Depósito de investigación de la Universidad de Sevilla

https://idus.us.es/

"This is an Accepted Manuscript of an article published by Elsevier in FOOD CHEMISTRY *on* 2016*, available* at:

https://doi.org/10.1016/j.foodchem.2016.03.037*."*

1 **TITLE:** Fluorescence excitation-emission matrix spectroscopy as a tool for determining quality of sparkling wines

3 **Running Title:** Multidimensional fluorescence to determine quality of sparkling wines

4 **AUTHORS:** Saioa Elcoroaristizabal[a], Raquel M. Callejón[*b], Jose M. Amigo[c], Juan A. Ocaña-González[d], M. Lourdes Morales[b], Cristina Ubeda[e]

6 **ADDRESS**

7 [a] Chemical and Environmental Engineering Department, Faculty of Engineering, University of the Basque Country, Alameda de Urquijo s/n, Bilbao, Spain

9 [b] Área de Nutrición y Bromatología, Fac. Farmacia, Univ. Sevilla, C/P. García Gonzalez no. 2, E-41012 Sevilla, Spain.

11 [c] Department of Food Sciences, Spectroscopy and Chemometrics, Faculty of Sciences, Univ. Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark.

13 [d] Dept. Química Analítica. Fac. Química. Univ. Sevilla. C/ P. García Gonzalez s/n, E-41012 Sevilla, Spain.

15 [e] Universidad Autónoma de Chile, Chile.

16 *Corresponding author email: rcallejon@us.es

17

18 **Abstract**

19 Browning in sparkling wines was assessed by the use of excitation–emission fluorescence spectroscopy combined with PARAllel FACtor analysis (PARAFAC). Four different *cava* sparkling wines were monitored during an accelerated browning process and subsequently storage. Fluorescence changes observed during the accelerated browning process were monitored and compared with other conventional parameters: absorbance at 420 nm (A_{420}) and the content of 5-hydroxymethyl-2-furfural (5-HMF). A high similarity of the spectral profiles for all sparkling wines analyzed was

observed, being explained by a four component PARAFAC model. A high correlation between the third PARAFAC factor (465/530 nm) and the commonly used non-enzymatic browning indicators was observed. The fourth PARAFAC factor (280/380 nm) gives us also information about the browning process following a first order kinetic reaction. Hence, excitation–emission fluorescence spectroscopy, together with PARAFAC, provides a faster alternative for browning monitoring to conventional methods, as well as useful key indicators for quality control.

Keywords: Browning; Sparkling wine; Heating; Storage; PARAFAC; Kinetic modeling.

38 ## 1. Introduction

39

40 Sparkling wine is a special wine whose most important characteristic is the
41 effervescence. This characteristic is due to the presence of CO_2 produced by a second
42 alcoholic fermentation of a still wine (Martínez-Rodríguez and Pueyo, 2009). The most
43 famous sparkling wines include *champagne* from France or *cava* from Spain among
44 others (Kemp, Alexandre, Robillard & Marchal, 2015). A special production method
45 named *Traditional* is employed to obtain these high quality sparkling wines in which
46 the second fermentation takes place in the bottle. Thus, *cava* is a premium sparkling
47 wine (designation of origin), which undergoes a biological ageing for at least 9 months
48 in contact with lees under anaerobic conditions in bottle (Commission Regulation,
49 2009). It is during its second fermentation process when *cava* wines develop their
50 complex organoleptic characteristics, which include aroma, colour, and their capacity of
51 creating foam. Among these, colour is of especial relevance since it is one of the first
52 sensory attributes observed by manufactures and consumers.

53

54 The grape phenolic compounds that remain in these wines following the elaboration
55 process are the primarily responsible for their colour, giving to the wines a yellowish or
56 even a brownish colour when oxidized (Buxaderas & López-Tamames, 2010).
57 However, after their elaboration, colour can also be affected during shipment and
58 commercial storage, where *cava* wines are usually exposed to uncontrolled temperature
59 conditions that may lead to an increase of non-enzimatic browning processes (Serra-
60 Cayuela, Jourdes, Riu-Aumatell, Buxaderas, Teissedre & López-Tamames, 2014).
61 Browning is an oxidative process involving sugars, lipids, amino acids and phenols (Li
62 & Guo, 2008), which decreases the sensorial quality of wines (loss of colour, flavour

63 and aroma, and increment of astringency) (Ferreira, Escudero, Fernández & Cacho,
64 1997). Thus, since quality is of prime relevance for *cava* wines, browning has to be
65 controlled during processing and storage. In this sense, several methods have been
66 suggested to quantify the degree of browning, based on the measurement of different
67 quality markers, by colorimetry, Ultra Violet-Visible (UV-Vis) spectroscopy and high
68 performance liquid chromatography (HPLC).

69 Tristimulus colorimetry using the *CIELab* or *Hunter Lab* color systems has been widely
70 used to measure the browning degree. Nevertheless, these methods are often influenced
71 by chemical (browning) as well as physical changes. Absorbance, in particular at 420
72 nm (A_{420}), has been also extensively used as a fast parameter for browning monitoring,
73 mostly in white wines (Pen, Duncan, Pocock & Sefton, 1998; Kallithraka, Salacha &
74 Tzourou, 2009), where an increase in the A_{420} parameter value is used to indicate
75 increased browning (Ibarz, Pagán & Garz, 2000). However, Serra-Cayuela, Aguilera-
76 Curiel, Riu-Aumatel, Buxaderas and López-Tamames (2013) demonstrated that the
77 value of the A_{420} parameter has low sensitivity and low specificity as a quality marker
78 of *cava* sparkling wines. Instead, they proposed the use of the 5-hydroxymethyl-2-
79 furfural (5-HMF) content as a more effective marker. This compound is an intermediate
80 product in the formation of brown pigments during the Maillard reaction, which
81 increases linearly with time and temperature following a zero-order reaction (Özhan,
82 Karadeniz & Erge, 2010). Nevertheless, laboratory analyses of this quality marker by
83 chromatographic methods are expensive, time and reagent-consuming as well as
84 destructive.

