TRABAJO FIN DE MASTER

PROGRAMA DE DOCTORADO ECOSISTEMAS AGRÍCOLAS SOSTENIBLES

Study of phenolic composition, biogenic amines and sensory analysis in eight white and rose sparkling wines made from alternative grape varieties

Leticia Martínez Lapuente

Tutores: Belén Ayestarán Iturbe y Zenaida Guadalupe Mínguez
Facultad de Ciencias, Estudios Agroalimentarios e Informática
Curso 2010-2011

UNIVERSIDAD DE LA RIOJA

publicaciones.unirioja.es
E-mail: publicaciones@unirioja.es

STUDY OF PHENOLIC COMPOSITION, BIOGENIC AMINES AND SENSORY ANALYSIS IN EIGHT WHITE AND ROSÉ SPARKLING WINES MADE FROM ALTERNATIVE GRAPE VARIETIES

ESTUDIO DE LA COMPOSICIÓN FENÓLICA, AMINAS BIÓGENAS Y ANÁLISIS SENSORIAL EN OCHO VINOS ESPUMOSOS BLANCOS Y ROSADOS ELABORADOS CON VARIEDADES DE UVA ALTERNATIVAS

LETICIA MARTÍNEZ LAPUENTE

JUNIO DE 2011

Trabajo listo para enviar a la revista Analytica Chimica Acta

ABSTRACT

This paper studied the sensory properties and the evolution of phenolic compounds and biogenic amines during the winemaking and ageing of white and rosé sparkling wines produced with different grape varieties: Verdejo, Viura, Malvasía, Albarín, Godello, Garnacha and Prieto Picudo. All base wines and thus sparkling wines showed low alcohol content, high acidity and low pH. The composition and content of phenolics and biogenic amines was independent on the winemaking technology (with or without prefermentative maceration) and on whether the grapes used were red or white. The cold-stabilization and clarification of the base wines before the *sparkle* stage produced a great decrease in the content of anthocyanins, more accused in Garnacha wines than in Prieto Picudo, catechin, proanthocyanidins and biogenic amines. The first months of aging on yeast lees entailed losses of all forms of polyphenols due to their reactivity and their adsorption by lees. However, part of the phenolics adsorbed in the first stages of aging were released during the last months due to the autolysis of the yeast cell walls. All final sparkling wines had low contents of biogenic amines and Garnacha rosé wines and Albarín white wines showed the highest values of total polyphenols. All wines obtained good punctuations in the sensory analysis but Prieto Picudo showed higher visual color intensity, red tones, olfactory intensity and foam quality than both Garnachas. Albarín and Verdejo showed higher visual color intensity and olfactory intensity than the rest of the whites, and Verdejo and Malvasía better foam quality. The results obtained in the present study indicated that all the analyzed varieties were suitable for the manufacturing of high quality sparkling wines, although Prieto Picudo, Albarín and Verdejo seemed to be the most promising.

Keywords: white and rosé sparkling wines, polyphenols, biogenic amines, sensory analysis, Verdejo, Viura, Malvasía, Albarín, Godello, Garnacha, Prieto Picudo.

RESUMEN

Este artículo estudia las características sensoriales y la evolución de los compuestos fenólicos y de las aminas biógenas durante el proceso de vinificación y envejecimiento de vinos espumosos blancos y rosados elaborados con diferentes variedades de uva: Verdejo, Viura, Malvasía, Albarín, Godello, Garnacha y Prieto Picudo. Todos los vinos base y sus correspondientes espumosos mostraron bajo grado alcohólico, elevada acidez y bajo pH. La composición y el contenido de los compuestos fenólicos y de las aminas biógenas fue independiente del proceso de elaboración seguido (con o sin maceración prefermentativa) y del color de la variedad de uva empleada. La estabilización por frío y la clarificación de los vinos base antes de la fase de tiraje produjo una disminución en el contenido de antocianos, más acusado en los vinos elaborados con Garnacha que en los obtenidos con Prieto Picudo, en el contenido de catequina, de proantocianidinas y de aminas biógenas. Durante los primeros meses de envejecimiento sobre lías se produjeron pérdidas en todas los tipos de polifenoles debido a su alta reactividad y a los fenómenos de adsorción por las levaduras. Sin embargo, parte de los compuestos fenólicos adsorbidos al inicio de la fase de tiraje fueron liberados durante los últimos meses de envejecimiento debido a la autolisis de las levaduras. Todos los vinos espumosos mostraron bajo contenido de aminas biógenas y los rosados de Garnacha y los blancos de Albarín presentaron los mayores valores de polifenoles totales. Aunque todos los vinos obtuvieron buenas puntuaciones en el análisis sensorial, los obtenidos con Prieto Picudo mostraron una mayor intensidad de color visual, de tonos rojos, de intensidad olfativa y de calidad de la espuma que los de Garnacha. Los espumosos de Albarín y Verdejo presentaron una mayor intensidad de color visual y olfativa que el resto de vinos blancos, mientras que Verdejo y Malvasía mostraron mayor calidad de la espuma. Los resultados obtenidos en este estudio indicaron que todas las variedades de

uva analizadas eran apropiadas para la elaboración de vinos espumosos de calidad, aunque Prieto Picudo, Albarín y Verdejo parecían ser las más prometedoras.

