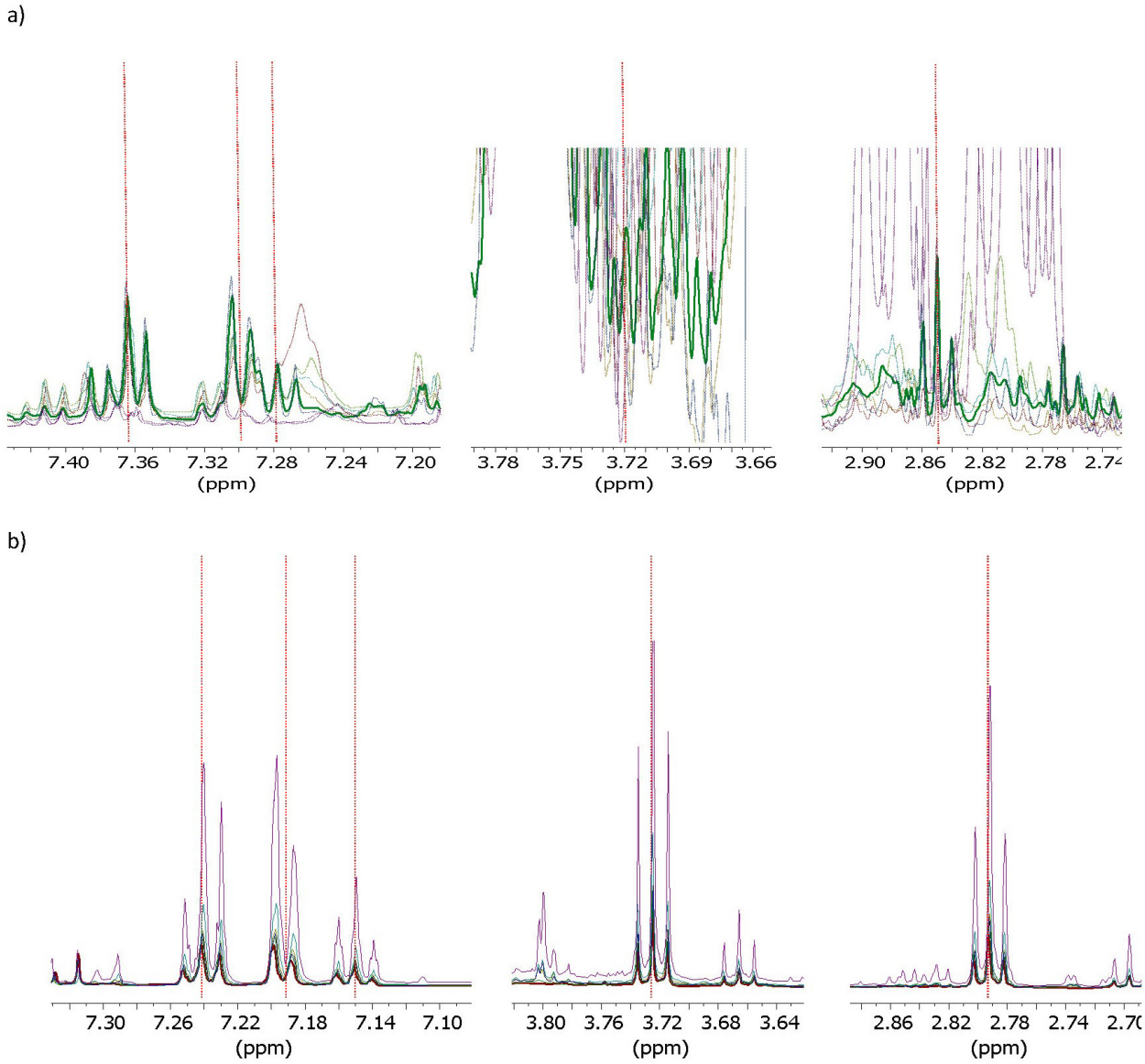
Supplementary Fig. S6: ^{1}H-NMR expansion spectra (700 MHz, MeOD-d_4, 25 °C) from 5.4 to 9.6 ppm of eight wine samples by SPE-NMR.Wines: **a**) 'Cabernet Sauvignon', **b**) mixture of 'Chenin Blanc' and 'Colombard', **c**) 'White Zinfandel', **d**) 'Petite Sirah', **e**) 'Merlot', **f**) 'Zinfandel', **g**) 'Nebbiolo' and **h**) 'Barbera'.



