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$_2$O, 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$_2$O, 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: 'H-NMR expansion spectra (700 MHz, D$_2$O, 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'.

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Wines:
- a) 'Cabernet Sauvignon'
- b) Mixture of 'Chenin Blanc' and 'Colombard'
- c) 'White Zinfandel'
- d) 'Petite Sirah'
- e) 'Merlot'
- f) 'Zinfandel'
- g) 'Nebbiolo'
- h) 'Barbera'
Supplementary Fig. S3: 1H-NMR expansion spectra (700 MHz, D$_2$O, 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: 1H-NMR expansion spectra (700 MHz, MeOD-\textsubscript{d4}, 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: ¹H-NMR expansion spectra (700 MHz, MeOD-d₄, 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 $\delta_H = 6.68$ ppm and $\delta_H = 7.01$ ppm (pseudo-doublet) 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 $\delta_H 6.68$ ppm (H7,8) and proton at $\delta_H 7.01$ ppm (H5,6) in the COSY spectra confirm de AA'XX' system of the aromatic ring.
- The quaternary carbons were assigned via $^1$H-$^1$C HMBC. The correlation from the aromatic protons to the quaternary carbons $\delta_C \sim 155.38$ (C9) and $\delta_C \sim 129.7$ (C4), indicated that the ring has an OH group attach to C9 and quaternary C4 in para position.
- The $^1$H-$^1$C HMBC correlation from the aromatic proton at $\delta_H 7.01$ ppm (H5,6) to a carbon $\delta_C \sim 38.12$ (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 $\delta_C \sim 38.12$ (C3) observed in color blue is a CH$_2$ group which protons appear at $\delta_H 2.70$ ppm, $t, J=7.2$ Hz.
- The correlation between the protons at $\delta_H -2.70$ ppm (H3) and proton at $\delta_H 3.67$ ppm (H2) in the COSY spectra, the multiplicity and the integrals in the $^1$H 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$_2$ ($\delta_H 3.67$ ppm, $\delta_C \sim 63.36$ 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.