The "Wine-T$_1$" NMR experiment for novel wine-metabolome fingerprinting with nuclear-spin relaxation

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Abstract. In agreement with the draft resolution OENO-SCMA 17-618 at step 5 "*Quantitation of glucose, malic acid, acetic acid, fumaric acid, shikimic acid and sorbic acid in wine using proton nuclear magnetic resonance spectroscopy* (^{1}H NMR)" said technique has been recently accepted within the OIV chair as a primary quantitative analytical technique for beverage analysis such as wine. However, poor chemical shift dispersion in ^{1}H NMR spectra severely penalizes quantification within overlapped or crowded regions. To outflank said penalization and quantify metabolites in signal overcrowding situations, the novel "Wine-T$_1$" experiment is proposed. The novel scheme comprises the addition of a second dimension, wherein the proton spin-lattice relaxation times (T$_1$-{^{1}H}) of each metabolite's spin-system is correlated to a chemical-shift dimension. The new experiment includes a water and ethanol signal pre-saturation module, prior to the T$_1$ saturation-inversion recovery dimension in order to maximize signal-to-noise ratio of wine metabolome NMR spectra. "Wine-T1" pulse sequence can be adapted to all commercial spectrometers (Bruker, Varian/Agilent, Jeol) and with acquisition times in the order of minutes, it should be considered as a fast repetition method to produce a robust metabolome fingerprint that has not been described before, to the best of our knowledge.

1. Introduction

An increased use of proton Nuclear Magnetic Resonance (^{1}H-NMR) technology for wine metabolomic analysis has been reported over the years due to improvements in high-throughput automations, NMR sensitivity and solvent-suppression routines [1]. For oenology, relevant data to obtain from the ^{1}H-NMR spectra includes signal assignment related to grape varieties, geographical origin of wine and year of vintage. Geographical discriminations between wines have been initially carried out by combining isotopic-Site-specific Natural Fractionation by Nuclear Magnetic Resonance (SNIF NMR)- and trace elements by Isotope Ratio Monitoring by Mass Spectrometry or NMR (irm-MS / irm-NMR) analysis [2]. For instance, discrimination between geographical regions of Spanish, Slovenian, French and Chinese wines with SNIF-IRMS technology [3–6] are some successful examples. Origin authentication by deuterium irm-^{2}H NMR as an official World Organization of Vine and Wine (OIV) method to said purpose presents at least three major limitations: a) the intrinsic ^{2}H low sensitivity (0.0155% of natural abundance relative to ^{1}H), b) narrow chemical-shift range of ^{2}H (couple of ppm's, such as its ^{1}H counterpart), producing in many cases, important signal overlap and c) ^{1}H-^{2}H solute-solvent exchanges. Said limitations lead in turn to have long acquisition times per experiment and detection of isotopic fractionation of only the most abundant metabolites, when non-conventional cryoprobes and high-magnetic fields are used [2].