Palabras clave: vinos espumosos blancos y rosados, polifenoles, aminas biógenas, análisis sensorial, Verdejo, Viura, Malvasía, Albarín, Godello, Garnacha, Prieto Picudo.

1. INTRODUCTION

Sparkling wines produced by the traditional method owe their peculiar characteristics to a double process of fermentation and to the ageing with yeast that takes place in the same bottle that reaches the consumer. The best known sparkling wines produced within this premium category are Champagne, from France, Talento, from Italy, and Cava, from Spain.

Although different factors such as variations in winemaking technology (length of time the wine is kept with yeast during ageing) and other viticultural characteristics (soil, vineyard yield, etc.) can affect sparkling wine composition, the grape variety used in its elaboration can be considered one of the most important [1]. In this sense, Spain has an important number of grape varieties which could present good characteristics to obtain sparkling wines with quality and distinctive profiles. Most of sparkling wines manufactured by the traditional method are produced with white grape varieties but the red varieties can also be used to produce rosé (partially macerated with skins) and *blanc de noir* (without maceration) sparkling wines. Some studies have evaluated the aptitude of Garnacha grape variety to elaborate Spanish sparkling wines [2, 3]; however no scientific researchers have been made to study the adequacy of other red varieties such as Prieto Picudo, an autochthonous variety from the northwest of Spain traditionally used for the production of semi-sparkling *aguja* rosé wines and with good characteristics for the winemaking of young and aged wines [4]. In the case of white sparkling wines, although there are many studies about Viura and Malvasía [5, 6, 7, 8, 9, 10, 11], to our knowledge, no studies have evaluated the oenological potential of other white varieties such as Godello, Albarín and Verdejo, frequently used for the

78 production of high-quality still white wines [12, 13, 14] and which present a priori an
79 acidity, aroma and mouth feel suitable for the production of sparkling wines.
80 Only the knowledge of the chemical composition and sensory properties of the
81 sparkling wines produced with these varieties will allow evaluating their adequacy for
82 the manufacturing of new types of high quality sparkling wines. In this sense, it is
83 obvious the influence of monomeric and polymeric phenolic compounds in the color,
84 taste and structure of any wine. In the case of sparkling wines, their quality will also
85 depend on the wine's capacity to create foam.
86 There is no information about the occurrence and evolution of phenolic compounds
87 during the different phases of the sparkling winemaking, i.e. production of base wines,
88 clarification and stabilization, second fermentation and post-fermentation aging on yeast
89 lees in bottles, and most studies are focused on the phenolic profile of the base wines or
90 the final products [2, 7, 9, 11, 15, 16, 17]. Moreover, as far as we know, there are very
91 few studies about phenolics on sparkling rosé wines [2, 3]. On the other hand, and as in
92 the case of phenolics, the grape variety selected by winemakers will be one of the most
93 important variable which will influence on the foam properties of the grape juices [18],
94 the base wines [19], and the sparkling wines [5].
95 Therefore, the present work was aimed to study the changes occurring in monomeric
96 and polymeric phenolic compounds during the winemaking and ageing processes as
97 well as the sensory quality of sparkling wines produced with different white and red
98 grape varieties: Verdejo, Viura, Malvasía, Albarín, Godello, Garnacha and Prieto
99 Picudo. Moreover, outstanding parameters such as biogenic amines were also studied as
100 sanitary control. Sparkling wines made by the traditional method, and also biologically

aged wines, are susceptible to the problem of biogenic amine formation, although there are very few studies of this in the literature [20, 21].

2. EXPERIMENTAL

2.1. Chemicals

All reagents were analytical grade unless otherwise stated. Malvidin-3-glucoside, isorhamnetin, isorhamnetin-3-glucoside, isorhamnetin-3-rutinoside, quercetin-3-O-galactoside, *trans*-ferulic acid, syringic acid, *trans*-caffeic acid, *trans*-*p*-coumaric acid, (+)-catechin, (-)-epicatechin, myricetin, myricetin-3-O-rhamnoside, quercetin, quercetin-3-glucopyranoside, quercetin-3-rutinoside, kaempferol, kaempferol-3-glucoside, *trans*-resveratrol and quercetin-3-arabinoglucoside were purchased from Extrasynthèse (Lyon, France). Agmatine sulfate, cadaverine, histamine, spermidine and phenylethylamine were purchased from Fluka (Buchs, Switzerland), and isoamylamine, diethylethoxymethylenemalonate (DEEMM), putrescine, tyramine, tryptamine, phloroglucinol, L-2-aminoadipic acid, sodium azide and boric acid were obtained from Sigma-Aldrich (Beerse, Belgium). Gallic acid, ethanol 96% (v/v), acetone, HPLC grade methanol, acetic acid glacial and sodium acetate were supplied by Scharlau (Barcelona, Spain). HPLC grade acetonitrile, ascorbic acid and ammonium di-hydrogen phosphate were obtained from Panreac (Barcelona, Spain), and sodium hydroxide and hydrochloric acid were supplied by Carlo Erba (Rodano, Milan, Italy). Trifluoroacetic acid, HPLC grade *o*-phoshoric 85% acid and HPLC grade acetic acid 50% were supplied by Fluka and Toyopearl gel HW-50F was obtained from Tosoh Corporation (Tokyo, Japan).