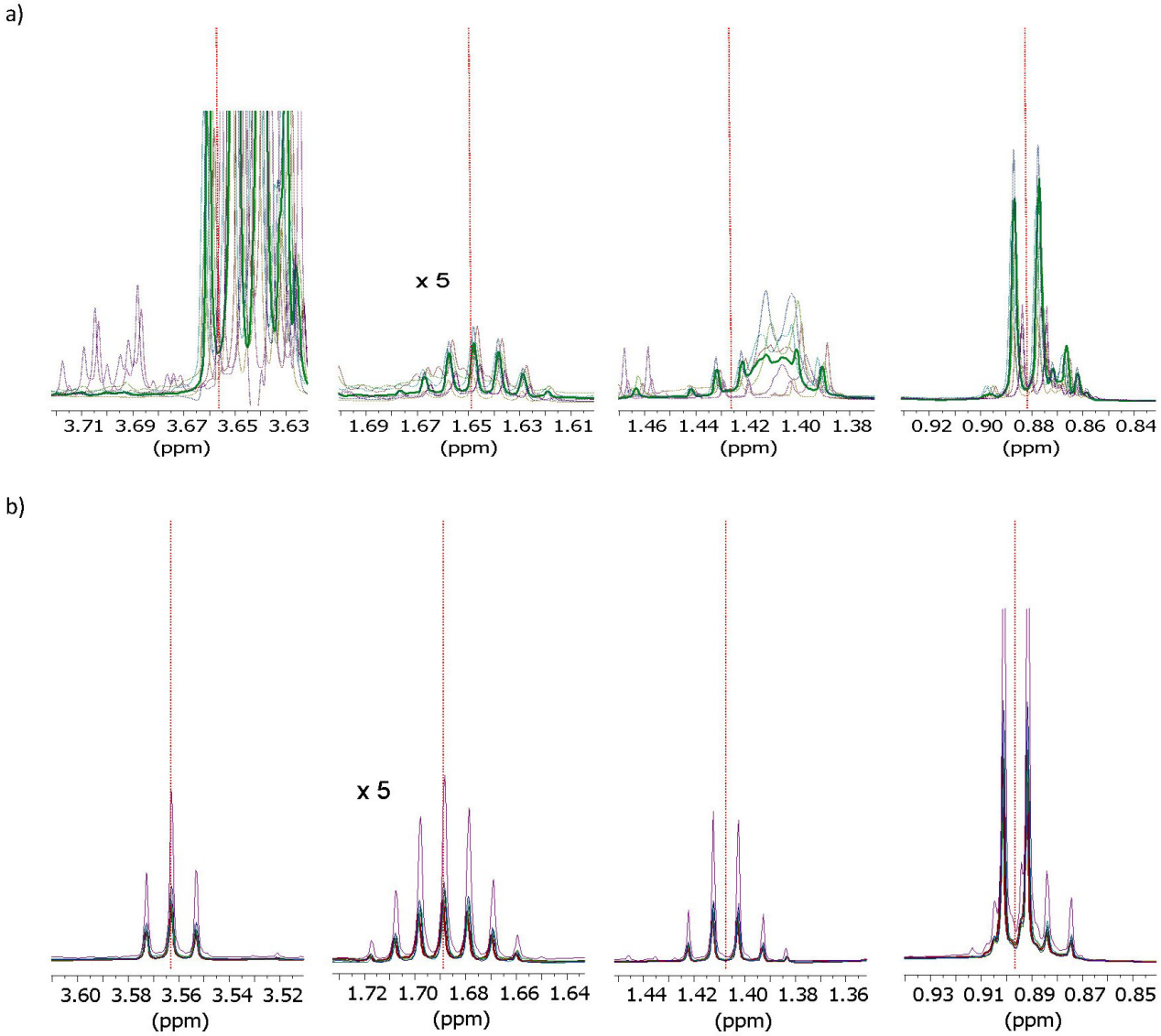
Supplementary Fig. S7: ^{1}H-RMN spectra (700 MHz, MeOD-d_4, 25 °C) of triplicate methanol extracts of a 'Cabernet Sauvignon' wine sample performed to evaluate SPE-NMR repeatability: **a**) Full overlapped spectra, **b**) overlapped expansions from 6.05 to 7.9 ppm and 2.76 to 2.88 ppm and **c**) stacked spectra of same expansions of **b**).



