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1 MULTISPECTRAL FLUORESCENCE SENSITIVITY TO ACIDIC AND POLYPHENOLIC CHANGES IN  
2 CHARDONNAY WINES – THE CASE STUDY OF MALOLACTIC FERMENTATION

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27 **Abstract :**

28 In this study, stationary and time-resolved fluorescence signatures, were statistically and  
29 chemometrically analyzed among three typologies of Chardonnay wines (A, B and C) with the  
30 objectives to evaluate their sensitivity to acidic and polyphenolic changes. For that purpose, a dataset  
31 was built using Excitation Emission Matrices of fluorescence (N=103) decomposed by a Parallel  
32 Factor Analysis (PARAFAC), and fluorescence decays (N=22), mathematically fitted, using the  
33 conventional exponential modeling and the phasor plot representation.

34 Wine PARAFAC component C4 coupled with its phasor plot g and s values enable the description of  
35 malolactic fermentation (MLF) occurrence in Chardonnay wines. Such proxies reflect wine  
36 concentration modifications in total acidity, malic/lactic and phenol acids. Lower g values among  
37 fresh MLF+ wines compared to MLF- wines are explained by a quenching effect on wine fluorophores  
38 by both organic and phenolic acids. The combination of multispectral fluorescence parameters opens a  
39 novel routinely implementable methodology to diagnose fermentative processes.

40

41 **Keywords :**

42 Malolactic fermentation - organic acids – traceability - PARAFAC components - fluorescence lifetime  
43 – phasor plot

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## 55 1. Introduction

56 Fluorescence spectroscopy techniques offer the advantage of a fast, robust and non-destructive  
57 characterization ability over expensive and time-consuming separative techniques. Their high  
58 sensitivity and specificity are important and allow the study of fluorescent compounds and chemical  
59 families among the complex chemistry present in food matrices and particularly wine. Over the past  
60 decade, much has been written on fluorescence usefulness in providing helpful classification  
61 indicators regarding cultural practices, varieties distinction, technological practices, regional  
62 authentication, adulteration and spoilage problematics (Airado-Rodríguez, Durán-Merás, Galeano-  
63 Díaz, & Wold, 2011; Cabrera-Bañegil, Valdés-Sánchez, Moreno, Airado-Rodríguez, & Durán-Merás,  
64 2019; Coelho, Aron, Roullier-Gall, Gonsior, Schmitt-Kopplin, & Gougeon, 2015; Elcoroaristizabal,  
65 Callejon, Amigo, Ocana-Gonzalez, Morales, & Ubeda, 2016; Mierczynska-Vasilev et al., 2021; Moret  
66 et al., 2019; Ranaweera, Gilmore, Capone, Bastian, & Jeffery, 2021; Robert-Peillard, Boudenne, &  
67 Coulomb, 2014; Saad, Bouveresse, Locquet, & Rutledge, 2016; Sadecka, Jakubikova, & Majek, 2018;  
68 Santos, Bosman, Aleixandre-Tudo, & du Toit, 2022; Wan, Pan, & Shen, 2012; Zhong et al., 2014).

69 From a practical point of view, fluorescence spectroscopy is classically used by winegrowers and  
70 winemakers to help them in their decision-making process. For instance, red grape maturity can be  
71 estimated using anthocyanins and chlorophyll steady state fluorescence proxies (Agati et al., 2013;  
72 Agati, Meyer, Matteini, & Cerovic, 2007). Trivellin *et al.* used the fluorescence lifetime variations of a  
73 metal/porphyrin complex to measure oxygen concentrations in wine fermentation and ageing tanks  
74 (Trivellin et al., 2018). This technology is widely used in wine industries for dissolved oxygen  
75 measurement during winemaking steps and wine bottling. The detection of wine yeasts and bacteria  
76 is also based on the steady state fluorescence response of labelled microorganisms, sorted by a flow  
77 cytometer and used by several oenological laboratories (Longin, Petitgonnet, Guilloux-Benatier,  
78 Rousseaux, & Alexandre, 2017). Other applications consisted in analysing the total steady state  
79 fluorescence of wines using Excitation Emission Matrices of fluorescence (EEMF) coupled to  
80 multivariate approaches in order to discriminate grape varieties, vintages, oenological or  
81 fermentation practices (Airado-Rodríguez, Galeano-Díaz, Durán-Merás, & Wold, 2009; Coelho et al.,  
82 2015). The measurement of fluorescence lifetimes of wine chemicals (resveratrol, flavylum and  
83 pyranoflavylum cations) when adsorbed on clay fining agents or adsorbents enabled the study of  
84 organic complexations and organic/inorganic interactions (Silva et al., 2019; Trigueiro et al., 2018).  
85 Some authors tried to relate wine fluorescence patterns to wine quality and to wine chemical  
86 composition (Coelho et al., 2015; Coelho, Gougeon, Perepelkine, Alexandre, Guzzo, & Weidmann,  
87 2019; Elcoroaristizabal et al., 2016; Saad et al., 2016; Sadecka et al., 2018).