2.2. Equipments

High performance liquid chromatography (HPLC) was performed using a modular 1100 Agilent liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with one G1311A quaternary pump, an on-line G1379A degasser, a G1316A column oven, a G1313A automatic injector, and a G1315B photodiode-array detector (DAD) controlled by the Chemstation Agilent software. All spectrophotometric measurements were carried out in quartz cuvettes in a UV-vis spectrophotometer (Shimadzu serie UV-1700 pharmaspec, China).

2.3. Vinifications and samples

Five monovarietal white sparkling wines and three monovarietal rosé sparkling wines were produced using the traditional method in the oenological station of Castilla y León. White sparkling wines were obtained with Verdejo and Viura grapes from the Rueda Denomination Origin (D.O.), Malvasía grapes from the Toro D.O., Albarín grapes from the Tierras de León D.O. and Godello grapes from the Bierzo D.O. Rosé sparkling wines were obtained with grapes of Garnacha from the Cigales D.O. and Prieto Picudo grapes from the Tierras de León D.O. Two different viticultural areas of Garnacha were used in this work and thus two different Garnacha wines were obtained, called Garnacha and Garnacha* respectively.

The elaboration of traditional sparkling wines involved two production stages: preparation of the base wine or *cuvee* and the *sparkle* stage. Therefore, base wines were prepared using the traditional white or rosé winemaking process. White grapes were destemmed-crushed and pressed to obtain juice, and red grapes were left to prefermentative maceration for 2 days before get the must. The fermentation processes took place in stainless steel tanks of 150 L by duplicate and from each of the eight base

147 wines a batch of sparkling wines was manufactured by the so-called *champenoise*,
148 *traditional* or *classic* method. Base wines were cold-stabilized and clarified with PVPP
149 and bentonite, and then the wines were bottled and the *tirage* liquor, formed by yeast *S.*
150 *cerevisiae* var. *bayanus*, sucrose and bentonite, was added. Second fermentation and
151 aging with yeast during nine months was carried out at approximately 15-16 ºC (cellar
152 temperature). Finally, sparkling wines were riddled and disgorged.
153 Samples were taken for analysis of the base wines (BW), after clarification and
154 stabilization of the base wines (CBW), and then after 3 months (T3M), 6 months (T6M)
155 and 9 months (T9M) of aging on yeast lees, previously having performed hand
156 disgorging. All of the analytical determinations were performed on wines previously
157 degasified. Sensory analysis was conducted in the final sparkling wines (T9M) since
158 nine months is the minimum time established by regulation (CE) Nº 606/2009 for aging.

159 **2.4. Determination of oenological parameters**

160 Oenological parameters were evaluated following official analysis methods [22]. The
161 color intensity and tonality of rosé wines was evaluated using the Glories parameters
162 [23]. The color of white wines was evaluated by the measurement of the absorbance at
163 420 nm.

164 **2.5. Analysis of monomeric phenolics**

165 Anthocyanins, hydroxycinnamic acids, flavonols, flavan-3-ols and gallic acid were
166 analyzed by HPLC-DAD with a direct injection of wine. Separation was achieved in an
167 ACE HPLC (5 C18-HL) particle size 5 μm (250 mm x 4.6 mm) column protected with a
168 guard column of the same material, according to the methodology described in Gómez-
169 Alonso *et al.* [24]. Quantification of non-commercial compounds was made using the
170 calibration curves belonging to the most similar compound: malvidin-3-glucoside for

171 the anthocyanins; quercetin-3-glucoside for myricetin-3-glucoside and quercetin-3-
172 glucuronide; caffeic acid for *cis*- and *trans*-caftaric acids (*cis*- and *trans*-caffeoyl-
173 tartaric acid); *p*-coumaric acid for *cis*- and *trans*-coutaric acids (*cis*- and *trans*-*p*-
174 coumaryl-tartaric acid); ferulic acid for *cis*- and *trans*-fertaric acids (*cis*- and *trans*-
175 ferulic-tartaric acid); and *trans*-resveratrol for its glucoside. Calibration curves were
176 obtained using the commercial standards in the concentrations normally present in
177 oenological products, obtaining regression coefficients (r^2) above 0.995 in all cases. The
178 content of non-acylated anthocyanins (A-Gluc) was calculated as the sum of
179 delphinidin, cyanidin, petunidin, peonidin and malvidin-3-glucosides; the content of
180 acetyl-glucoside anthocyanins (A-Ac) as the sum of delphinidin, cyanidin, petunidin
181 and malvidin-3-(6-acetyl)-glucosides; the content of coumaryl-glucoside anthocyanins
182 (A-Cm) included delphinidin, petunidin, and malvidin-3-(6-*p*-coumaryl)-glucosides.
183 The sum of A-Gluc, A-Ac and A-Cm was referred to as total monomeric anthocyanins
184 (T-A). Total hydroxycinnamic acids (T-HA) were calculated as the sum free acids, i. e.,
185 caffeic, ferulic and coumaric acid, and hydroxycinnamates, i. e., *trans*-caftaric (*trans*-
186 caffeoyl-tartaric acid), *cis*-caftaric (*cis*-caffeoyl-tartaric acid), *trans*-coutaric (*trans*-*p*-
187 coumaryl-tartaric acid), *cis*-coutaric (*cis*-*p*-coumaryl-tartaric acid) and *trans*-fertaric.
188 Total esterified hydroxycinnamic acids (E-Ac) were calculated as the sum of *trans*-
189 caftaric, *cis*-caftaric, *trans*-coutaric, *cis*-coutaric and *trans*-fertaric. Total flavonol
190 content (T-Flavo) was calculated as the sum of myricetin-3-glucoside, quercetin-3-
191 rutinoside, quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-glucuronide,
192 isorhamnetin-3-rutinoside, kaempferol-3-glucoside, isorhamnetin-3-glucoside,
193 myricetin, quercetin, kaempferol and isorhamnetin. All analyses were performed in
194 duplicate.