Supplementary Fig. S8: **a**) Control chart of the first component PC1 that explains 94 % of the data variability of the Fig.S7 spectra and **b**) cliff plot showing the explained variance of the PCA model with a principal component.



Supplementary Fig. S9: Overlapped ^{1}H-NMR spectra of phenethyl alcohol signals in the eight wine samples: **a**) DA-NMR and **b**) SPE-NMR.

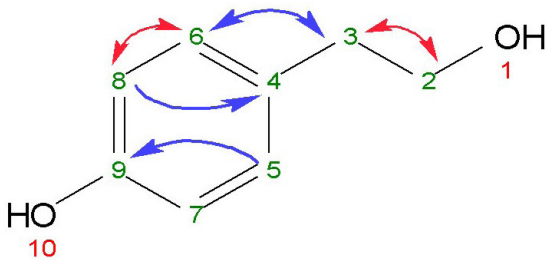


Supplementary Fig. S10: Overlapped ^{1}H-NMR spectra of isoamyl alcohol signals in the eight wine samples: **a**) DA-NMR and **b**) SPE-NMR. The regions around 1.6-1.7 ppm were multiplied by a factor of five.

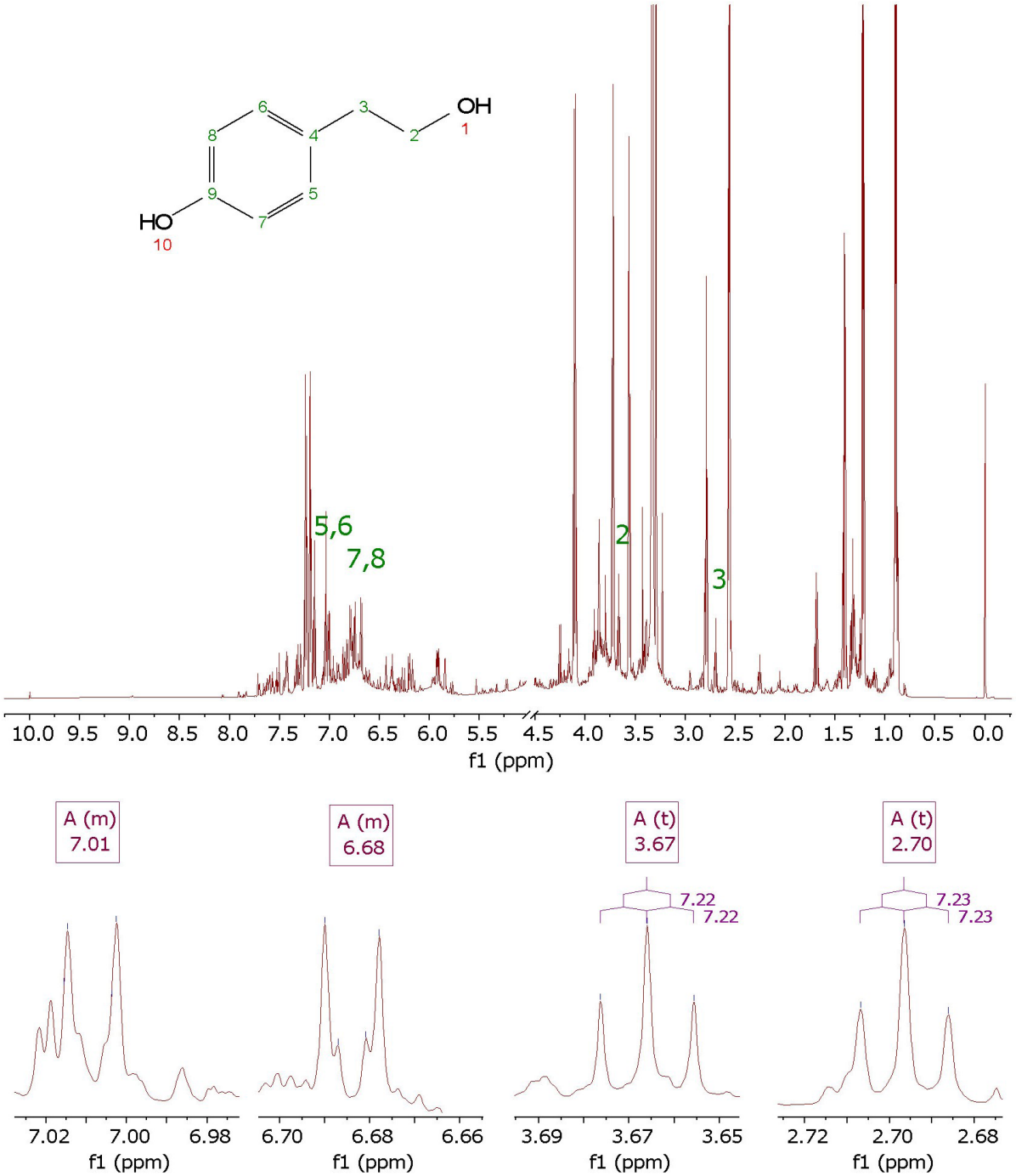
Supplementary work flow S11: Structural elucidation of tyrosol using 1D and 2D NMR spectra in 'Merlot' wine.