85

86 Therefore, finding fast and accurate methods for monitoring the extent of browning
87 reaction as well as alternative quality markers would be of utmost importance for *cava*

88 producers. In this sense, fluorescence spectroscopy has been more and more applied in
89 the last decades as a fast, non-destructive and environmentally safe analyzing method in
90 food science, due to its high sensitivity and specificity (Andersen, Wold & Engelsen,
91 2009).
92
93 In wines, several substances exhibit intrinsic fluorescence (stilbenes, anthocyanins,
94 amino acids, vitamins, flavanols and tannins), but most of them are related to
95 polyphenols (Sádecká & Tóthová, 2007). This offers a valuable alternative of wine
96 characterization and monitoring. Dufour, Letort, Laguet, Lebecque and Serra (2006)
97 demonstrated the potential use of direct single front-face fluorescence measurements
98 combined with chemometric methods for discriminating different French and German
99 wines according to variety, typicality and vintage (i.e. ageing). Later, Airado-Rodríguez,
100 Galeano-Díaz, Durán-Meras and Wold (2009) employed fluorescence Excitation–
101 Emission Matrix (EEM) spectroscopy linked to a resolution method such as PARAllel
102 FACtor analysis (PARAFAC) for fingerprinting of red wines, where the main groups of
103 fluorescent compounds detected were also tentatively identified by high performance
104 liquid chromatography. Further, Airado-Rodríguez, Durán-Meras, Galeano-Díaz and
105 Wold (2011) explored the feasibility of the autofluorescence of wine for the purpose of
106 discrimination of wines according to the appellation of origin. Moreover, their
107 PARAFAC analysis revealed four groups of fluorophores in red wines, assigning two of
108 them to benzoic-like phenolic acids and phenolic aldehydes, and to monomeric
109 catequins and polymeric proanthocyanidin dimers, respectively.
110 On the other hand, fluorescence has been pointed out as an alternative tool to assess the
111 progress of browning in foodstuff (Park & Kim, 1983) in the same way as the browning
112 index at 420 nm (Morales, Romero & Jiménez-Pérez, 1996). In this sense,

113 FLuorescence Relative Index (FLRI) values (measured using maximum emission and
114 excitation wavelengths at 493 and 400 nm, respectively) were introduced by Cohen,
115 Birk, Mannheim and Saguy (1998) to monitor the quality deterioration of apple juice
116 during thermal processing. Later, front-face fluorescence spectroscopy was applied to
117 study the development of Maillard browning in milk during thermal processing
118 (Schamberger & Labuza, 2006), pointing out a high correlation between the emission
119 spectra and the 5-HMF content as well. Furthermore, Zhu, Baoping, Eum and Zude
120 (2009) used front-face fluorescence excitation–emission matrix combined with
121 chemometric methods as a sensitive indicator of the non-enzymatic browning in
122 thermally processed apple juices, suggesting also that fluorescence spectra could be
123 used to predict the 5-HMF concentration.

124

125 The aim of this study is to assess the browning in sparkling wines by the use of
126 fluorescence excitation–emission matrix spectroscopy combined with PARAFAC
127 analysis. Four different *cava* sparkling wines were monitored during an accelerated
128 browning process at a temperature of 65 °C and subsequently storage. In order to assess
129 the potential use of the proposed methodology, the fluorescence trends observed during
130 the accelerated browning were tested and compared with those obtained by means of
131 different common quality parameters, such as the A_{420} and the 5-HMF content.
132 Furthermore, the fluorescence monitoring was studied to determine whether any
133 fluorophore could be used as an aging marker for quality controlling in *cava* sparkling
134 wines.

135

136 2. Materials and methods

137

138 *2.1 Sparkling wine samples*

139 A set of four commercial *cava* sparkling wines (*Brut*, *Brut Reserva*, *Brut Gran Reserva*
140 and *Semiseco*) were purchased in local supermarkets, coming from several *cava* brands.
141 These *cava* wines, mostly produced from a blending of three grape varieties (*macabeu*,
142 *xarel·lo*, and *parellada*), were selected based on two criteria: sugar content and ageing,
143 to cover most of types of marketed *cava* wines. Thus, *Brut* (sugar content < 12 g L^{-1})
144 and *Semiseco* categories (sugar content between 32 to 50 g L^{-1}) were selected according
145 to the sugar content; whereas *Reserva* and *Gran Reserva cava* wines corresponding to
146 the *Brut* category were chosen based on the ageing periods. The term *Reserva* applies to
147 wines that have been kept in contact with the lees for at least 15 months, while *Gran*
148 *Reserva* refers to wines that have been kept in contact with the lees for at least 30
149 months. These two qualities of *cava* wines have a different price in the market due to
150 the fact that the longer ageing time the better quality and higher cost of production.

151 The enological parameters of each type of *cava* wine at the initial sampling point (time
152 zero) are shown as Supplementary Material (Table I). The total sugar content, alcohol
153 content, pH, free and total sulfur dioxide were measured using the established standard
154 methods (OIV, 2009).