195 **2.6. Analysis of proanthocyanidins**

196 Wine samples were directly fractionated by gel permeation chromatography (GPC) on a
197 Toyopearl gel HP-50F column as described by Guadalupe *et al.* [25]. A first fraction
198 (F1) was eluted with ethanol/water/trifluoroacetic acid (55:45:0.05, v/v/v), and a second
199 fraction (F2) was recovered by elution with acetone/water (60:40, v/v) and it was taken
200 to dryness under vacuum. Fractionation was performed in triplicate and phloroglucinol
201 adducts were analyzed in F2 fractions by reversed-phase HPLC as described by
202 Kennedy and Jones [26]. The column was an ACE HPLC (5 C18-HL) particle size 5μm
203 (250 mm x 4.6 mm) protected by a guard column containing the same material.
204 Proanthocyanidins cleavage products were estimated using their response factors
205 relative to (+)-catechin which was used as the quantitative standard (r^2 of 0.999 in the
206 range of concentrations 0-500 mg L^{-1}). Total proanthocyanidin content (PA) in mg L^{-1}
207 was calculated as the sum of all the subunits: extension subunits (phloroglucinol
208 adducts) and terminal subunits (catechin, epicatechin and epicatechin-gallate). The
209 percentage of the terminal subunits catechin (%Cat), epicatechin (%Epi) and
210 epicatechin-gallate (%Epigal) was calculated over the total proanthocyanidin content.
211 To calculate the apparent mean degree of polymerization (mDP), the sum of all subunits
212 was divided by the sum of the terminal subunits.

213 **2.7. Analysis of biogenic amines**

214 Chromatographic separation was performed in an ACE HPLC column (5 C18-HL)
215 particle size 5 μm (250 mm x 4.6 mm) thermostatized at 16 ºC through the binary
216 gradient (solvent A: 25 mM acetate buffer pH= 5.8 with 0.02% sodium azide; solvent
217 B: mixture of acetonitrile and methanol 80:20) as described Gómez-Alonso *et al.* [27].
218 In the proposed conditions, 9 biogenic amines as aminoenone derivatives were

219 separated, identified and quantified in a single injection. Calibration curves were
220 obtained using the commercial standards in the concentrations normally present in
221 oenological products, obtaining regression coefficients (r^2) above 0.995 in all cases.
222 Total biogenic amines were calculated as the sum of histamine, agmatine, spermidine,
223 tyramine, putrescine, tryptamine, cadaverine, phenylethylamine and isoamylamine. All
224 analyses were performed in triplicate.

225 **2.8. Sensory analysis**

226 Sparkling wines after nine months of aging on yeast lees were analyzed for sensory
227 profiling and panellists rated the sparkling wines for visual, olfactory, gustatory and
228 foam quality conformance to sparkling wine typology.

229 The sensory analysis was carried out by a tasting panel made up of twelve persons, all
230 expert tasters from the Regulatory Councils of various Spanish Origin Designations and
231 wineries. Tasters defined the descriptors used in the sensory analysis according to the
232 methodology described in González-San José *et al.* [28] and they were then trained to
233 quantify them using structured numerical scales. The training was carried out in
234 accordance with UNE-87-020-93 Norm, corresponding to ISO 4121:1987 Norm.
235 Tasters selected 8 attributes for the olfactory, 7 for the gustative and 4 for the visual
236 phase, which were agreed upon as best for describing the sensory characteristics of the
237 sparkling wines. A structured numerical scale of seven points was used, with 1
238 representing absence of sensation and 7 a very high intense perception. In visual aspect,
239 special attention was paid not only to the color but also to the observation of foam
240 characteristics, which many consumers consider to be one of the most important
241 characteristics of a sparkling wine. To determine the foam quality, 5 descriptors
242 previously defined by Gallart *et al.* [29] were evaluated on a scale of 1 to 3. Judges

243 rated two groups of sparkling wines in two different sessions. In the first session they
244 were given the five white sparkling wines, in the second, they were given the three rosé
245 sparkling wines. In order to ascertain judges' consistency one sample was replicated in
246 each session. Serving temperature was 10±2 ºC in new flute glasses (100 mL) with no
247 faults or marks. Each bottle was opened slowly, with the cork held in the hand and
248 without shaking the bottle. To avoid air bubble formation, the wine was poured slowly
249 into the glass.