In the other hand, high-resolution ^{1}H-NMR spectroscopy has recently been accepted and routinely used in the direct study of liquid foods such as fruit juices, beer and wine, with high-throughput instrumentation, in a faster way with respect irm-NMR schemes and in most of the cases with a magnetic field of 9.4 Teslas (400 MHz proton frequency) [7–9]. Furthermore, in agreement with the OIV draft resolution project OENO-SCMA 17-618, currently at step 5 "Quantitation of glucose, malic acid, acetic acid, fumaric acid, shikimic acid and sorbic acid in wine using proton nuclear magnetic resonance spectroscopy (^{1}H-NMR)", said technique has been recently accepted within the OIV scientific chair as a promising primary quantitative analytical technique for beverage analysis such as wine. Particularly, ^{1}H-NMR can be seen as a non-targeted metabolomics technique, wherein minimal sample preparation is required for identification and quantification of various compounds in wine, in a non-invasive way by means of isotropic chemical-shift, signal integrations and signal's fine structure analysis of each metabolite [1, 10]. However, poor chemical shift dispersion and weak intensities of several resonances in ^{1}H-NMR spectra, severely penalizes identification within overlapped crowded regions. In particular, the aromatic regions of wine spectra are difficult to assign due to these
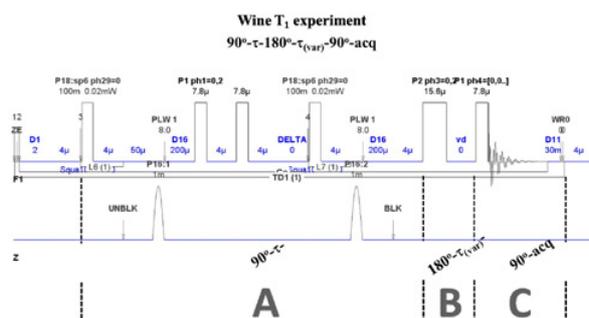
Figure 1. The novel "Wine-T_1" NMR pulse sequence, which consists in a saturation-inversion recovery scheme to compute proton spin-lattice relaxation values of metabolites in wine. It consist in three main blocks: A) ($90° - \tau$) Multipresaturation of intense water-to-ethanol signals with a home-made shaped pulse (see Methods). B) ($180° - \tau_{var}$) an inversion recovery module applied with a variable delay list (vd) going from 10 milliseconds to five seconds that produces all experimental points representing signal attenuation due to the saturation-inversion recovery process of the magnetization. C) ($90° -$ acq) Detection of attenuated signal promoted by the spin-lattice relaxation delay, point B.

inconveniences and overall, the above mentioned OIV resolution project proposes the quantification of no more than 6 metabolites. Assignment within crowded regions can be partially alleviated by the addition of a second dimension, generated by the correlation of a spin system with its covalently-bounded or spatial neighbours, by means of respectively Correlation SpectroscopY (COSY), TOtal Correlation Spectroscopy (TOCSY) and Nuclear Overhauser Effect SpectroscopY (NOESY) NMR schemes [11], as some of the most common techniques to increase the chemical shift dispersion within a spectra. However, said techniques need an evolution t_1 period related to spectral resolution. Longer t_1 increments will produce better resolved spectra at longer experimental times. For that, a compromise has to be met between experimental time consuming and spectral resolution. Routine users must take into account that wine metabolomic profiles obtained with 2D-shift correlation schemes will have a lower signal to noise ratio or longer experimental times with respect a standard 2D-scheme, as a multi presaturation module to suppress water and ethanol signals has to be done in order to increase signal to noise ratio of weak metabolites. Even said performances, weak signal intensity, severe signal overlap or the lack of coupling information between different spin segments of molecules within a metabolome, often leads to ambiguous or incomplete assignments.

For that, the present work shows for the first time a novel NMR high-resolution technique that correlates ^{1}H-NMR chemical shifts of wine spin systems enhanced by a water-to-ethanol multi-presaturation module, with the proton spin-lattice relaxation times (T_1-$\{^1H\}$) of each detected metabolite, computed in a second dimension, with attractive experimental times: The Wine-T_1 experiment. Present scheme intends to have an accurate interpretation of slight changes of each constituent of the complex mixture of molecules within wine samples, with respect differences of T_1 values per resonance, independently if spin systems appear in severe crowded regions within the chemical shift dimension. The central idea of the
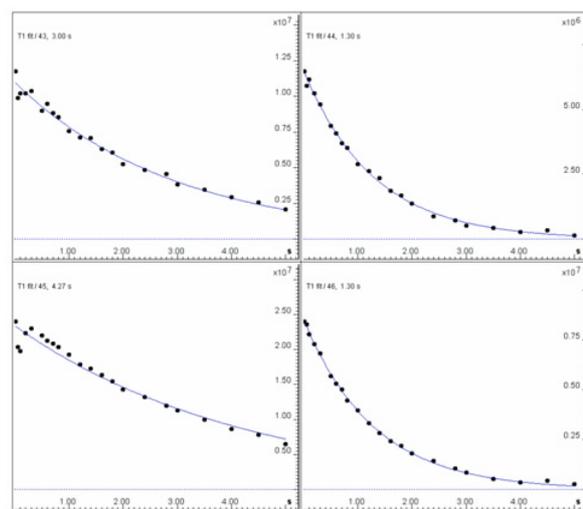
Figure 2. Signal attenuation and mathematical fitting process [12] of an arbitrary set of ^{1}H resonances from Ancon Gran Reserva wine sample (year of vintage 2009), obtained with the Wine-T_1 experiment. Accurate fitting of signal attenuation detected at a particular chemical shift, produces the expected $T_1\{^1H\}$ values used as metabolomic fingerprint (*vide infra*).