155 The accelerated browning test was carried out in total darkness conditions for each *cav*a
156 wine (4 series). Once the bottles were opened, 10 mL of wine were aliquoted into 20
157 mL amber vials and were degassed under a N_2 stream. All vials, expect those belonging
158 to time zero (initial sampling point), were subjected to heating at a constant temperature
159 of 65 ± 1 °C in an oven (Selecta, Barcelona Spain). Sampling points were at 48 h
160 intervals over a period of 10 days, i.e. after 0, 2, 4, 6, 8 and 10 days (6 sampling points).
161 Three replicates were taken for each type of *cava* at each sampling time point. Hence,
162 the heating experiment resulted in a total of 72 samples (6 sampling points x 4 cava

163 series x 3 replicates). After that, to monitor the evolution of heated samples during
164 storage, the heated samples were stored at room temperature for a further 10 days, and
165 measured at 2, 4, 6, 9 and 10 days, giving a total of 60 samples (5 sampling points x 4
166 cava series x 3 replicates). All samples, 132 in total, were stored at 4 ºC until analysis.
167 The sampling procedure is shown as Supplementary Material (Table II).

168

169 *2.2 Analytical procedures*

170 *2.2.1. Absorbance spectroscopy*

171 The absorbance spectrum of each sample was measured in the range 200–700 nm
172 (spectral resolution of 1 nm) in a Shimadzu® UV-3600 spectrophotometer (Duisburg,
173 Germany), using a 10 mm path length quartz cuvette and double-distilled water as
174 reference. Absorbance values at 420 nm (A_{420}) were multiplied 1000-fold and expressed
175 as milli-absorbance units (mAU).

176

177 *2.2.2. 5-HMF quantification*

178 5-HMF was determined in all the samples according to the standard method of OIV
179 (2009). HPLC analysis was performed with LaChrom® WWR-Hitachi (Barcelona,
180 Spain) liquid chromatograph with a quaternary L-7100 pump connected to an L-7455
181 diode array detector (DAD). The column was a Luna C18, 5 μm, 250 x 4.6 mm and
182 guard precolumn 4.0 x 3.0 mm from Analytical Phenomenex (Torrance, CA, USA).
183 Detection was carried out at 280 nm. The injection volume was 10 μL and the
184 separation was obtained at a flow rate of 1.2 mL min^{-1} with an isocratic method. The
185 mobile phase consisted of 80% water, 18% methanol and 2% acetic acid, previously
186 degassed in an ultrasound. Samples were analyzed in duplicate previously filtered
187 through a 0.45 μm PTFE membrane filter (Merck, Darmstadt, Germany).

188 Quantification was carried out by using an external calibration curve in the range
189 between 0.5 and 400 mg L^{-1}. A calibration curve at 10 levels and two replicates per
190 level was built using the least-squares method. The response of the 5-HMF standard
191 was linear within the concentration range tested, with a determination coefficient of R^2=
192 0.9999. Standard solutions were prepared using a hydro-alcoholic matrix (12 % v/v). 5-
193 HMF standard was purchased from Sigma-Aldrich (Madrid, Spain).
194

195 *2.2.3. Fluorescence analysis*

196 Fluorescence measurements were recorded using a Varian Cary-Eclipse fluorescence
197 spectrophotometer (Varian Iberica, Madrid, Spain), equipped with two Czerny-Turner
198 monochromators, and a Xenon discharge lamp pulsed at 80 Hz with a half peak height
199 of ~2 μs (peak power equivalent to 75 kW). A high-performance R298 photomultiplier
200 was employed for collection of the fluorescence spectra. Standard quartz cells of 1 cm
201 path length were used to carry out the measurements in a peltier thermostatted (25.00 ±
202 0.05 °C) cuvette holder. The spectrometer was interfaced to a computer with Cary-
203 Eclipse software for Windows 98/NT for spectral acquisition and exportation.
204 The fluorescence Excitation-Emission Matrix (EEM) landscapes were obtained by
205 recording the emission spectra from 300 to 700 nm (every 4 nm), while excitation
206 wavelengths were ranging between 250 and 650 nm (every 5 nm). For these
207 measurements, excitation and emission slits were both set at 5 nm, and scan rate was
208 fixed to 600 nm min^{-1}. The system was wavelength calibrated every day by means of
209 the water Raman peak to account for possible wavelength drift of the instrument. EEM
210 fluorescence landscapes were registered by triplicate for each type of *cava* at each
211 sampling time point.
212

213 *2.3 EEM data modeling*

214 EEM data modeling was performed by using the PLS_Toolbox 7.9.5 (Eigenvector
215 Research Inc., Wenatchee, WA) working under Matlab v.8.5.0 environment (The
216 Mathworks Inc., Natick, MA).

217 First, EEM landscapes were preprocessed to reduce the effects of Rayleigh and Raman
218 scattering and avoid the so-called “inner-filter effects”. In this sense, the specific bands
219 of scattering were removed by replacing them with missing data (Elcoroaristizabal, Bro,
220 García & Alonso, 2015), and the corresponding correction factor accounting for its
221 inner effect was calculated by using the absorbance spectrum of the sample.