250 **2.9. Statistical procedures**

251 Significant differences between analytical determinations were studied by an analysis of
252 variance (ANOVA) and they were set up to at least $p < 0.05$ (95% confidence level). In
253 sensory analysis, the within judges reproducibility in rating two replicated wines was
254 tested by an ANOVA, and the sensory data were then analysed by Generalized
255 Procrustes Analysis (GPA) on the mean ratings for olfactory, gustatory and quality
256 foam attributes. A permutation test was made to study the agreement between tasters,
257 obtaining a good consistency (87.43%).

258 ANOVA evaluations were performed using the Statistica 8.0 program for Microsoft
259 Windows (Statsoft Inc., Tulsa, Oklahoma) and the GPA and correlation analyses by
260 using the Senstools Version 3.3.2. program (Utrecht, The Netherlands).

261 **3. RESULTS AND DISCUSSION**

262 **3.1. Oenological parameters**

263 Table 1 shows the oenological parameters of the base wines (BW) and the final
264 sparkling wines after 9 months of aging on yeast lees (T9M). For sparkling
265 winemaking, grapes were harvested at low sugar content and thus the base wines
266 obtained showed low alcohol content (between 10.3% and 11.8 % vol), high acidity (>

267 7.0 g L^{-1} TH_2) and low pH (< 3.1). Volatile acidity ranged from 0.1 to 0.3 g of acetic
268 acid per liter, which is acceptable for any wine. The absorbance at 420 nm, which is
269 customarily employed as a measure of browning of white wines, was low in all base
270 wines and no significant differences were found between them. Regarding color
271 intensity and hue in rosé wines, Prieto Picudo base wines showed significantly higher
272 values than Garnacha. In general, all CI values were lower than those usually found in
273 still rosé wines [30]. This was due to the fact that the grapes used for the elaboration of
274 sparkling wines have to be harvested at low maturity and consequently the phenolic
275 maturity is low, which make difficult the extraction of phenolic compounds.
276 Relative to the *sparkle* stage, the alcoholic content increased in all the wines because of
277 the second fermentation in bottle. Total acidity in the final sparkling wines was quite
278 high although it decreased due to the precipitation potassium tartrate during cold
279 stabilization. The low pH and high acidity values of the final wines indicated that a
280 priori the grape varieties studied were suitable for the elaboration of sparkling wines
281 because low pH and high acidities have a positive impact on the sensorial evaluation of
282 this wine category, especially in the freshness characteristics of sparkling wines. With
283 regards to browning and color, final white sparkling wines showed almost half of the
284 value of absorbance at 420 nm than base wines. These results contrasted with those
285 obtained by Ibern-Gómez *et al.* [7] who found in Cavas an increase of browning
286 (measured as the absorbance at 420 nm) during ageing due to the oxidation of phenolic
287 compounds in spite of the reducing atmosphere existent within the bottle. Values of
288 color intensity decreased in all rosé wines during the winemaking process. This descent,
289 which was more accused in both Garnacha varieties (76-79%) than in Prieto Picudo
290 (65%), was observed to occur in two winemaking stages (data not shown): firstly,

291 during the clarification-stabilization processes, and secondly, during wine ageing in
292 contact with lees, in which phenolic compounds responsible for color would probably
293 have an affinity to adhere to the cell walls of lees or to combine with other wine
294 constituents. The CI values found in the final sparkling wines were in the range
295 described by other authors for other rosé sparkling wines made of Monastrell and Trepat
296 [31] and quite higher than those found by Pozo-Bayón *et al.* [3] for a rosé sparkling
297 wine made of Garnacha.

298 **3.2. Monomeric phenolics**

299 3.2.1. Monomeric anthocyanins

300 Changes in total anthocyanin (T-A) and non-acylated anthocyanin (A-Gluc)
301 concentration during the different stages of rosé sparkling winemaking are shown in
302 Figure 1. No significant differences were observed in the total content of anthocyanins
303 among base wines. All of them showed low contents of these compounds (~15 mg L^{-1})
304 in comparison with still rosé wines [30] because, as explained before, grapes were
305 harvested at low phenolic maturity. Glucoside anthocyanins were the major
306 anthocyanins in all the wines, varying between 85% in Prieto Picudo to 93% in
307 Garnachas. Differences between both varieties were also observed in the content of *p*-
308 cumarylated and acetylated anthocyanins, and thus the content of acetylated
309 anthocyanins was higher than the *p*-cumarylated forms in Prieto Picudo base wines but
310 the opposite occurred in both Garnachas (data not shown). Malvidin-3-glucoside was
311 the main anthocyanin in all the wines and represented around 70% of total
312 anthocyanins; hence, its derivatives were also the main anthocyanins in the acetylated
313 and coumaroylated forms (data not shown). The content of all forms of anthocyanins
314 was drastically reduced during two stages of the wine elaboration: the process of cold-

315 stabilization and clarification of the base wines and the first six months of ageing
316 sparkling wine in contact with lees. Decreases during clarification-stabilization were
317 attributed to the cold treatment and to the adsorption of phenolic material by the
318 bentonite and PVPP; decreases during aging, which may be attributed to the adsorption
319 of these compounds to the cell walls of lees [32, 33, 34] or to their combination with
320 other wine compounds [35], were in good agreement with the results previously
321 observed with the CI values. In this sense it is important to point out two phenomena.
322 Firstly, the greatest looses in the anthocyanic compounds occurred in the Garnacha
323 varieties and it was mainly due to the clarification stage. This fact, again in accordance
324 with the lower CI values obtained in the Garnacha final wines, seemed to indicate that
325 that Prieto Picudo was more stable in terms of color and anthocyanic compounds.
326 Secondly, the content of total anthocyanins decreased in both varieties during the first
327 six months of aging on yeast lees but it slightly increased during the last three months in
328 the sparkling wines made from Prieto Picudo. This probably occurred because as wine
329 aging progresses, a seemingly slow autolytic process of yeast occurs [36, 37] and the
330 phenolic adsorbed in the first stages of aging are released. With regard to the
331 anthocyanin concentration of the final sparkling wines, Prieto Picudo showed
332 significantly higher contents than Garnacha wines although the values found in the
333 present study were considerably higher than those described by Pozo Bayón *et al.* [3] in
334 a rosé Garnacha sparkling wine.