Wine-T_1 pseudo 2D-experiment is to separate NMR chemical shift resonances on the basis of their proton spin-lattice relaxation times [12,13]. Measurements of the T_1 $\{^1H\}$ values relay on the basis of the recently reported Saturation-Inversion recovery process [14]. The last implies the computing of series of 1D ^{1}H spectra with a pre-filter block $\mathbf{90° - \tau - 180° - \tau}$ prior to acquisition. First $90° - \tau$ saturation block is done to multipresaturate water and ethanol intense signals, whilst the inversion $180° - \tau$ block promotes the evolution of signal intensity of each proton as a function of τ (Fig. 1). Signal attenuation promoted by the second $180 - \tau$ inversion block can be fitted according to Bloch equations in order to obtain T_1-$\{^1H\}$ values (Fig. 2).

Computed T_1 $\{^1H\}$ values per wine sample not only extends another variable to identify metabolites in a wine sample, mostly in regions with severe resonances' overlap within the standard proton spectra. The present experiment intends to reveal the basis of the correlations between differences in spin-relaxation of metabolites, with respect differences of grape varieties, geographical origin of wine and year of vintage in wine samples.

2. Materials and methods

2.1. Wine samples

Mexican wines of the Llano Colorado, San Vicente, Valle de Guadalupe, Baja California, México, presenting differences in terms of grape varieties and year of vintage were analysed for the present study, and hereafter identified as follows: Ancon San Vicente- Gran Reserva (AGR, year of vintage 2009, Nebbiolo + Caubernet Sauvignon); J2:10 – Reserva (J210, year of vintage 2014, Nebbiolo + Caubernet Sauvignon); Merlot 2016 (M16, year of vintage 2016, Merlot); Merlot 2017 (M17, year of vintage 2017, Merlot); Nebbiolo 2016 (N16, year of vintage 2016, Nebbiolo); Nebbiolo 2017 (N17, year of vintage 2017, Nebbiolo); Syrah 2016 (S16, year of vintage

2016, Syrah) and Syrah 2017 (S17, year of vintage 2017, Syrah). Sample preparation for NMR studies comprised the addition of 100 uL of a mixture of D_2O and chemical-shift reference sodium 3-(trimethylsilyl)-propionate-2, 2, 3, 3-d$_4$ (TSP), phosphonate buffer KH_2PO_4 0.1% and 2% NaN_3 to 900 uL of wine sample, whereas pH was finally adjusted to a value of 3.1 for all samples. Samples were finally versed in standard 5 mm NMR tubes.

2.2. Nuclear Magnetic Resonance (NMR) spectroscopy

All spectra were recorded on a Bruker 600 AVANCE III HD equipped with a 5 mm ^{1}H/ D TXI probehead with *z*-gradient. 1D-^{1}H experiments with water-to-ethanol solvent presaturation was carried out with a NOESY experiment and a home-made shape-pulse multipresaturation module, centring the transmitter frequency at 4.69 ppm (water) and shifting the decoupler frequency between 3.51 ppm (CH$_2$-ethanol) and 1.04 ppm (CH$_3$-ethanol) for accurate multipresaturation of all signals, during both relaxation delay (5 seconds) and mixing time (100 ms), with a 0.000115 W power level irradiation. A total of 32 transients were collected into 28 K complex data points with a spectral width of 9615 Hz and acquisition times of 1.5 seconds produce experimental times of 2′41″.