The AA´XX´ system at δ_H = 6.68 ppm and δ_H = 7.01 ppm (*pseudo-doublets*) suggested a di-substituted phenyl moiety with hydroxyl group in *para* position (**Supplementary Fig. S12**), that could by consistent with tyramine, tyrosol or tyrosine. Interpretation of 2D NMR spectra, i.e., ed-HSQC (edited -Heteronuclear Single Quantum Coherence) (**Supplementary Fig. S15)**, HMBC (Heteronuclear Multiple Bond Correlation) (**Supplementary Fig. S16)** and COSY (COrrelation SpectroscopY) (**Supplementary Fig. S14)**, allowed identification of tyrosol structure. For instance, the key correlations are:

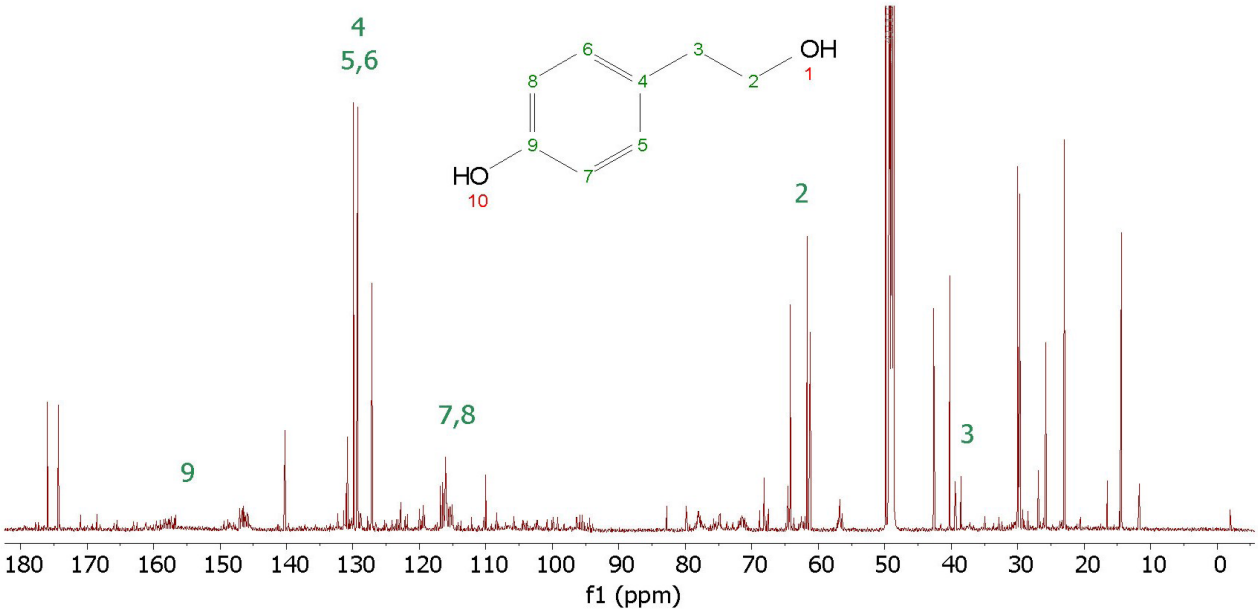
- The correlation between the proton at δ_H 6.68 ppm (H7,8) and proton at δ_H 7.01 ppm (H5,6) in the COSY spectra confirm de AA´XX´ system of the aromatic ring.
- The quaternary carbons were assigned via ^1H-^{13}C HMBC. The correlation from the aromatic protons to the quaternary carbons δ_C ~ 155.38 (C9) and δ_C ~ 129.7 (C4), indicated that the ring has an OH group attach to C9 and quaternary C4 in *para* position.
- The ^1H-^{13}C HMBC correlation from the aromatic proton at δ_H 7.01 ppm (H5,6) to a carbon δC ~38.12 (C3) indicates the C3 is connected to C4.
- In the multiplicity-edited HSQC experiments, the amplitude of CH_2 signals is negative (signals in blue color) compared to those of CH and CH_3 groups (signals in red color). Carbon δ_C ~38.12 (C3) observed in color blue is a CH_2 group which protons appear at δ_H 2.70 ppm, t, *J*=7.2 Hz.
- The correlation between the protons at δ_H = 2.70 ppm (H3) and proton at δ_H 3.67 ppm (H2) in the COSY spectra, the multiplicity and the integrals in the ^1H spectra and the blue color in the edited-HSQC confirm that are two connected CH_2 groups, discarding the tyrosine.
- The chemical shift of the last CH_2 (δ_H 3.67 ppm, δ_c ~ 63.36 ppm) is the expected to be near a OH group and discard the tyramine where a CH_2 near a NH_2 is expected around 42 ppm.