335 3.2.2. Hydroxybenzoic and hydroxycinnamic acids

336 The concentration of hydroxycinnamic acids and hydroxycinnamates during the
337 different stages of the sparkling wine elaboration is shown in Figure 2. The compounds
338 not shown in the figure were detected in very low concentrations. Thus, the content of

339 gallic acid was below 2.4 mg L^{-1} and free hydroxycinnamic acids were also detected in
340 very low concentrations (< 1.2 mg L^{-1} for caffeic acid, < 0.63 mg L^{-1} for coumaric acid
341 and < 0.5 mg L^{-1} for ferulic acid). These results are in good agreement with those
342 obtained in other studies for different white (*blanc de blanc* and *blanc de noir*) and rosé
343 sparkling wines [2, 7, 15].
344 Both Garnacha base wines had the greatest content of total hydroxycinnamic acids (T-
345 HA) (~90 mg L^{-1}), followed by far by Albarín and Viura (~30 mg L^{-1}) and lastly by
346 Malvasía, Verdejo, Godello and Prieto Picudo (< 20 mg L^{-1}). Total hydroxycinnamates
347 (E-Ac) were the predominant acids in all the wines, representing over 95% of total
348 hydroxycinnamic acids, and there were the esters of caffeic acids the major compounds.
349 Hydroxycinnamic acid composition and content was found to be independent on the
350 winemaking technology employed for the elaboration of the white or rosé wines and on
351 whether the grapes used were red or white. Thus, Garnacha rosé base wines contained
352 largely the highest content of all forms of hydroxycinnamic acids while Prieto Picudo
353 rosé wines showed values in the range found for the white wines. The most abundant
354 hydroxycinnamic found in Albarín, Viura, Godello, Malvasía and both Garnachas was
355 the *trans*-caftaric acid but it was its *cis*- isomer the major on Verdejo and Prieto Picudo.
356 With the exception of Viura, the stabilization and clarification of the white base wines
357 produced a decrease in the content of total hydroxycinnamic acids, both in the free and
358 esterified forms. On the contrary, and unlike observed with monomeric anthocyanins,
359 the stabilization and clarification of rosé base wines did not produce any effect on the
360 content of hydroxycinnamic acids.
361 As observed by other authors [37], the second fermentation and aging increased free
362 hydroxycinnamic acid formation (data not shown) through forms esterified with tartaric

363 acid. The *sparkle* stage produced important differences among wines in the evolution of
364 total hydroxycinnamic acids and they were due to differences in their esterified forms.
365 The first three months of aging produced a decrease in the content of total
366 hydroxycinnamic acids in all the wines. However, this descent was much more accused
367 in the varieties that showed the highest content of these compounds in the clarified base
368 wine (CBW); hence, both Garnacha wines, with initial values of hydroxycinnamic acids
369 of 90 mg L^{-1}, suffered a decrease of around 20% while Viura, Albarín and Malvasía
370 (initial values between 15 and 30 mg L^{-1}) suffered a decrease of around 15%. Lastly, the
371 values of total hydroxycinnamic acids dismissed less than 10% in Godello and Verdejo,
372 which reached the lowest values of these compounds (~10 mg L^{-1}). Following this
373 tendency, the content of total acids decreased around 45% in the next three months of
374 aging in the wines with the highest contents, the Garnachas, while it was maintained in
375 the rest. Finally, the last three months of aging produced an increase of 70% in total
376 hydroxycinnamic acids in both Garnacha wines but its content was again maintained in
377 the rest. Taking into account these differences, it seemed that the evolution of the
378 hydroxycinnamic acids was directly related with its concentration rather than with any
379 other factor. The first months of aging on yeast lees entailed losses of esters of
380 hydroxycinnamic acids due to their interaction with other wine compounds to give more
381 stable pigments [38] and/or because they can also be metabolised and adsorbed by yeast
382 lees [39, 40]. In this sense, it was observed that the highest the initial values of
383 hydroxycinnamic acids in the base wines, the highest the adsorption or transformation
384 phenomena occurred. Finally, and like it was observed with anthocyanins, adsorbed
385 hydroxycinnamic acids were released as yeast cell walls were degraded due to the