88 Among the qualitative criteria enhancing wine quality, malolactic fermentation (MLF) is often  
89 achieved in cellars by endogenous or exogenous lactic acid bacteria, reducing acidity by conversion  
90 of malic acid into lactic acid and increasing organoleptic properties by generating new volatile  
91 compounds (Bauer & Dicks, 2017). MLF is a fermentative process that brings a reorganisation of the  
92 chemical composition of wines and contributes to flavour complexity. During this fermentative  
93 process, the phenolic profile of wines evolves and depends on the strains of lactic bacteria realizing  
94 MLF (T. Hernández, Estrella, Pérez-Gordo, Alegría, Tenorio, & Moreno-Arribas, 2007). MLF has been  
95 shown to influence the concentrations of hydroxycinnamic acids and their derivatives, increasing the  
96 concentrations of caffeic/p-coumaric/ferulic acids and decreasing those in their precursors such as  
97 caftaric/coutaric/fertaric acids (Cabrita, Torres, Palma, Alves, Patão, & Costa Freitas, 2008; Teresa  
98 Hernández, Estrella, Carlavilla, Martín-Álvarez, & Moreno-Arribas, 2006; T. Hernández et al., 2007;  
99 Martínez-Pinilla, Martínez-Lapuente, Guadalupe, & Ayestarán, 2012). MLF can also be accompanied  
100 by a wood contact time in wines providing novel sensory attributes to Chardonnay wines (González-  
101 Centeno, Chira, & Teissedre, 2019). Additionally MLF could distinguish cool-climate and warm-  
102 climate wine producing regions in the world (Bartowsky, 2005). In those regions, differences in malic  
103 lactic consumption and derived metabolisms during berry maturation phase, would increase the  
104 chemical variability on grapes and therefore on MLF+/-Chardonnay wines on a world scale  
105 (Bartowsky, 2005; Ruffner, 1982). For that reason, the use of high-throughput fluorescence  
106 spectroscopic techniques to diagnose an elevated number of diverse wine samples combining  
107 chemometric tools, appears to be a good alternative to evaluate both MLF+/- wine discrimination  
108 and associated chemical modifications.

109 In this paper, we discuss the application of multispectral fluorescence with chemometrics to  
110 differentiate Chardonnay wines based on their acidic modification once malolactic fermentation was  
111 achieved (MLF+) or not (MLF-). For that purpose, Excitation Emission Matrices of fluorescence  
112 (N=103) were decomposed by a Parallel Factor Analysis (PARAFAC) and fluorescence lifetime decays  
113 (N=22) were mathematically fitted, using the conventional exponential modeling and the phasor plot  
114 representation. The chemical significance of MLF discrimination among Chardonnay wines by  
115 multispectral fluorescence was evidenced by organic acids and polyphenol concentration  
116 measurement on the analyzed wines.

## 117 **2. Materials and Methods**

### 118 **2.1 Wine Samples**

119 One hundred and three Chardonnay wines were analyzed in this study gathering three typologies of  
120 Chardonnay wines, named as A, B and C wines. Twelve fresh wines during vintage 2021 from two

121 Burgundian wineries, Jean Claude Boisset at Nuit Saint Georges (named as A) and Domaine de  
122 l'Université de Bourgogne at Marsannay La Côte (named as B), were sampled before (MLF-) and after  
123 (MLF+) malolactic fermentation. Ninety-one Chardonnay wines were sampled during the  
124 "Chardonnay du Monde 2018" wine contest (named as C) organized in March 2018 at Chateau des  
125 Ravatys (Saint Lager, France), originating from 18 countries of the world, with no information  
126 concerning the MLF achievement. Table Supplementary Information (S.I.) 1, 2 & 3 gathers  
127 information collected for the different analyzed wines.

## 128 **2.2 Chemical analyses**

129 Each wine was analyzed by the OenoFoss™ (Foss, Nanterre, France) analyser for classical oenological  
130 parameters: ethanol, total acidity, glucose, pH and volatile acidity. A Beckman® P/ACE 5000 capillary  
131 electrophoresis (Beckman Coulter, Brea, CA, USA) equipped with a UV detector was used to  
132 determine the amount of the four main organic acids in wine : tartaric acid, malic acid, lactic acid and  
133 citric acid following the methodology recommended by Organisation Internationale de la Vigne et du  
134 Vin (OIV, 2011). Polyphenolic compounds in wines were separated and quantified using an Acquity  
135 Waters UHPLC-DAD-fluorometer (Waters Corporation, Milford, MA, USA), using a previously  
136 described methodology (Coelho et al., 2019).

## 137 **2.3 Wine Fluorescence measurements**

138 All wines were diluted forty times with ultrapure water and poured in a 1 cm path-length quartz  
139 cuvette prior to steady state and time resolved spectroscopic measurements. For time-resolved  
140 fluorescence, five MLF+ wines and five MLF- wines were also diluted forty times with ultrapure  
141 water.

### 142 **2.3.1 Steady state fluorescence**

143 Excitation-Emission Matrices of Fluorescence (EEMF) were acquired on a Horiba Aqualog (Horiba  
144 Scientific, Kyoto, Japan) unit by setting the excitation wavelengths from 225 nm to 600 nm (3 nm  
145 interval) and the emission wavelengths from 200 to 600 nm (3.22 nm). Each EEMF was corrected  
146 daily for Rayleigh scattering and inner filtering and normalized to 1ppm quinine sulfate solution,  
147 using the constructor software (Aqualog software). All EEMF were gathered in a Chardonnay wine  
148 database and mathematically analyzed with a PARAFAC model using the Matlab (Mathworks, Natick,  
149 MA, USA) drEEM box (Murphy, Stedmon, Graeber, & Bro, 2013).

### 150 **2.3.2 Time resolved fluorescence**

151 Fluorescence decay measurements were performed using time-correlated single-photon counting  
152 (TCSPC) with a Horiba Jobin Yvon FluoroMax-4 (Horiba Scientific, Kyoto, Japan). Excitation was  
153 generated using a 950 ps pulsed 280 nm LED head source (DD-280L, pulse duration <1.5 ns) with a  
154 fixed working frequency of 1 MHz. We used a 280 nm diode instead of a 315 nm diode to be as close  
155 as possible to the maximum excitation with the pulsed light sources at our disposal. A Ludox®  
156 solution (Sigma Aldrich, Saint Louis, MI, USA) solution was used to daily calibrate the impulsional  
157 response function of the instrument. The fluorescence signal collected at emission maximum  
158 corresponding to PARAFAC component C4: 440 nm. In TCSPC the time difference between each  
159 photon detection and the corresponding excitation pulse is measured and stored into a histogram,  
160 showing the fluorescence decay after an excitation pulse. A total of 10000 photons were counted in  
161 the peak of each histogram before stopping the acquisition. Histograms were 4096 gates (4096  
162 channels = 224.746 ns). The fluorescence decay was analyzed using the conventional exponential  
163 decay fitting function by the “DAS6 Analysis” analysis software (Horiba Scientific, Kyoto, Japan) and  
164 the phasor plot representation using the MAPI software (IRI, USR 3078 CNRS, BCF, available on  
165 request: <http://biophotonique.univ-lille1.fr/spip.php?rubrique60>) (Leray, Padilla-Parra, Roul, Hélot,  
166 & Tramier, 2013).