The new experiment Wine T_1, composed by a:

$$90° - \tau - 180° - \tau_{(var)} - 90° - acq \qquad (1)$$

pulse sequence consist in a first $90° - \tau$ module to multipresaturate water and ethanol intense signals, such as for the above mentioned 1D-^{1}H experiments. The second inversion recovery $180° - \tau_{(var)}$ module consist in the inversion of the generated *xy* spin coherences followed by a variable delay list, consisting in 22 points from 10 ms to 5 seconds, whereas residual magnetization that survive the relaxation delay module, is acquired within the third $90° - acq$ module of the Wine-T_1 experiment (see Fig. 2). All Wine-T_1 experiments were carried out with 8 transients of 15 K complex, having recycling delays of 5 seconds and with acquisition times of 800 ms produce experimental times of 1 h 3′. Validation of the method was done by analysing each wine batch by triplicate. Fitting of saturation-inversion-recovery decay curves with mono- and bi-exponential fitting was done with the Bruker Biospin software Dynamics Center, using a least-square fitting routine with an incorporated Monte Carlo error estimation analysis, whereas all mathematical treatment was done as described in previous reports [12].

3. Results and discussion

Although standard 1D proton experiments allows the assignment of some isolated major resonances like acetates, pyruvic, succinic and lactic acids, $\alpha - \beta$ glucose or fructose signals and major alcohols such as isobutanol, isopentanol, propanol or methanol (Fig. 3), there are still an important number of unassigned signals within the spectra due to signal overlap or weak signal intensity, such as the aliphatic region comprised between 3.5 to 4.5 ppm, as well as the aromatic region between 6 to 8 ppm. The Wine-T_1 experiment is an attempt to increase the number of assigned metabolites by dispersing the
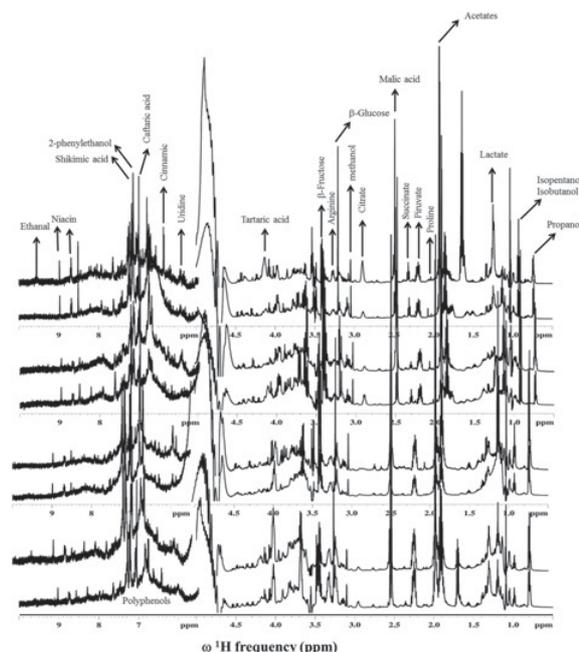

Figure 3. Proton one-dimensional Nuclear Magnetic Resonance (NMR) spectra with multipresaturation of water and ethanol signals. From bottom to top: AGR, J210, M16, M17, N16, N17, S16 and S17. The set of assigned resonances within the stacked plots were obtained by a NMR- Multivariate Statistical Analysis preliminary approach, carried out with Mexican wines [1] and confirmed with respect orthogonal literature related to Port wines [15].

overlapped chemical shifts within the T_1-{^{1}H} dimension (Fig. 4).