386 autolytic process, but this only occurred in the wines that showed high quantities of
387 these compounds.
388 With regard to the acid composition of the final sparkling wines, Garnacha base wines
389 contained largely the highest content of all forms of hydroxycinnamic acids (> 70 mg L^{-1}
390 of T-HA), followed by a great distance by Albarín and Viura (~20 mg L^{-1} of T-HA),
391 and finally by the rest of the wines (8-12 mg L^{-1} of T-HA). Therefore, and with the
392 exception of both Garnachas, all the wines showed similar contents than those described
393 in bibliography for Chardonnay, Pinot Noir, Macabeo, Parellada and Xarel.lo white
394 sparkling wines, and higher contents than Trepat or Monastrell white sparkling wines
395 [2, 7, 15]. As it was observed in the base wines, it was again the *trans*-caftaric acid the
396 most abundant hydroxycinnamic found in Albarín, Viura, Godello, Malvasía and both
397 Garnachas while it was the *cis*- form the most abundant in Verdejo and Prieto Picudo.
398 These results contrasted with other studies in which the *trans*-caftaric form is found to
399 be the most abundant in all the cases [2, 7, 15].
400 3.2.3. Flavonols and catechin
401 The content of total flavonols both in white and rosé base wines was below 2.5 mg L^{-1}
402 (data not shown) and this was again independent on the winemaking technology and the
403 grape used. In the final sparkling wines, total flavonols could only be quantified in both
404 Garnacha and Prieto Picudo wines, which reached values of 2.14, 1.73 and 1.08 mg L^{-1},
405 respectively.
406 The only flavan-3-ol detected in the wines within the quantification limits was (+)
407 catechin (Figure 2). Among base wines, Albarín and both Garnachas showed the highest
408 values of catechin, followed by far by Prieto Picudo and lastly by Viura, Godello,
409 Malvasía and Verdejo. Contrary to what was observed with other phenolics, the

410 clarification and stabilization process did not affect to the content of catechin. The
411 *sparkle* stage produced an important decrease of catechin in the wines that showed the
412 higher content of this compound, i. e., both Garnachas, Prieto Picudo and Albarín. This
413 decrease may be due to the fact that original monomeric flavan-3-ols are converted into
414 more stable polymers by reacting with other flavanols, anthocyanins, and small
415 molecules such as pyruvic acid and vinylphenol.
416 The average level of catechin in Viura, Godello, Malvasía and Verdejo final white
417 sparkling wines was within the range reported by other authors in *blanc de blanc*
418 Chardonnay, Macabeo, Parellada and Xarel.lo, *blanc de noir* Pinot Noir, Trepat and
419 Monastrell, and rosé Garnacha sparkling wines [2, 15]. However, the content found in
420 the Albarín white sparkling wines and in both Garnachas and Prieto Picudo rosé
421 sparkling wines was considerably higher.
422 **3.3. Proanthocyanidins**
423 The evolution of total proanthocyanidins (PA) content as well as % of catechin, %
424 epicatechin, % epicatechin-gallate terminal subunits and mean Degree of
425 Polymerization (mDP) during sparkling winemaking is shown in Table 2.
426 As it was observed with catechin, the content of total proanthocyanidins was
427 considerably higher in Albarín and Garnacha base wines than in the rest of the base
428 wines. This fact indicated again that the content of these compounds was independent
429 on whether the grapes used were red or white, and on the winemaking technology
430 employed for the elaboration of the rosé or white wines (with or without
431 prefermentative maceration), which was somehow expected as this kind of compounds
432 are better extracted with a certain content of alcohol. The mean degree of
433 polymerization (mDP), which is sometimes related with the astringent and bitter

434 character of proanthocyanidins [41], was found to be quite similar in all the base wines
435 and showed a value around 6.5. With the exception of Malvasía, the terminal units were
436 mainly comprised of catechin in all base wines, and epicatechin and epicatechin-gallate
437 were found in lower quantities. Catechin is the primary terminal subunit described in
438 the grape skin and epicatechin and epicatechin-gallate are usually reported in grape
439 skins in much lower quantities [42, 43, 44], suggesting that the contribution of PA from
440 grape skins was higher than the contribution of PA from grape seeds in the wine
441 samples evaluated.
442 The clarification and stabilization reduced significantly the content of PA in all base
443 wines, and it was the Garnacha the wine which showed the most pronounced loss
444 (~45%). Decreases in PA content were attributed to cold stabilization because tannins
445 were associated with the tartrate precipitates, in agreement with previous studies [45].
446 The mDP was maintained during clarification-stabilization but this stage clearly
447 modified the percentage of terminal subunits in all the wines; hence, epicatechin-gallate
448 was the major terminal subunit in all the wines after clarification-stabilization.
449 As observed with other phenolics, two different phases were distinguished during the
450 *sparkle* stage. The content of PA decreased in all the wines during the first six months
451 of aging, from 9% in Verdejo to 55% in Albarín, to increase thereafter during the last
452 three months, from 5% in Viura to 82% in Albarín. The drop in the tannin levels was
453 explained on the basis of their reactivity and their adsorption by lees. On the one hand,
454 proanthocyanidins are highly reactive compounds which are involved in condensation
455 and polymerization reactions as well as precipitations [46]. On the other hand, some
456 studies seem to indicate that tannins are adsorbed on lees in preference to monomeric
457 phenols, even with low quantities of lees [47]. The PA increase during the last months

458 of aging was attributed to the release of this kind of polyphenol during the autolysis of
459 the yeast cell walls. With regards to the percentages of catechin, epicatechin and
460 epicatechin-gallate terminal subunits, they showed different tendencies during aging of
461 lees, but with the exception of Godello, epicatechin-gallate continued being the
462 predominant subunit in all wines. Although there are no studies on sparkling wines,
463 these results contrasted with bibliography since concentrations of gallates as terminal
464 units in wines are usually low or even undetectable [43, 48]. In general, and unlike it
465 occurred with monomeric polyphenols, the sparkling winemaking reduced the
466 differences in the PA concentrations observed in the initial base wines. Consequently,
467 all the sparkling final wines obtained in this study contained similar concentrations of
468 proanthocyanidins and similar to those obtained by Jordão *et al.* [17] in white
469 Portuguese sparkling wines.