#### 167 • 2.3.2.1. Exponential decay fitting

168 If a uniform population of fluorophores are excited at a single excitation wavelength, the  
169 fluorescence intensity decay after an excitation pulse is described by a mono-exponential function,  
170 as follows:

$$171 \quad F(t) = F_0 \cdot e^{-t/\tau} \quad (\text{Equation 1})$$

172 Where F(t) is the fluorescence intensity measured at time t, F<sub>0</sub> is the initial fluorescence intensity and  
173 τ is the fluorescence lifetime.

174 For complex samples, due to different fluorescent subpopulations and different chemical  
175 environments, a multi-exponential fitting procedure should be used to estimate as best as possible  
176 the number of fluorescent subpopulations contributing to the fluorescence decay curve. Such fitting  
177 procedures are validated by a “χ<sup>2</sup>” (CHISQ) close to 1 (Moore, McCabe, & Craig, 2017).

178 In this study, we used a double exponential fitting function for all Chardonnay wines fluorescence  
179 decays, as follows:

$$180 \quad F(t) = B_1 \cdot e^{-t/\tau_1} + B_2 \cdot e^{-t/\tau_2} \quad (\text{Equation 2})$$

181 With F<sub>1</sub> = B<sub>1</sub> / (B<sub>1</sub>+ B<sub>2</sub>) and F<sub>2</sub> = B<sub>2</sub> / (B<sub>1</sub>+B<sub>2</sub>)

182 F(t) is the fluorescence intensity measured at time t, B<sub>1</sub> and B<sub>2</sub> are the pre-exponential factors, F<sub>1</sub> and  
183 F<sub>2</sub> the fractional intensities expressed in percentage, respectively for the subpopulations with  
184 fluorescence lifetimes τ<sub>1</sub> and τ<sub>2</sub> (τ<sub>1</sub> < τ<sub>2</sub>).

185 • **2.3.2.2. Phasor plot representation**

186 The phasor plot is a graphical representation of all the raw TCSPC data in a vector space obtained  
187 by the mathematical Fourier transform decomposition. Each wine fluorescence decay curve is  
188 converted into [g ; s] coordinates, calculated by the cosine and sine transforms of the measured  
189 fluorescence intensity decay I(t), defined by:

190 
$$g_{i,j}(\omega) = \frac{\int_0^T I(t) \cdot \cos(n\omega t) dt}{\int_0^T I(t) dt} \text{ (Equation 3)}$$

191 
$$s_{i,j}(\omega) = \frac{\int_0^T I(t) \cdot \sin(n\omega t) dt}{\int_0^T I(t) dt} \text{ (Equation 4)}$$

192

193 Where ω is the laser repetition angular frequency, n is the harmonic frequency and T is the finite  
194 width of the temporal measurement window. For a mono-exponential decay, the [g ; s] coordinates  
195 are located on a semicircle centred on a point [0.5;0] with a radius 0.5. Whereas for a multi-  
196 exponential decay, the [g ; s] coordinates will no longer be located on the semicircle but rather  
197 inside this semicircle. In the semicircle, the short fluorescence lifetimes are close to the coordinates  
198 [1;0], and the long fluorescence lifetimes approach the origin [0;0].

199 Fluorescein (fluorescence lifetime: 4.1 ns) was used in the phasor plot representation for calibrating  
200 measurements between values obtained by exponential decay fitting and phasor plot analysis.

201 **2.4. Statistical analysis**

202 The measured oenological parameters, PARAFAC components Fmax values, fluorescence lifetimes  
203 and phasor plot g and s values of Chardonnay wines were statistically analyzed with a variance  
204 analysis (p-value <0.05) to evaluate the significance of differentiation between MLF+ and MLF- wines.  
205 This was conducted with Origin pro 8.0 software (OriginLab, Northampton, MA). Two types of  
206 multivariate analysis were carried out with SIMCA software (Umetrics, AB, Umea, Sweden): principal  
207 component analysis (PCA) with PARAFAC components Fmax with and without g and s values, and  
208 partial least square analysis (PLS) to evaluate the discrimination of MLF achievement in Chardonnay  
209 wines based on PARAFAC components Fmax values and g and s values obtained for the three  
210 typologies of Chardonnay wines (N=22).

211



### 212 3. Results and discussion

#### 213 3.1 Wine chemical composition

214 Table 1 illustrates the chemical constituents that statistically differentiate wines according to MLF  
215 achievement among the 103 Chardonnay wines. Citric acid and pH were not significantly different.  
216 Tartaric acid, volatile acidity and glucose appeared to be significantly different between the two  
217 groups at 95% of confidence interval. Mean values of glucose concentrations were comprised  
218 between 2.59 and 3.03 g.L<sup>-1</sup>, indicating that all wines are dry white wines. Total acidity, malic acid  
219 and lactic acid are the only three parameters that are significantly different at 99 % of interval of  
220 confidence. Mean values of ethanol and malic acid are higher in MLF- wines compared to MLF+  
221 wines, respectively. Inversely, total acidity and lactic acid mean values are higher in MLF+ wines  
222 compared to MLF- wines. The great variability of provenance among the Chardonnay contest wines  
223 (Table S.I.3), led to a statistical differentiation of alcohol content in wines, principally due to grape  
224 maturity variability among all countries, but no differentiation in ethanol content is generally  
225 admitted. Results from fresh wines highlight the same tendency. Hence only the Total acidity and  
226 Malic/Lactic content of wines are truly statistically differentiate those wines. Additionally, for  
227 Chardonnay contest wines, the distinction between MLF+ and MLF- wines should be attenuated by  
228 the fact that malic and lactic acid could be added up to 4 g.L<sup>-1</sup> (expressed in tartaric acid equivalent)  
229 in musts and wines for acidification purposes according to OIV regulations (OIV, 2017). Some wines  
230 could have been bottled and stabilized while malolactic fermentation was still in progress.