From Fig. 3, it can be observed that most of the assigned resonances within the 1D-^{1}H NMR fingerprint are well-resolved, intense and easily-identified isolated resonances. In contrast, an important set of unassigned overlapped resonances from 1D-^{1}H NMR spectra comprising regions between 1 to 1.5 ppm, 6.5 to 7.5 ppm and the most crowded region between 3.5 to 4.5 ppm, are observed. For that it is desirable to extend the dispersion of overlapped resonances by the addition of a second dimension. Figure 4 shows the benefits to disperse overlapped chemical shifts within the addition of the T_1-{^{1}H} dimension obtained with the Wine-T_1 experiment. Respectively, AGR, J210, M16, M17, N16, N17, S16 and S17 present a couple or tens of different T_1-{^{1}H} values for crowded regions that can allow to identify a complete set of unassigned metabolites by their differences in proton spin-lattice relaxation values.
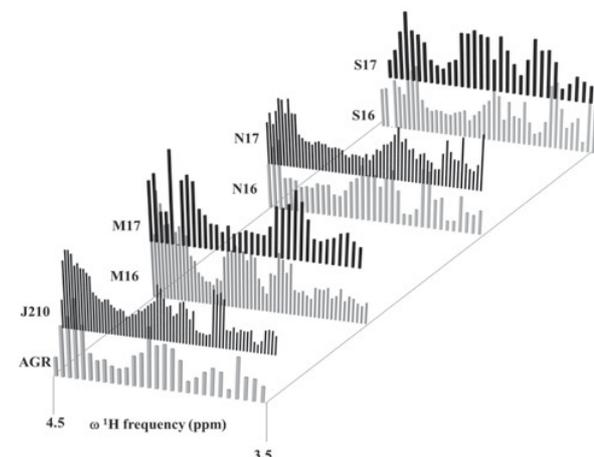
For the most critical shift crowding comprising the ^{1}H-NMR spectral region between 3.5 and 4.5 ppm (Fig. 3), the novel Wine-T_1 approach reveals an attractive way to disentangle a distribution of metabolites encumbered in the above mentioned frequency region (Fig. 5). The T_1-{^{1}H} distribution over said frequency range could be regarded as an additional wine fingerprint due to its characteristic pattern, regardless the number of total computed spin-lattice relaxation values per wine sample, defined at said aliphatic region. In contrast, the number of unambiguous assignments obtained directly from the ^{1}H spectra between 3.5 and 4.5 ppm, until know is restricted to only tartaric acid (Fig. 3). Overall, we claim that large-scale application
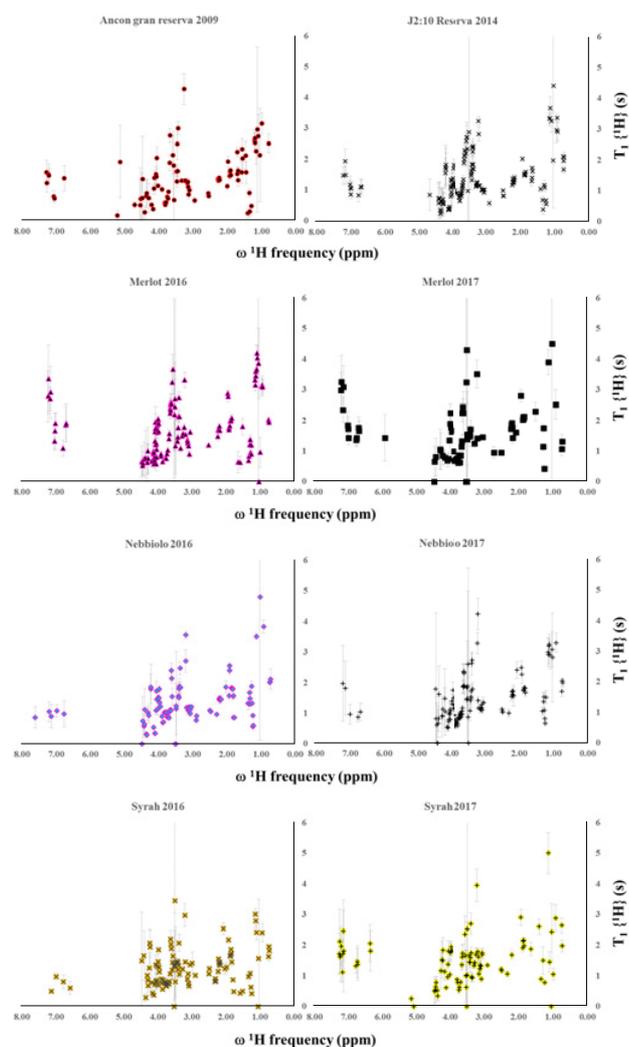
Figure 4. Pseudo two-dimensional Wine T_1 NMR experiment and computed proton spin-lattice relaxation times (T_1-{^{1}H}), of the complete set of resonances associated to metabolites found in the following wine samples (from top to bottom and from left to right): AGR, J210, M16, M17, N16, N17, S16 and S17.