222 Then, the resulting corrected EEM data were subjected to PARAlell FACtor analysis
223 (PARAFAC) (Bro, 1997) in order to develop qualitative and quantitative models of the
224 degree of browning for each type of *cava* (4 series). To model the set of fluorescence
225 data for each sparkling wine, the EEM landscapes of the 33 samples (11 samples
226 replicated 3 times) were arranged in a three-dimensional structure ($\underline{\mathbf{X}}$) of size 33 × 101
227 × 81 (samples × number of emission wavelengths × number of excitation wavelengths).
228 This three-way array $\underline{\mathbf{X}}$ was then decomposed by PARAFAC modeling as indicated in
229 equation 1:

230 $$\underline{\mathbf{X}}^{(I \times JK)} = \mathbf{A}(\mathbf{C} \odot \mathbf{B})^T + \underline{\mathbf{E}}^{(I \times JK)} \quad (1)$$

231 where $\odot$ is the Khatri-Rao product. The decomposition of $\underline{\mathbf{X}}$ for a number of factors (F)
232 is usually accomplished through Alternating Least Squares (ALS), by minimizing the
233 sum of squares of the residuals $\underline{\mathbf{E}}$. In the case of EEMs, the loading matrices **B**, and **C**,
234 contain the spectral excitation and emission profiles of the factors (fluorophores), and
235 the score matrix **A**, contains information about the relative contribution of each factor in
236 every sample. There are multiple criteria to determine the proper number of factors in
237 the model which are necessary to reconstruct the data. In this work, the CORe

238 CONsistency DIAgnostic test (CORCONDIA), which is 100% for a completely trilinear
239 model (Bro & Kiers, 2003), and the percentage of variance explained by the model,
240 have been used. Additionally, non-negative constraint for all modes (concentrations and
241 both spectral profiles) was applied to obtain meaningful solutions.

242

243 **3. Results and Discussion**

244

245 *3.1 Fluorescence landscapes and potential fluorophores of cava sparkling wines*

246 As an example, Fig. 1 shows the fluorescence landscapes (after removing the scattering
247 areas, denoted in the Figure as the white stripes) belonging to each *cava* category before
248 (t = 0 days, Fig. 1 top), and after being subjected to the heating process (t = 10 days,
249 Fig. 1 bottom). As it can be observed, the EEM landscapes obtained for all varieties of
250 *cava* sparkling wines show similar profiles (Fig. 1 top), containing several fluorophores
251 that are clearly overlapped in both excitation and emission dimensions. These
252 fluorescence profiles show a maximum around 370/455 nm, a second peak at 280/380
253 nm, and a shoulder around 445/525 nm of excitation and emission wavelength,
254 respectively. Some of these features are similar to those observed recently by Azcarate,
255 Araújo Gomes, Alcaraz, Ugulino de Araújo, Camiña & Goicoechea (2015) in white
256 wines which presented excitation/emission maxima at 340/445 nm.

257

258 Additionally, a preliminary assessment of the EEM landscapes before and after the
259 heating, allows us to confirm an a priori difference by looking at the areas where the
260 potential compounds appear between samples. Thus, for example, the peak at 280/380
261 nm seems to have disappeared after the heating (Fig. 1 bottom).

262

263 Another important feature of these samples is that the EEM landscapes seem to be quite
264 similar independently to the *cava* variety. This was also confirmed after decomposing
265 the EEM landscapes into the main fluorescence contributions by using PARAFAC
266 analysis. Specifically, the best PARAFAC model built for each *cava* variety was found
267 to be the one with four factors, giving final models that explain more than 99% of the
268 variance and with a core consistency over zero (Table III Supplementary Material).
269 Both parameters indicated that the model was reliable and that it corresponded to the
270 inherent chemical behavior of the *cava* sparkling wines.

271

272 Fig. 2 shows the PARAFAC loadings (excitation / emission profiles) of each main
273 fluorophore obtained for each *cava* series. The high similarity of the spectral profiles
274 obtained for the four different series suggests that these potential fluorescence
275 fingerprints could be used as indicators independently of the type of *cava*.

276

277 The first factor (blue in Fig. 2) has a maximum excitation centered around 395 nm and
278 an emission maximum at 485 nm, approximately. This compound has not yet been
279 reported in *cava* sparkling wines. The pair of excitation/emission wavelengths
280 corresponding to the maximum fluorescent intensity for the second factor (red in Fig. 2)
281 is 365/440 nm. This peak could be related to oxidation products, Maillard products and
282 Nicotinamide Adenine Dinucleotide in the reduced form (NADH) (Christensen,
283 Nørgaard, Bro & Engelsen, 2006). NADH is formed in the fermentation processes that
284 take place during the production of these wines (Zamora, 2009). In contrast, the
285 oxidation and Maillard products may be present due to the browning processes observed
286 by others authors (Ibern-Gomez, Andres-Lacueva, Lamuela-Raventós, Buxaderas,
287 Singleton & Dela Torre-Boronat, 2000) during ageing and storage of these wines.