231 *Table 1 : Physicochemical parameters of wines for the 103 analyzed Chardonnay wines samples (A+B+C).*

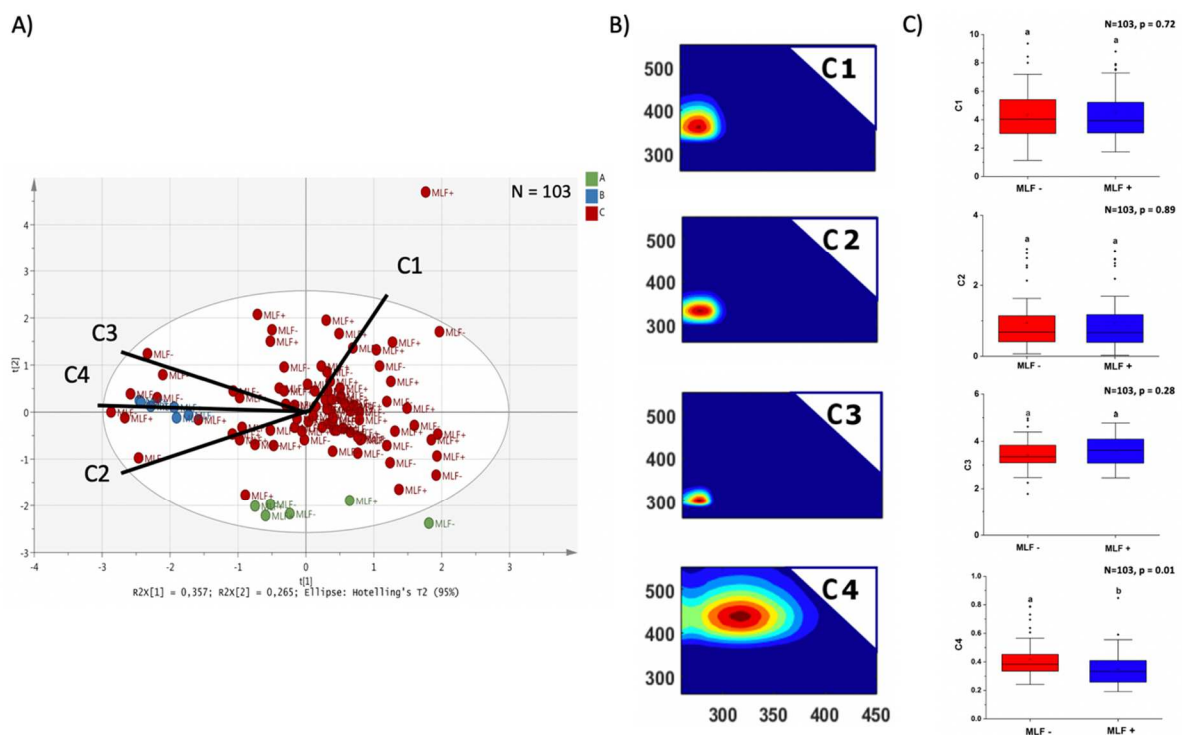
	MLF- wines	MLF+ wines
233 <b>Alcohol (%v,v)</b>	13.28 (0.83)	12.98 (0.67)*
234 <b>pH</b>	3.48 (0.14)	3.46 (0.14)
<b>Glucose (g.L<sup>-1</sup>)</b>	2.59 (1.87)	3.03 (1.62)
235 <b>TA (g.L<sup>-1</sup>)</b>	4.04 (0.42)	3.72 (0.34)**
<b>Malic acid (g.L<sup>-1</sup>)</b>	2.56 (0.71)	0.52 (0.53)**
236 <b>Lactic acid (g.L<sup>-1</sup>)</b>	0.39 (0.24)	3.59 (1.10)**
<b>Tartaric acid (g.L<sup>-1</sup>)</b>	1.81 (0.57)	2.05 (0.54)*
237 <b>Citric acid (g.L<sup>-1</sup>)</b>	0.50 (0.79)	0.61 (0.85)
<b>N</b>	50	53

238 *TA: total acidity expressed in tartaric acid equivalent per liter; N: number of wine samples; figures in*  
239 *parentheses, standard deviations; \*\*: significant with p-value < 0.01 between the two groups of wines (MLF+/-);*  
240 *\* : significant with p-value < 0.05 between the two groups of wines (MLF+/-).*

241

242 **3.2 PARAFAC components describing Chardonnay wine diversity and sensitivity towards MLF**  
 243 **achievement on full sample (A+B+C)**