Figure 5. Histogram distribution of spin-lattice relaxation computed values (T_1-{^{1}H}) with the Wine- T_1 approach, within the frequency range between $3.5 – 4.5$ ppm of wine samples AG, J210, M16, M17, N16, N17, S16 and S17. To refer the exact $T_1 − {^1H}$ value, see Fig. 4.
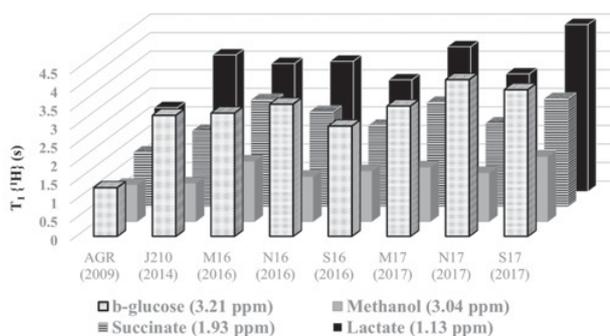
Figure 6. Histogram distribution of T_1-{^{1}H} values of β-glucose (3.21 ppm); methanol (3.04 ppm); succinate (3.21 ppm) and lactate (1.13 ppm) and dependence with respect the year of vintage of Ancon Gran Reserva (2009); J2:10 (2014); Merlot, Nebbiolo and Syrah (2016) and Merlot, Nebbiolo and Syrah (2017).

of the Wine-T_1 experiment at the wine industry, combined with the current advances in targeted and non-targeted NMR – multivariate statistical analysis (MSA), opens the way to have a robust metabolomics wine fingerprint for authentication of geographical origin, grape variety, year of vintage and barrel-/bottle-aging times. Scaling-up the spin-lattice relaxation experiment herein presented will allow to define the precise metabolites that define histogram presented in Fig. 5.

Limitations of the Wine-T_1 experiment lay mainly in the poor prediction or high data dispersion of T_1-{^{1}H} values close to the intense water and ethanol signals that are prior suppressed with the first $90 − \tau$ saturation module of the pulse sequence. Suppression of the water and ethanol signal is carried out by applying a modulated shaped pulse during the relaxation delay (variable D1, Fig. 1) and mixing time (variable "DELTA", Fig. 1). However, it is well know the disturbances that solvent suppression schemes promotes within vicinal frequencies and the increment of these artefacts when several signals are simultaneously suppressed [16]. Triple suppression

provokes the T_1-{^{1}H} values of resonances, with close vicinity (± 0.15 ppm) to the suppressed signals at 4.7, 3.5 and 1.02 ppm, were importantly over or under estimated (see error bars within Fig. 4).

Finally, Table 1 summarizes the proton spin-lattice relaxation values (T_1-{^{1}H}) of those isolated resonances unambiguously assigned, for the full set of wine samples (Fig. 3). General trend between T_1-{^{1}H} and year of vintage is that faster spin-lattice relaxation times were observed for longer aged samples (AGR 2009) in most of the cases. Despite the last observation is not linearly increased for younger wines for most of the assigned metabolites, longer T_1-{^{1}H} values for 2017 wines were observed with respect "*Grand cru*" wines (2009) for β-glucose, methanol, succinate and lactate spin systems (Fig. 6). Further validations must have to be performed in order to conclude that assigned signals at 3.21, 3.04, 1.93 and 1.13 ppm could serve as a year of vintage fingerprint, such as demonstrated in Fig. 6.

Interestingly, for some resonances it was observed a trend between grape variety and T_1-{^{1}H}. For instance,

Table 1. Proton spin-lattice relaxation values (T$_1$-{^{1}H}, in seconds), obtained with the Wine-T$_1$ NMR experiment of assigned resonances (Fig. 3) in wine samples. The legend "NI" indicates "Not Identified".