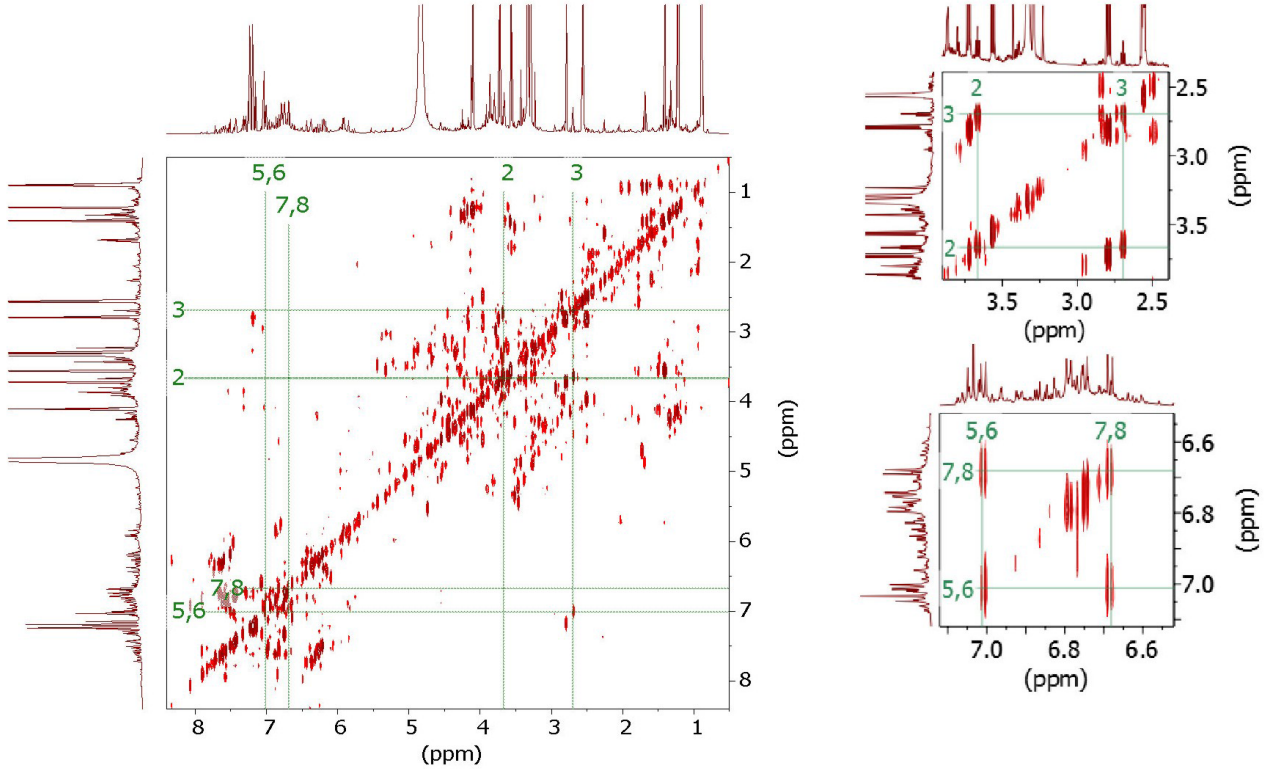
Selected HMBC (blue arrows) and COSY (red arrows) key correlations of tyrosol.
(The numbering used is only for assignment purposes).