244 Fresh wines from two wineries (A and B), and Contest Chardonnay wines (C) were described by their  
 245 EEMFs (examples of each group shown in Figure S.I.1). A wine PARAFAC model generates 4 PARAFAC  
 246 components C1, C2, C3 and C4 describing all the analyzed wines (Figure 1). The PARAFAC model was  
 247 pre-validated with a core consistency of 80% and further split half validated with four independent  
 248 splits on the 4 component PARAFAC model, according to previously published procedures (Murphy et  
 249 al., 2013). Figure 1A shows that the first two components of the PCA model cover 62.2 % of the  
 250 fluorescence variability among the 103 analyzed wines. At a first glance, Chardonnay wines were  
 251 heterogeneously distributed in terms of MLF achievement among these wines. Chardonnay wines  
 252 from the two wineries A and B were clearly differentiated by means of their steady-state  
 253 fluorescence. A less evident distinction appeared between fresh wines and wines from the  
 254 Chardonnay wine contest. Such differentiation could be related to some particular attributes already  
 255 discussed in literature (Coelho et al., 2015; Suci, Zarbo, Guyon, & Magdas, 2019) gathering the  
 256 vintage effect (2020 for the fresh wines compared to 2015-2017 for the contest wines) and the  
 257 winemaking techniques (stabilization, fining, filtration, bottling and surely the bottle ageing enabling  
 258 to differentiate Chardonnay fresh wines from bottled contest wines). Even if it was not the objective  
 259 of the study, some wine origins were rather well discriminated among wines from the typology C,  
 260 particularly when representing Fmax values of PARAFAC C3 against C4 (Figure S.I.2).



261

262 *Figure 1 : Biplot representation of the first two components of a PCA model describing Fmax values of PARAFAC*  
263 *components for Chardonnay fresh wines from wineries A (green) and B (blue) and from Chardonnay wine*  
264 *contest C (red) (A). PARAFAC components EEMFs that were used as loadings for the PCA model (B). Analysis of*  
265 *variance of the four PARAFAC components Fmax values depending on the malolactic fermentation achievement*  
266 *(MLF+) or not (MLF-) (C). Different letters above box plot (a,b) represent significant differences (p=0.05)*  
267 *between the fermentative conditions.*

268

269 EEMFs of the four PARAFAC components were generated with a PARAFAC model validated with a  
270 core-consistency diagnostic and a split-half validation made on four independent splits. They were in  
271 accordance with results from recent studies carried out on wines (Coelho et al., 2015;  
272 Elcoroaristizabal et al., 2016; Ranaweera et al., 2021; Suciú et al., 2019). We further study the ability  
273 of PARAFAC components to differentiate MLF achievement among wines, even though a global  
274 heterogeneity and variability from the wine provenance were previously discussed. Figure 1C shows  
275 the boxplot of the four PARAFAC components Fmax values. Within this variability and heterogeneity  
276 of Chardonnay wines: C1, C2 and C3 were not sensitive enough to distinguish the MLF achievement  
277 (same letter “a” between groups above the box plots). Nevertheless, PARAFAC component C4 Fmax  
278 values appeared to be statistically higher in MLF+ wines (0.41 +/- 0.14) compared to MLF- wines  
279 (0.34 +/- 0.12).

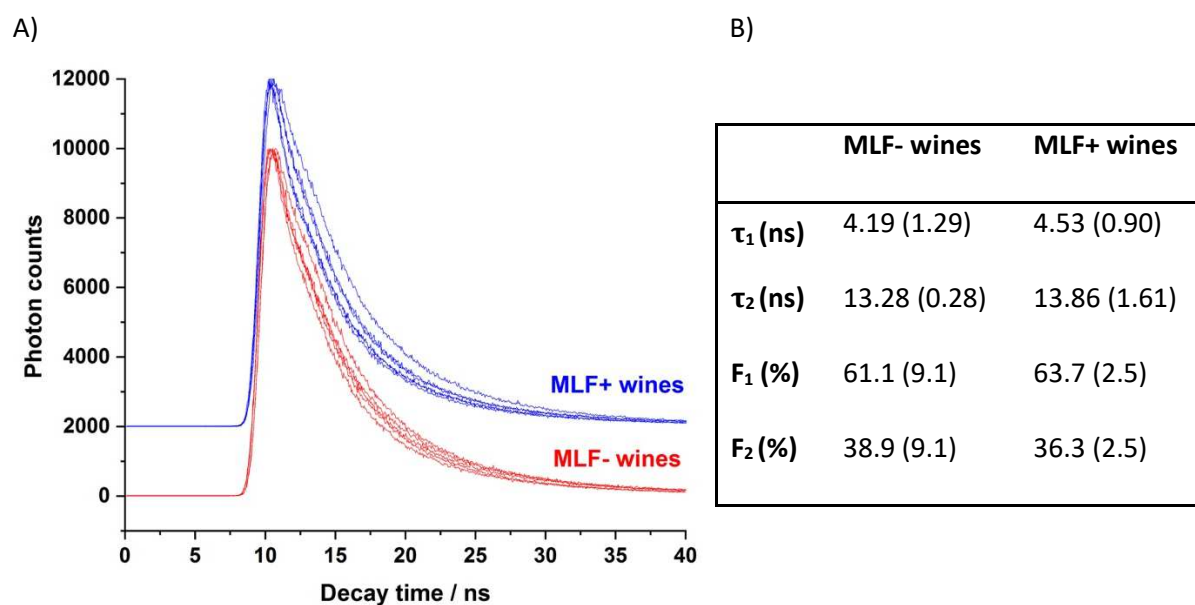
280 Previous studies highlighted this point among non-flavonoid compounds and more particularly  
281 tartaric esters of phenolic acids that are metabolized during MLF due to esterase activities present in  
282 several malolactic bacteria and liberating free phenolic and hydroxycinnamic acid derivatives (Cabrita  
283 et al., 2008; Collombel, Melkonian, Molenaar, Campos, & Hogg, 2019; Pérez-Martín, Seseña,  
284 Izquierdo, & Palop, 2013). As an example, tartaric acid has already been indicated as a good  
285 candidate with optical similarities and well correlated with PARAFAC component C4 but obviously  
286 this is not the only one ~~sole~~ (Roullier-Gall et al., 2017).