	AGR (2009)	J210 (2014)	M16 (2016)	N16 (2016)	S16 (2016)	M17 (2017)	N17 (2017)	S17 (2017)
Propanol (0.74 ppm)	2.51 ± 0.29	1.98 ± 0.06	1.95 ± 0.11	2.02 ± 0.07	1.96 ± 0.05	1.31 ± 0.22	1.97 ± 0.12	1.98 ± 0.19
Isobutanol (0.92 ppm)	3.15 ± 0.34	3.36 ± 0.13	3.15 ± 0.22	3.83 ± 0.21	2.41 ± 0.28	2.52 ± 0.47	3.28 ± 0.31	2.88 ± 0.43
Isope ntanol (1.04 ppm)	2.74 ± 0.7	2.96 ± 0.41	4.04 ± 0.41	3.51 ± 0.09	2.42 ± 0.28	3.9 ± 0.4	3.06 ± 0.56	2.43 ± 0.91
Lactate (1.13 ppm)	2.26 ± 0.41	3.68 ± 0.37	3.45 ± 0.15	3.51 ± 0.09	3.01 ± 0.17	3.9 ± 0.4	3.17 ± 0.25	4.5 ± 0.67
Ace tate (1.33 ppm)	0.297 ± 0.15	0.39 ± 0.15	0.835 ± 0.15	1.67 ± 0.14	0.975 ± 0.13	1.14 ± 0.13	1.04 ± 0.15	0.894 ± 0.05
Piruvate (1.85 ppm)	NI	1.62 ± 0.03	2.01 ± 0.05	1.8 ± 0.04	1.87 ± 0.03	2.12 ± 0.07	1.18 ± 0.07	2.16 ± 0.1
Succinate (1.93 ppm)	1.46 ± 0.09	2.04 ± 0.07	2.87 ± 0.11	2.55 ± 0.06	2.18 ± 0.11	2.8 ± 0.2	2.24 ± 0.29	2.9 ± 0.26
Malic acid (2.49 ppm)	0.8 ± 0.05	0.832 ± 0.02	1.2 ± 0.03	1.09 ± 0.03	1.07 ± 0.02	0.957 ± 0.07	1.01 ± 0.04	1.17 ± 0.03
Citrate (2.89 ppm)	0.664 ± 0.1	0.595 ± 0.1	NI	0.912 ± 0.07	1.02 ± 0.07	NI	1.31 ± 0.05	1.35 ± 0.02
Methanol (3.04 ppm)	0.991 ± 0.03	1.03 ± 0.05	1.62 ± 0.08	1.21 ± 0.03	1.36 ± 0.02	1.46 ± 0.08	1.31 ± 0.05	1.75 ± 0.05
β-glucose (3.21 ppm)	1.31 ± 0.04	3.26 ± 0.14	3.32 ± 0.25	3.56 ± 0.11	2.97 ± 0.37	3.51 ± 0.46	4.22 ± 0.33	3.95 ± 0.53
β-fructose (3.4 ppm)	1.62 ± 0.20	1.72 ± 0.13	1.77 ± 0.21	1.8 ± 0.23	1.43 ± 0.13	1.49 ± 0.34	1.46 ± 0.3	1.44 ± 0.3
Tartaric acid (3.94 ppm)	1.02 ± 0.09	1.01 ± 0.07	0.89 ± 0.11	0.904 ± 0.06	0.901 ± 0.06	0.991 ± 0.09	0.898 ± 0.08	0.88 ± 0.06
Caftaric acid (7.05 ppm)	0.717 ± 0.13	1.2 ± 0.28	1.33 ± 0.3	0.905 ± 0.37	0.979 ± 0.11	1.84 ± 0.4	0.939 ± 0.3	1.81 ± 0.9
2-phe nyle thanol (7.2 ppm)	1.47 ± 0.23	1.5 ± 0.28	3.35 ± 0.37	1.05 ± 0.32	0.495 ± 0.04	3.25 ± 0.57	1.79 ± 0.88	1.12 ± 0.33

the isopentanol signal (1.04 ppm) present equivalent relaxation values for pure Merlot (4.04 ± 0.41; 2016/3.9 ± 0.4; 2017), Nebbiolo (3.51 ± 0.09; 2016/3.06 ± 0.56; 2017) and Syrah (2.42 ± 0.28; 2016/2.43 ± 0.91; 2017) regardless their year of vintage. Equivalent observations were warned for T$_1$-{^{1}H} values detected at the 2-phenylethanol signal: Merlot (3.35 ± 0.37; 2016/3.25 ± 0.57; 2017), Nebbiolo (3.51 ± 0.09; 2016/3.06 ± 0.56; 2017). Again, further and extensive validations are compulsory to verify observed trends.