288 The third factor (black in Fig. 2) is a peak centered around 465 and 530 nm,
289 respectively. This fluorophore could be related to vitamin B_2 or riboflavin (270, 382,
290 442/518 of excitation/emission maxima) (Christensen et al., 2006). Finally, the fourth
291 factor (green in Fig. 2) has an excitation maximum around 280, with a shoulder at 350
292 nm, and the emission one centered at 380 nm. This fluorophore could match with
293 stilbenes compounds such as *trans*-piceid and *trans*-resveratrol (Vitrac, Monti,
294 Vercauteren, Deffieux & Mérillon, 2002), with excitation/emission pairs at 290/390 nm
295 and 300/390 nm respectively. These compounds have been previously reported in *cava*
296 sparkling wines (Andrés-Lacueva, Ibern-Gómez, Lamuela-Raventós, Buxaderas & De
297 la Torre-Boronat, 2002). Similarly, amino acids such as tryptophan, present in *cava*
298 wines (Puig-Deu, López-Tamames, Buxaderas & Torre-Boronat, 1999), emits at 357
299 nm with an excitation maxima at 280 nm (Christensen et al., 2006), and this could be
300 related to the observed shoulder at 350 nm. Also gallic and protocatechuic acids,
301 detected in *cava* wines (Satué-Gracia, Andrés-Lacueva, Lamuela-Raventós & Frankel,
302 1999) emitting at around the 280/360 nm pair could contribute to this fluorophore
303 (Coellho, Aron, Roullier-Gall, Gonsior, Schmitt-Kpplin & Gougeon, 2015). In this
304 regard it is important to emphasize that each PARAFAC factor probably corresponds to
305 a related fluorescent molecule group, and not necessarily to a single fluorescent
306 molecule (Morales et al., 1996).
307
308 *3.2 Monitoring of the browning evolution*
309 Each *cava* series was analyzed to monitor the development of browning by using the
310 absorbance at 420 nm (A_{420}) and the 5-HMF content. Additionally, the score values
311 corresponding to each PARAFAC factor are plotted against the heating time in order to
312 study possible information contained in fluorescence data with respect to the browning

313 process. In this sense, it was observed that the evolution of the score values of the third
314 PARAFAC factor (F3, black in Fig. 2) increased linearly over time, as well as the
315 absorbance at 420 nm and the 5-HMF content, showing zeroth-order kinetics described
316 by equation 2:

317 $$Y = Y_0 + kt \qquad (2)$$

318 where Y is the absorbance at 420 nm (mAU), the 5-HMF content (mg L^{-1}) or the F3
319 score value (a.u.), Y_0 is the initial value of the absorbance (mAU), the initial 5-HMF
320 content (mg L^{-1}) or the initial F3 score value (a.u.), k is the velocity constant (expressed
321 as mAU day^{-1} for A_{420}, mg L^{-1} day^{-1} for 5-HMF, a.u. day^{-1} for F3),and t is time (in
322 days).

323 For each *cava* variety, the parameters calculated for the zero*th*-order kinetics of A_{420}, 5-
324 HMF content and the third factor calculated by PARAFAC at 65°C are shown in Table
325 1.

326

327 From these results, all the indicators suggest that the browning velocity constant
328 decreases with the ageing (from *Gran Reserva* to *Reserva*) and increases with the sugar
329 content (from *Brut* to *Semiseco*). Indeed, since the rate of 5-HMF formation is sugar
330 dependent (Cámara, Alves & Márquez, 2006), the 5-HMF formation is highly
331 correlated (r=0,998) with the initial sugar content. Thus, the sparkling wines of higher
332 quality (*Gran Reserva*, *Reserva*) seem to be less affected by browning processes.

333

334 Additionally, the fourth PARAFAC factor (F4, green in Fig. 2) may also give us
335 information about the browning process. Interestingly, the scores of F4 seem to follow a
336 first-order kinetic as it can be observed in Fig. 3. Hence, the scores of Factor 4 describe
337 a first-order kinetic equation as follows:

338 Ln Y = Ln Y_0 – kt (3)

339 where Y is the score value of F4 in each sample (a.u.), Y_0 is the initial value of the F4
340 score (a.u.), k is the velocity constant (expressed as a.u. day^{-1}), and t is time (in days).
341 The parameters calculated for the first-order kinetics of each sparkling wine at 65°C are
342 shown in Table 1.

343

344 According to the literature, this fourth PARAFAC factor may reflect the degradation of
345 polyphenols during the browning process of *cava* wines. In fact, Coelho et al. (2015)
346 observed a similar trend in white wines treated with different concentration of sulfur
347 dioxide. The intensity of 280/340 nm excitation/emission pair decreased in those wines
348 with lower sulfur dioxide dosage. They related this phenomenon with the degradation of
349 some phenolic compounds due to oxidative browning.

350

351 *3.3. Storage after heating process*

352 The storage evolution after the heating process was also studied analyzing 5 samples in
353 triplicate during a 10 day period. In this sense, it is interesting to investigate the
354 evolution of the 5-HMF content over the storage and after finishing the heating period.
355 5-HMF is a furanic compound which forms as an intermediate in the Maillard reaction
356 as well as from direct dehydration of sugars under acidic conditions (caramelisation)
357 during thermal treatments applied to foods (Campo & Fogliano, 2011). Consequently, it
358 is greatly formed during heating (t=10 days) as shown in Table 2. However, 5-HMF
359 changes to other secondary products at the final stage of Maillard reaction. Thus, 5-
360 HMF slightly decreased during the subsequent storage (*Brut* and *Reserva*) or did not
361 show a significant difference after the heating process (*Gran Reserva* and *Semiseco*)
362 (Fig. 3).

This behavior is also reflected by other indicators such as the third fluorescence PARAFAC factor (F3) as shown in Fig. 3. Indeed, the increase in the F3 score values during heating is probably due to the appearance of neoformed fluorescent compounds, according to Ait Ameur (2006) and Sahar, ur Rahman, Kondjovan, Portanguen and Dufour (2016). The later decreased during storage can be linked to intermediate products such as 5-HMF as it has stated previously (Zhu et al., 2009). Thus, to gain an insight into this process, the correlations between all browning indicators are studied in the following section.

3.4. Correlation between browning indicators

As it has been mentioned above, the third PARAFAC factor (F3) shows a similar trend to the one followed by the browning index (A_{420}) and the 5-HMF content. In this way, linear regression analysis was employed to determine the relationships between these browning indicators as shown in Table 3.