### 287 **3.3 Fluorescence lifetime decay of PARAFAC component C4 in Chardonnay wines**

#### 288 **3.3.1. Exponential decay fitting analysis of sample C**

289 To interpret the discrimination between MLF- and MLF+ wines in terms of physico-chemistry of wine  
290 fluorophores behaving like the PARAFAC component C4 (Ex 315 nm/Em 440 nm) we investigate the  
291 fluorescence decays of wine fluorophores emitting at 440 nm when excited at 280 nm and not 315  
292 nm for all this study. Figure 2 shows the fluorescence decays of MLF- and MLF+ Chardonnay wines,  
293 taken randomly from the Chardonnay contest wine series (C). They all present at least a bi-  
294 exponential decrease of intensity with short lifetimes  $\tau_1$  comprised between mean values of 4.19 ns  
295 and 4.53 ns and longer fluorescence lifetimes  $\tau_2$  comprised between 13.28 ns and 13.86 ns.  
296 Regardless of MLF achievement, fractional intensities were higher for the short fluorescence lifetime

297  $\tau_1$  comprised between 61.1 and 63.7 % compared to 36.3 and 38.9 % for lifetime  $\tau_2$  showing this  
 298 general tendency for Chardonnay wines at constant pH equal to 5. This heterogeneous distribution of  
 299 fluorescence lifetimes indicated that fluorescence decay is not attributed to sole monomeric  
 300 fluorescent compound but also to condensed structures, which have already been associated with  
 301 flavonoids dimers (Bergmann, Barkley, Hemingway, & Mattice, 1987) and for polyphenolic  
 302 associations with proteins (Mueller-Harvey et al., 2012). Such chromophoric macromolecular  
 303 compounds derived from flavonoids or non-flavonoids should be present in Chardonnay white wines  
 304 (Coelho et al., 2017). The four mathematical parameters ( $\tau_1$ ,  $\tau_2$ ,  $F_1$  and  $F_2$ ) present no statistical  
 305 difference between MLF- and MLF+ wines, indicating that the fluorophores should reveal intimately  
 306 close environments related to an abundant phenolic population. Those wine emitting moieties are  
 307 characterized by a majority of short fluorescence lifetimes and they should be associated in larger  
 308 structures covalently bound or attached by interaction as reflected by the contribution of longer  
 309 fluorescence lifetimes.



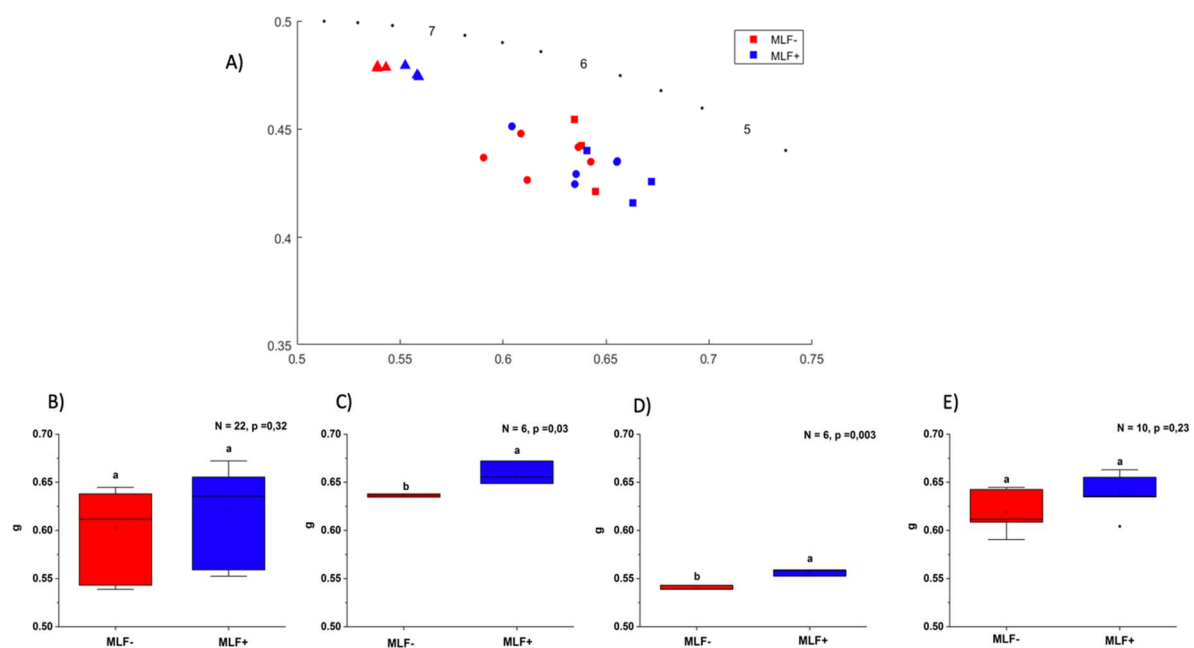
310 *Figure 2 : Decay of fluorescence measured at pH=5 of MLF+ (blue) and MLF- wines (red) sampled during*  
 311 *Chardonnay wine contest C with an excitation wavelength set as 280 nm and emission set as 440 nm. The*  
 312 *baseline of the blue curve is shifted to better observe changes in fluorescence decay (A). Mean values of decay*  
 313 *lifetimes  $\tau_1$  and  $\tau_2$ , expressed in ns and their associated fractional intensities  $F_1$  and  $F_2$  expressed in*  
 314 *percentage, as indicated in equation 2, at a fixed pH=5. Values in parenthesis indicate the mean standard*  
 315 *deviations for the five MLF+ wines (N=5) and MLF- wines (N=5) (B).*

316

### 317 3.3.2. Phasor plot analysis

318 For the following part, we use the (g,s) coordinates of wines to characterize fluorescence lifetime  
 319 decays of wines from wineries A (N=6) and B (N=6) and from Chardonnay wine contest C (N=10). This  
 320 type of analysis is commonly used in biology and medicine but has never been reported in food or