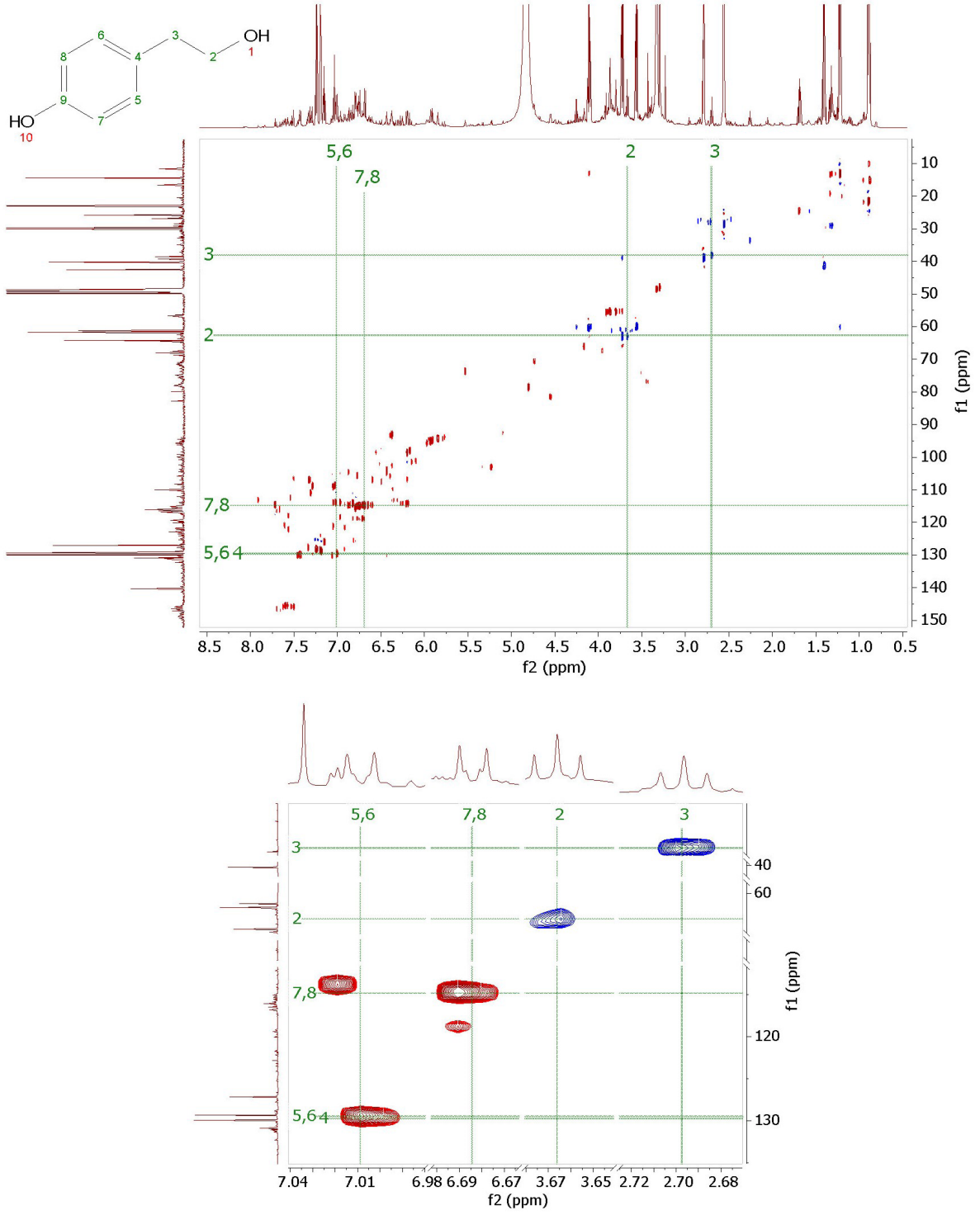
Supplementary Fig. S12: ^{1}H-NMR spectrum (700 MHz, MeOD-d_4, 25 °C) of Merlot wine extracted by SPENMR. Signal assignments of tyrosol Top full spectrum. Bottom expanded signals of tyrosol.