The obtained parameters indicate a high correlation between the third PARAFAC factor (F3) and the commonly used non-enzymatic browning indicators. Indeed, these models suggest that the 5-HMF content could be derived from the known F3 score values (R^2> 0,89) with high accuracy ($p < 0.001$). Moreover, high determination coefficients (R^2 = 0,959 (*Semiseco*), 0,907 (*Brut*), 0,984 (*Reserva*) and 0,889 (*Gran Reserva*) with $p<0.001$) were found between the 5-HMF content and the F4 evolution. This confirms that this fluorophore could be also used as an alternative indicator of the browning process. Furthermore, since the F4 trends follows a first order reaction, monitoring of this factor could be more sensitive to study the extent of browning development.

388 ## 4. Conclusions

389 The feasibility of fluorescence excitation-emission spectroscopy coupled to PARAFAC
390 modeling to monitor the browning process of four different *cava* sparkling wines has
391 been successfully proven. Moreover, the potential use of similar fluorescence
392 fingerprints of cava sparkling wines has been also demonstrated.

393 To sum up, this approach provides a fast alternative method to the conventional ones, as
394 well as key indicators for quality control of sparkling wines. Specifically, monitoring
395 the fluorophores located at the pairs 465/530 nm 280/380 nm provides us useful
396 information about the chemical changes undergone during browning in the same way as
397 the conventional quality markers.

398

399 ## 5. Acknowledgements

400 The authors want to thank CONICYT for the financial support provided through project
401 FONDECYT 11140275 and the Universidad Autónoma de Chile. Saioa
402 Elcoroaristizabal wants to thanks UPV/EHU for the grant received from the program
403 "*Contratación de Doctores Recientes*".

404

405

406

407

408

409 **6. References**

410 Airado-Rodríguez, D., Galeano-Díaz, T., Durán-Merás, I., & Wold, J.P.
411 (2009).Usefulness of Fluorescence Excitation-Emission Matrices in combination with
412 PARAFAC, as Fingerprints of Red Wine. *Journal of Agricultural and Food Chemistry,*
413 57, 1711–1720.

414 Airado-Rodríguez, D., Durán-Merás, I., Galeano-Díaz, T., & Wold, J.P. (2011). Front-
415 face fluorescence spectroscopy: A new tool for control in the wine industry. *Journal of*
416 *Food Composition and Analysis, 24,* 257-264.

417 Ait Ameur, L. (2006). Evolution de la qualité nutritionnelle des protéines de biscuits
418 modèles au cours de la cuisson au travers d'indicateurs de la réaction de Maillard:
419 intérêt de la fluorescence frontale. PhD thesis Institut National Agronomique Paris
420 Grignon, France. <http://pastel. paristech. org/2269>.

421 Andersen, C.M., Wold, J.P., & Engelsen, S.B. (2009). Autofluorescence Spectroscopy
422 in Food Analysis. In: S. Ötles (Ed.), *Handbook of Food Analysis Instruments,* (pp. 347
423 364). Boca Raton: CRC Press.

424 Andrés-Lacueva, C., Ibern-Gómez, M., Lamuela-Raventós, R.M., Buxaderas, S., & de
425 la Torre-Boronat, M.C. (2002). Cinnamates and Resveratrol Content for Sparkling Wine
426 Characterization. *American Journal of Enology and Viticulture, 53*, 147-150.

427 Azcarate, S.M., Araújo Gomes, A., Alcaraz, M.R., Ugulino de Araújo, M.C., Camiña,
428 J.M., & Goicoechea, H.C. (2015). Modeling excitation–emission fluorescence matrices
429 with pattern recognition algorithms for classification of Argentine white wines
430 according grape variety. *Food Chemistry, 184,* 214-219.

431 Bro, R. (1997). PARAFAC. Tutorial and applications. *Chemometrics and Intelligent*
432 *Laboratory Systems, 38,* 149-171.

433 Bro, R., & Kiers, H.A.L. (2003). A new efficient method for determining the number of
434 components in PARAFAC models. *Journal of Chemometrics, 17,* 274-286.
435 Buxaderas, S., & López-Tamames, E., (2010). Managing the quality of sparkling wines,
436 in: A.G. Reynolds (Ed.), *Managing Wine Quality: Oenology and Wine Quality* (pp.
437 553-588). Cambridge: Woodhead Publishing Limited.
438 Cámara, J.S., Alves, M.A., & Marques, J.C. (2006). Changes in volatile composition of
439 Madeira wines during their oxidative ageing. *Analytical Chimica Acta, 563,* 188-197.
440 Capuano, E., & Fogliano, V. (2011). Acrylamide and 5-hydroxymethylfurfural (HMF):
441 A review on metabolism, toxicity, occurrence in food and mitigation strategies. *Food*
442 *Science and Technology, 44,* 793-810.
443 Christensen, J., Nørgaard, L., Bro, R., & Engelsen, S.B. (2006). Multivariate
444 Autofluorescence of Intact Food Systems. Chemical Reviews, 106, 1979-1994.
445 Coelho, C., Aron, A., Roullier-Gall, C., Gonsior, M., Schmitt-Kopplin, P., & Gougeon,
446 R.D. (2015). Fluorescence Fingerprinting of Bottled White Wines Can Reveal
447 Memories Related to Sulfur Dioxide Treatments of the Must. *Analytical Chemistry, 87,*
448 8132-8137.
449 Cohen, E., Birk, Y., Mannheim, C.H., & Saguy, I.S. (1998). A rapid method to monitor
450 quality of apple juice during thermal processing. *Lebensmittel-Wissenschaft &*
451 *Technologie, 31,* 612-616.
452 Dufour, E., Letort, A., Laguet, A., Lebecque, A., & Serra, J.N., (2006). Investigation
453 of variety, typicality and vintage of French and German wines using front-face
454 fluorescence spectroscopy. *Food Chemistry, 563,* 292-299.
455 Elcoroaristizabal, S., Bro, R., García, J.A., & Alonso, L. (2015). PARAFAC models of
456 fluorescence data with scattering: A comparative study. *Chemometrics and Intelligent*
457 *Laboratory Systems, 142,* 124-130.