321 wine science to our knowledge. This approach better assesses heterogeneous fluorescence as  
 322 encountered in wine environments (Ranjit, Malacrida, Jameson, & Gratton, 2018; Stringari, Cinquin,  
 323 Cinquin, Digman, Donovan, & Gratton, 2011) and to depict physico-chemical interactions of  
 324 fluorophores due to changes in pH, metal and metabolite concentration (Battisti, Digman, Gratton,  
 325 Storti, Beltram, & Bizzarri, 2012; Celli, Sanchez, Behne, Hazlett, Gratton, & Mauro, 2010; Martelo,  
 326 Fedorov, & Berberan-Santos, 2015). In our experimentation, the phasor plot approach helps to  
 327 visualize the entire dataset of wine fluorescence decays dataset for MLF+/- wines. From the phasor  
 328 plot (Figure 3) MLF+ wines appear to shift to higher g values. In order to validate this observation, we  
 329 apply a variance analysis with a confidence interval of 95%. Mean values for the three typologies of  
 330 wines are represented in the boxplot representations (Figure 3B, 3C, 3D and 3E). When all  
 331 Chardonnay wines (N=22) are represented, g values are not statistically different between MLF- and  
 332 MLF+ wines due to a high variability, even if a trend to higher g mean values is observed (from 0.60  
 333 to 0.62 in Figure 3B). This variability comes essentially from unfollowed fermentative processes  
 334 during wine elaboration as observed in Figure 3E. Additional variability is brought by great  
 335 discrepancies observed for g-values from winery A (Figure 3C) and winery B (Figure 3D) presenting  
 336 mean g values of 0.65 and 0.55, respectively. Interestingly, for these fresh wines, regardless of the  
 337 winery origin, MLF+ wines present systematically higher g mean values, meaning that wine  
 338 fluorescence lifetimes decrease after the malolactic fermentation. This suggests the possibility of an  
 339 input of wine quenchers during malolactic fermentation. This will be discussed in the next part in  
 340 relation to wine acidic and polyphenolic composition (Table S.I.4).



341  
 342 *Figure 3: Phasor plot of fresh Chardonnay wines in winery A (squares), winery B (triangles) and Chardonnay*  
 343 *contest wines C (circles) analyzed before and after malolactic fermentation, with an emission wavelength set at*  
 344 *440 nm for a fixed excitation at 280 nm (A). Chardonnay wines g values for all analyzed wines A+B+C (partly)*

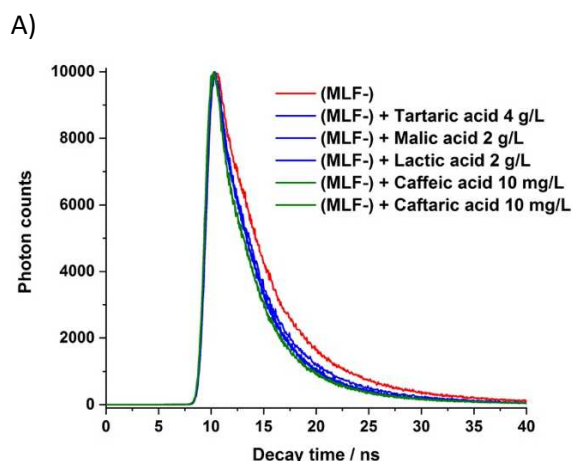
345 (N=22) (B), fresh wines from winery A (N=6) (C), fresh wines from winery B (N=6) (D) and a selection of  
346 Chardonnay contest wines (N=10) (E). Different letters above box plot (a,b) represent significant differences  
347 ( $p=0.05$ ) between the fermentative conditions.

348

### 349 **3.4. Discussion of potential quenchers occurring during malolactic fermentation in Chardonnay** 350 **wines**

351 In order to deepen wine fluorescence lifetime changes upon malolactic fermentation,  $g$  and  $s$  values  
352 were plotted for the 22 Chardonnay analyzed wines upon total acidity expressed in tartaric acid  
353 equivalent. A low correlation between  $g$  values and total acidity of each wine was found (correlation  
354 coefficient  $r=0.36$  and coefficient of determination  $R^2=0.1278$ , Figure S.I.3), strengthening the role  
355 of organic acid in the fluorescence lifetimes decreasing with an increase of organic acids in wines  
356 upon MLF. Nevertheless, the increase in total acidity upon malolactic fermentation is not suspected  
357 due to the metabolization of a stronger acid, malic acid into lactic acid. Generally, pH tends to  
358 increase with MLF but in our study the correlation was not good (correlation coefficient  $r=0.06$  and  
359 coefficient of determination  $R^2=0.0039$ , Figure S.I.4). Nevertheless, the study of wine pH on wine  
360 fluorophores should be carefully detailed in the future due to deprotonation of polyphenolic  
361 moieties, changing drastically the fluorophores lifetimes. We also look at the correlation with  
362 polyphenolic compounds without satisfactory results meaning that their concentration does not  
363 influence wine fluorescence lifetime measurement (results not shown here).

364 In order to understand the increase of  $g$  values during MLF, we supplement one MLF- fresh wine with  
365 potential acidic and polyphenolic quenchers at their determined concentrations in wines. Results are  
366 presented in Figure 4. It appears that all organic acids present a quenching effect towards  
367 fluorescence decay in a similar order of magnitude for concentrations ranging from 2 to 4 g/L. With  
368 increasing concentration of tartaric acid in wine from 4 to 5 and to 6 g/L,  $g$  values of MLF- wine  
369 increase at 0.700, 0.710, 0.715. This is in accordance with the positive correlation of  $g$  values of wines  
370 as a function of their total acidity (shown previously in Figure S.I.3). Interestingly caffeic acid and  
371 caftaric acid when supplemented at 10 mg/L in MLF- wine present a quite stronger quenching effect,  
372 as visualized by a  $g$  value reaching up to 0.737.