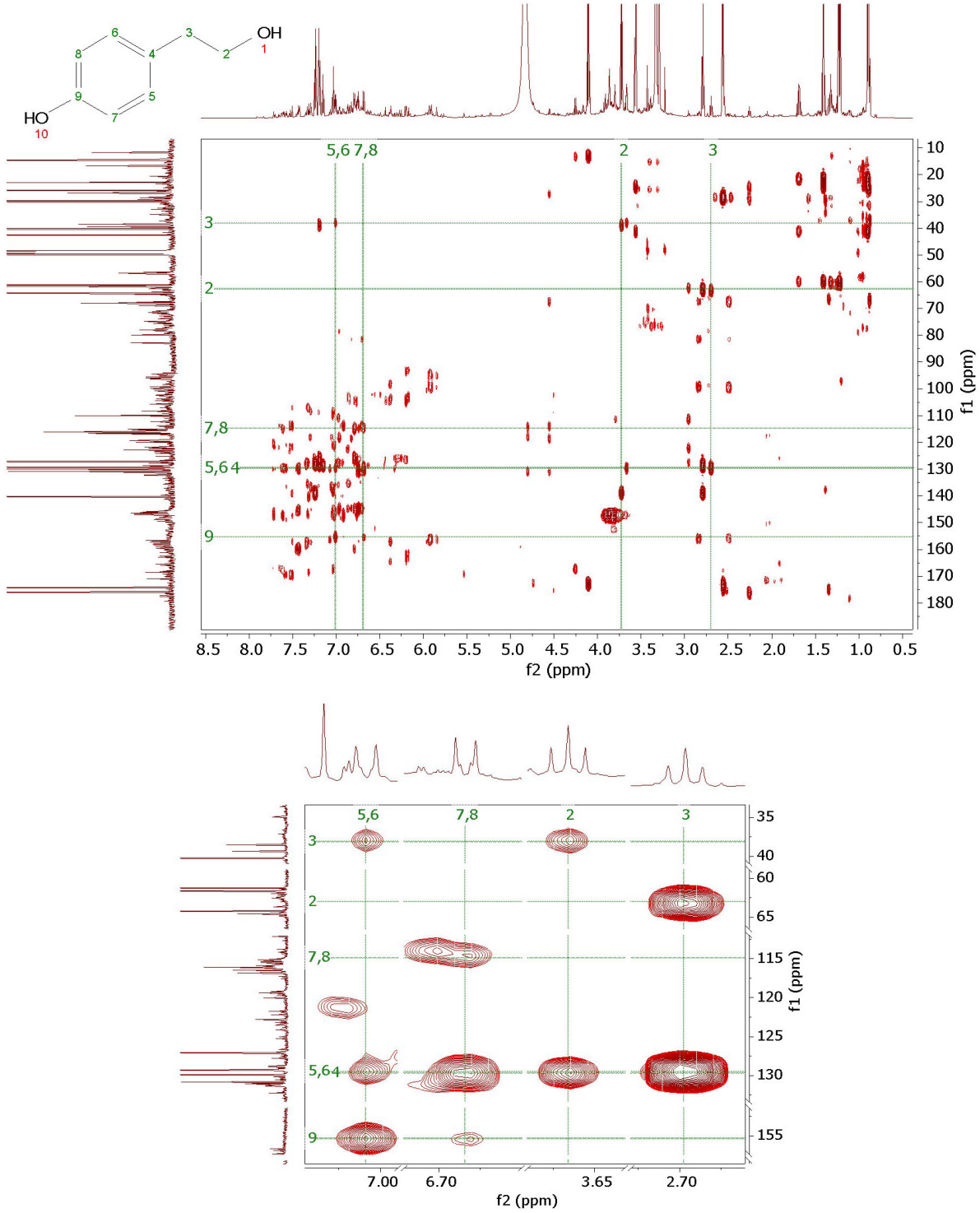
Supplementary Fig. S13: ^{13}C NMR spectrum (175 MHz, MeOD-d_4, 25 °C) of wine extracted by SPE-NMR. Signal assignments of tyrosol (performed with the correlation observed in 2D experiments).



Supplementary Fig. S14: COSY spectrum of wine extracted by SPE-NMR (right) full spectrum and (left) expanded regions of tyrosol signals.



Supplementary Fig. S15: HSQC spectrum of wine extracted by SPE-NMR (top) full spectrum and (bottom) expanded regions of tyrosol signals.



Supplementary Fig. S16: HMBC spectrum of wine extracted by SPE-NMR (top) full spectrum and (bottom) expanded regions of tyrosol signals.