458

459 Ferreira, V., Escudero, A., Fernández, P., & Cacho, J. F., (1997). Changes in the profile
460 of volatile compounds in wines stored under oxygen and their relationship with the
461 browning process. *Zeitschrift für Le0bensmittel-Untersuchung und –Forschung, 205*,
462 392-396.

463 Ibern-Gomez, M., Andres-Lacueva, C., Lamuela-Raventos, R.M., Buxaderas, S.,
464 Singleton, V.L., & De la Torre-Boronat, M.C. (2000). Browning of Cava (sparkling
465 wine) during aging in contact with lees due to the phenolic composition. *American*
466 *Journal of Enology and Viticulture, 51,* 29-36.

467 International Organisation of Vine and Wine (OIV) (2009). Compendium of
468 international methods of analysis; OIV-MA-AS315-05B. París, France.

469 Kallithraka, S., Salacha, M.I., & Tzourou, I., (2009). Changes in phenolic composition
470 and antioxidant activity of white wine during bottle storage: Accelerated browning test
471 versus bottle storage. *Food Chemistry, 113,* 500–505.

472 Kemp, B., Alexandre, H., Robillard, B., & Marchal, R., (2015). Effect of production
473 phase on bottle-fermented sparkling wine quality. *Journal of Agricultural and Food*
474 *Chemistry, 63,* 19–38.

475 Li, H. Guo, A., & Wang, H., (2008). Mechanisms of oxidative browning of wine, *Food*
476 *Chemistry, 108*, 1-13.

477 López-Tamames, R., (2014). Kinetics of Browning, Phenolics, and
478 5Hydroxymethylfurfural in Commercial Sparkling Wines. *Journal of Agricultural and*
479 *Food Chemistry, 62*, 1159−1166.

480 Martínez-Rodríguez, A.J., & Pueyo, E., (2009). Sparkling wines and yeast autolysis, in:
481 A.V. Moreno-Arribas, & M.C. Polo (Eds.), *Wine chemistry and Biochemistry* (pp. 61–
482 80). New York: Springer.

483 Morales, F.J., Romero, C., & Jiménez-Pérez, S. (1996). Fluorescence associated with
484 Maillard reaction in milk and milk-resembling systems. *Food Chemistry, 57,* 423-428.
485 Official Journal of the European Union, Commission Regulation (EC). L193 (2009).
486 Özhan, B., Karadeniz, F., & Erge, H.S., (2010). Effect of storage on nonenzymatic
487 browning reactions in carob pekmez. *International Journal of Food Science and*
488 *Technology, 45*, 751-757.
489 Park, C. K., & Kim, D.H. (1983). Relationship between fluorescence and antioxidant
490 activity of ethanol extracts of Maillard browning mixture. *Journal of the American Oil*
491 *Chemists' Society, 60,* 22-26.
492 Peng, Z., Duncan, B., Pocock, K.F., & Sefton, M.A., (1998). The effect of ascorbic acid
493 on oxidative browning of white wines and model wine. *Australian Journal of Grape*
494 *and Wine Research, 4,* 127-135.
495 Puig-Deu, M., López-Tamames, E., Buxaderas, S., & Torre-Boronat, M.C. (1999).
496 Quality of base and sparkling wines as influenced by the type of fining agent added pre-
497 fermentation. *Food Chemistry, 66,* 35-42.
498 Sádecká, J., & Tóthová, J., (2007). Fluorescence Spectroscopy and Chemometrics in
499 the Food Classification−a Review. *Czech Journal of Food Science, 25,* 159-173.
500 Schamberger, G.P., & Labuza, T.P. (2006). Evaluation of front- face fluorescence for
501 assessing thermal processing of milk. *Journal of Food Science, 71,* 69-74.
502 Satué-Gracia, M.T. Andrés-Lacueva, C., Lamuela-Raventós, R.M., & Frankel, E.N.
503 (1999). Spanish Sparkling Wines (Cavas) As Inhibitors of in Vitro Human Low-Density
504 Lipoprotein Oxidation. *Journal of Agricultural and Food Chemistry, 47,* 2198-2202.
505 Serra-Cayuela, M., Jourdes, M., Riu-Aumatell, M., Buxaderas, S., Teissedre, P.L., &
506 Ibarz, A., Pagán, J., & Garza, S., (2000). Kinetic models of non-enzymatic browning in
507 apple puree. *Journal of the Science of Food and Agriculture, 80,* 1162-1168.