4. Conclusions

For the first time to the best of our knowledge, proton spin-lattice relaxation times were used as wine metabolomics profiles in an effort to increase the number of assigned metabolites in wine samples, mostly in encumbered regions. For that, it is presented the novel Wine-T$_1$ NMR experiment, optimized to obtain T$_1$-{^{1}H} values from 1D-^{1}H NMR spectra with a previous triply solvent suppression module. The Wine-T$_1$ was coded in a Bruker NMR platform, but this experiment can be adapted to Jeol or Varian/Agilent environments, upon request. It has been demonstrated that Wine-T$_1$ experiments notably increases the dispersion of overlapped regions within a second spin-lattice dimension, that allowed to identify tens of novel identities in situations of severe signal overlap. Finally, preliminary observations strongly suggest that T$_1$-{^{1}H} values could serve as probe to disentangle slight differences between wines' year of vintages or grape varieties.

References

[1] R. Godelman, F. Fang, E. Humpfer, B. Schütz, M. Bansbach, H. Schäfer, M. Spraul, J. Agric. Food Chem. **61**, 5610 (2013)

[2] T. Jézéquel, V. Joubert, P. Giraudeau, G.S. Remaud, S. Akoka, Magn. Reson. Chem. **55**, 77 (2017)

[3] G.J. Martin, M. Mazure, C. Jouitteau, Y.L. Martin, L. Aguile, P. Allain, Am. J. Enol. Vitic. **50**, 409 (1999)

[4] N. Ogrinc, I.J. Kosir, M. Kocjancic, J. Kidric, J. Agric. Food Chem. **49**, 1432 (2001)

[5] J.E. Giménez-Miralles, D.M. Salazar, I. Solana, J. Agric. Food. Chem. **47**, 2645 (1999).

[6] J. Wei, X. Jie, L. Xiang, W. De-liang, G. Yang, W. Lu, Int. J. Food Sci. Technol. **50**, 774 (2015)

[7] A.M. Gil, I.F. Duarte, M. Godejohann, U. Braumann, M. Maraschin, M. Spraul, Anal. Chim. Acta **488**, 35 (2003)

[8] I.F. Duarte, M. Godejohann, U. Braumann, M. Spraul, A.M. Gil, J. Agric. Food Chem. **51**, 4847 (2003)

[9] A. Ramos, H. Santos, Annu. Rep. NMR Spectrosc. **37**, 179 (1999)

[10] L. Mannina, A. Sobolev, S. Viel, PNAS **66**, 1 (2012)

[11] J. Keeler, *Understanding NMR Spectroscopy*, 2nd edn (Wiley ISBN 978-0-470-74608-0, 2002), p. 96

[12] J.E. Herbert-Pucheta, D. Prim, J.M Gillet, J. Farjon, Chem. Phys. Chem. **17**, 1 (2016)

[13] J.E. Herbert-Pucheta, H. Colaux, G. Bodenhausen, P. Tekely, J. Phys. Chem. B **115**, 15415 (2011)

[14] H. Wang, M. Zhao, J.L. Ackerman, Y. Song, J. Magn. Reson. **274**, 137 (2017)

[15] M. Nilsson, I.F. Duarte, C. Almeida, I. Delgadillo, B.J. Goodfellow, A.M. Gil, G.A. Morris, J. Agric. Food Chem. **52**, 3736 (2004)

[16] S. De Sanctis, W.M. Malloni, W. Kremer, A.M. Tomé, E.W. Lang, K.P. Neidig, H.R. Kalbitzer, J. Magn. Reson. **210**, 177 (2011)