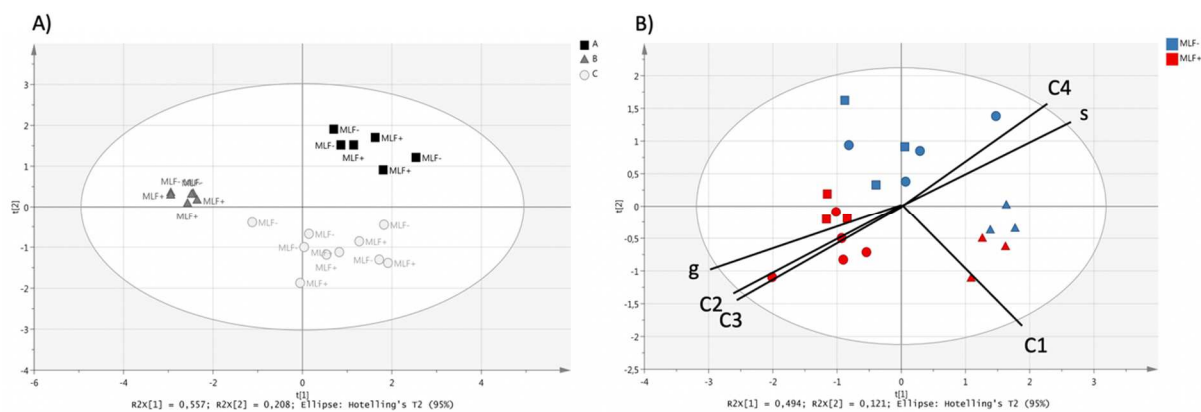
B)

Supplemented wines	g	s
(MLF-) wine	0.442	0.638
(MLF-) wine + Tartaric acid 4 g/L	0.416	0.700
(MLF-) wine + Malic acid 2 g/L	0.404	0.731
(MLF-) wine + Lactic acid 2 g/L	0.402	0.736
(MLF-) wine + Caffeic acid 10 mg/L	0.391	0.737
(MLF-) wine + Caftaric acid 10 mg/L	0.392	0.737

373 *Figure 4: Fluorescence decays measured at pH=5 of a Chardonnay wine from winery A that has not achieved*  
 374 *realize malolactic fermentation (MLF-) (red) and its supplemented wines with potential quenchers of wine*  
 375 *fluorescence: organic acids (blue) and polyphenols (green) at realistic concentrations that could be encountered*  
 376 *in Chardonnay wines (A). Table presenting g and s values from the phasor plot analysis of (MLF-) Chardonnay*  
 377 *wine and supplemented wines fluorescence decays (B).*

378 **3.5. MLF distinction in Chardonnay wines (sample A+B+C) using multispectral fluorescence and**  
 379 **chemometric analysis**

380 Chardonnay wines multispectral fluorescence parameters generated by PARAFAC modeling and  
 381 phasor plot analysis (PARAFAC components Fmax, g and s values) were interpreted with a  
 382 multivariate analysis in order to discuss MLF achievement among wines depending on their  
 383 typologies. Results are illustrated in Figure 5. The three categories of Chardonnay wines were  
 384 clustered separately in Figure 5A, along PC1 (55.7%) and PC2 (20.8%), but with no visible separation  
 385 in relation with MLF achievement. In order to gain significance in MLF distinction, a PLS-DA model  
 386 was attempted on the same dataset (Figure 5B). The PLS model fits with a pretty good predictive  
 387 ability  $Q^2Y = 0.632$  (N=21, with an exclusion of one MLF- Chardonnay contest wine). Results show a  
 388 clear discrimination of MLF- wines (blue) and MLF+ wines (red) regardless of the three typologies of  
 389 wines. The loadings of the models are represented and confirmed the importance of values from  
 390 PARAFAC component C4 Fmax values and g values for the optimization of the MLF separation among  
 391 Chardonnay wines. Such combined multispectral data could be used in future for a better  
 392 traceability among Chardonnay wines, particularly related to fermentative processes during  
 393 winemaking. Further research could deepen multispectral fluorescence in wines to diagnose  
 394 microbiological and chemical processes naturally occurring in wines that could impact its final  
 395 quality. Such developments could be applied to other fermented food products and to research on  
 396 adulteration and authentication issues.



397  
 398 *Figure 5: Biplot representation of the multispectral fluorescence Chardonnay wines from fresh wines from*  
 399 *winery A (squares), winery B (triangles) and Chardonnay contest wines C (circles) obtained by a Principal*  
 400 *component analysis (A) and by a Partial Least Square – Discriminant Analysis oriented on MLF achievement (B).*

401  
 402 **Conclusions**

403 In conclusion, the combination of steady-state and time-resolved fluorescence techniques could be  
 404 used to study the biochemical differences in Chardonnay wines. This study reveals that PARAFAC  
 405 component C4 is a good biomarker index for MLF realization in wines. Chardonnay wine fluorophores  
 406 with similar optical characteristics to the PARAFAC component C4 have been characterized at pH=5,  
 407 by two fluorescence lifetimes:  $\tau_1$  (4.19 - 4.53 ns) and  $\tau_2$  (13.28 to 13.86 ns), respectively with two  
 408 fractional intensities: F1 (61.1-63.7) and F2 (38.9-36.3). By applying a phasor plot analysis, wine  
 409 fluorescence decays could be easily described by their (g,s) coordinates. g values appeared to  
 410 systematically increase under malolactic fermentation on Chardonnay wines. Such an increase has  
 411 been discussed by a quenching effect on wine fluorophores, occurring after acidic and polyphenolic  
 412 modifications after MLF achievement in Chardonnay wines. When combining multispectral  
 413 fluorescence to chemometric analysis, (MLF+) wines were clearly separated from (MLF-) wines,  
 414 regardless of the origin of wines. Results from this study could motivate further wine research to  
 415 combine multidimensional fluorescence measurements to gain in traceability towards fermentative  
 416 processes occurring during winemaking.

417  
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425

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