508 Serra-Cayuela, A., Aguilera-Curiel, M.A., Riu-Aumatell, M., Buxaderas, S., & López
509 Tamames, E., (2013). Browning during biological aging and commercial storage of
510 Cava sparkling wine and the use of 5-HMF as a quality marker. *Food Research*
511 *International, 53,* 226-231.
512 Sahar, A., ur Rahman, U., Kondjoyan, A., Portanguen, S., & Dufour, E. (2016).
513 Monitoring of thermal changes in meat by synchronous fluorescence spectroscopy.
514 *Journal of Food Engineering, 168,* 160-165.
515 Vitrac, X., Monti, J.P., Vercauteren, J., Deffieux, G., & Mérillon, J.M. (2002). Direct
516 liquid chromatographic analysis of resveratrol derivatives and flavanonols in wines with
517 absorbance and fluorescence detection. Analytical Chimica Acta, 458, 103-110.
518 Zamora, F. (2009). Biochemistry of Alcoholic Fermentation. In: A.V. Moreno-Arribas,
519 M.C. Polo (Eds.), *Wine chemistry and Biochemistry* (pp. 3-26). New York: Springer.
520 Zhu, D., Baoping, J., Eum, H.L., & Zude, M. (2009). Evaluation of the non-enzymatic
521 browning in thermally processed apple juice by front-face fluorescence spectroscopy.
522 *Food Chemistry, 113,* 272-279.

523

524

525

Figure captions

Fig. 1. Excitation–emission landscapes obtained for *cava* sparkling wines subjected to an accelerated browning process. t = 0 days (top) corresponds to the starting sampling point (without heating) and t = 10 days (bottom) is related to the samples measured after 10 days of heating at 65°C. SS = *Semiseco*, B= *Brut*, R= *Reserva*, and GR= *Gran Reserva*.

Fig. 2. PARAFAC excitation and emission profiles obtained for each *cava* sparkling wine. Factor 1 in blue, Factor 2 in red, Factor 3 in black and Factor 4 in green. Continuous line for *Semiseco*, discontinuous line for *Brut*, double line for *Brut Reserva* and dotted line for *Gran Reserva cava* models.

Fig. 3. (A) Evolution of the scores of PARAFAC factor 4 during the browning process. SS = Semiseco, B= Brut, R= Reserva, and GR= Gran Reserva. Evolution of the scores of PARAFAC factor 3 (blue) and 5-HMF content (orange) during heating and storage for: (B) Brut and (C) Gran Reserva cava wines.

Tables

Table 1. Zero-order kinetic parameters (A_{420}, 5-HMF and F_3) and first-order kinetic parameters (F4 expressed as Ln) for each sparkling wine (n=18, 6 sampling points in triplicate*).

Parameter	**A_{420}**					**5-HMF**				
Cava	**Y_0**	**k**	**R^2**	**S**	**p**	**Y_0**	**k**	**R^2**	**S**	**p**
SS	91	66	0,954	52,3	4,08E-12	1,08	24,74	0,941	23,2	1,84E-7
B	96	49	0,947	42,0	1,25E-11	1,21	5,77	0,941	5,4	1,81E-7
R	72	46	0,883	60,4	7,12E-9	1,86	5,34	0,980	2,9	9,03E-10
GR	97	33	0,958	24,6	1,79E-12	2,45	1,31	0,895	1,7	3,37E-6
Parameter	**F3**					**Ln**				
Cava	**Y_0**	**k**	**R^2**	**S**	**p**	**Y_0**	**k**	**R^2**	**S**	**p**
SS	576	85	0,963	51	1,07E-10	6,94	0,454	0,964	0,32	5,70E-13
B	566	81	0,990	24	1,95E-14	6,53	0,293	0,981	0,15	3,41E-15
R	453	79	0,983	32	6,06E-13	6,80	0,238	0,983	0,11	1,48E-15
GR	581	54	0,857	67	7,66E-7	6,75	0,139	0,966	0,09	3,33E-13

Note: Y_0 is the initial value of the absorbance (mAU), the initial 5-HMF content (mg L^{-1}) or the initial F3 score value (a.u.), k is the velocity constant (expressed as mAU day^{-1} for A420, mg L^{-1} day^{-1} for 5-HMF, a.u. day^{-1} for F3), R^2 is the coefficient of determination of the linear model, S is the standard error of the regression and p is the significant associated probability value.

Table 2. 5-HMF content (mg L^{-1}) after heating (final value after 10 days) and storage (final value after other 10 days) and the initial value (starting point at 0 days). Average value ± standard deviation.

***Cava* category**	***Semiseco***	***Brut***	***Brut Reserva***	***Brut Gran Reserva***
Acronym	**SS**	**B**	**R**	**GR**
Initial (t=0)	1,08 ± 0,02	1,21± 0,01	1,86± 0,04	2,45± 0,04
Heating (t=10)	241,24 ± 0,12	50,63± 0,34	51,52± 0,27	13,19± 0,08
Storage (t=10)	237,95 ± 2,80	35,18± 0,05	29,69± 0,53	13,83± 0,11

Table 3. Linear regression parameters obtained between the browning indicators. R^2 = determination coefficient, S= standard error of the regression, and p= probability value.

Model	**x= A_{420} vs. y= 5-HMF**			**x= F3 vs. y= A_{420}**			**x= F3 vs. y= 5-HMF**		
Parameters	**R^2**	**S**	**p**	**R^2**	**S**	**p**	**R^2**	**S**	**p**
SS	0,992	8,06	2,21E-18	0,971	41,66	1,04E-13	0,989	9,87	5,78E-17
B	0,868	7,83	1,96E-8	0,875	64,37	1,21E-08	0,914	6,33	6,31E-10
R	0,900	6,17	2,00E-09	0,896	56,97	2,80E-09	0,993	1,67	1,59E-18
GR	0,814	2,16	3,12E-07	0,866	44,14	2,20E-08	0,892	1,64	3,81E-9

FIGURES