470 **3.4. Total phenolics**

471 The evolution of total phenolics in wines during the different stages of the sparkling
472 wine elaboration is shown in Figure 3. The content of total polyphenols was calculated
473 as the sum of flavonoids (anthocyanins, flavonols, catechin and proanthocyanidins) and
474 non flavonoids (hydroxycinnamic acids (free and esterified forms) and hydroxybenzoic
475 acids). Among rosé wines, Garnacha base wines showed the highest content of total
476 phenols (> 210 mg L^{-1}) followed by far by Prieto Picudo (~ 90 mg L^{-1}). In white wines,
477 Albarín base wines showed the highest content of total phenols (~ 135 mg L^{-1}) while the
478 rest of the wines showed concentrations from 70 to 95 mg L^{-1}. With the exception of
479 Garnacha base wines, proanthocyanidins were the major phenols in all base wines (from
480 60% in Prieto Picudo to 80% in Godello), followed by hydroxycinnamic acids (from
481 15% in Prieto Picudo to 30% in Viura), anthocyanins in Prieto Picudo (~ 15%), catechin

(2-8%) and lastly flavonols and gallic acid (< 2%). In both Garnacha base wines, hydroxycinnamic acids showed a similar content than proanthocyanidins and both represented about 42% of total polyphenols.

In final sparkling wines after 9 months of aging, both the contents and proportions of wine polyphenols were quite similar to those observed in the initial base wines. Garnacha rosé wines showed again the highest values of total polyphenols (115-120 mg L^{-1}), followed by Albarín (~ 85 mg L^{-1}), Viura and Prieto Picudo (65-70 mg L^{-1}), Verdejo (~ 57 mg L^{-1}), and Malvasía and Godello (45-50 mg L^{-1}). With the exception of Garnachas, the content of proanthocyanidins represented more than 65% of total phenols in all the wines, followed by far by hydroxycinnamic acids (20-30%), catechin (2%-10%) and the rest of phenols (< 2%). In both Garnacha rosé sparkling wines, the content hydroxycinnamic acids was significantly higher than the proanthocyanidins; hence, total hydroxycinnamic acids represented around 60% of total phenols in the final wines while the content of proanthocyanidins was around 35%.

3.5. Biogenic amines

Figure 4 shows the biogenic amine concentration in base wines before and after clarification and stabilization. Table 3 shows the content of biogenic amines in sparkling wines during aging on yeast lees. Putrescine was by far the most abundant amine in all base wines analyzed (56-72% of the total content) followed from a great distance by agmatine, spermidine, tryptamine, cadaverine, phenylethylamine and histamine (Figure 4a). Putrescine is also described in bibliography as the major amine both in the must [49] and in the wine [50]. The clarification and cold stabilization of the base wines caused a significant reduction in the content of total biogenic amines (Figure 4b), and there were the putrescine and spermidine the most affected in all the wines

while phenylethylamine or tryptamine concentration was unaffected. Other authors also observed that the use of clarifying agents can reduce amine levels [51, 52] but there is no much literature on the subject. Regarding the *sparkle* stage, total biogenic amine concentration remained constant during the first three months of aging on yeast lees. These results suggested that during the second fermentation in the bottle, *S. cerevisiae* var. *bayanus*, which was added in the *tirage* liquor, was unable to produce biogenic amines. However, an increase of total biogenic amines was observed in all the analyzed wines during the following three months of aging and it was attributed to the release of amino acids during yeast autolysis [53], which can act as precursors of biogenic amines. Finally, it is important to point out that the contents of histamine, tyramine and phenylethylamine in all the final sparkling wines elaborated in this study were ten times lower than the toxic levels described in bibliography [54]. Therefore, none of the wines could cause the undesirable physiological effects usually associated to the presence of these compounds.

3.6. Sensory analysis

Sparkling wines after 9 months of aging were submitted to a panel of expert tasters for sensory profiling. Firstly, the within judges reproducibility was evaluated by mean of replicated sparkling wines and replications were demonstrated not to be source of variation. Generalized Procrustes Analysis (GPA) was then applied to sensory data to ascertain consistency among the twelve judges and provide information on relationship between sparkling wines and attributes.

In the visual phase, Albarín and Verdejo sparkling wines showed significantly higher visual color intensity, green and yellow tones than the other white sparkling wines (data not shown). These results were in agreement with the oenological parameters as Albarín

530 and Verdejo sparkling wines showed higher absorbance at 420 nm than the rest of the
531 white wines. Among rosé sparkling wines, Prieto Picudo showed higher visual color
532 intensity and red and tones than rosé sparkling wines made of Garnacha (data not
533 shown). These results were again correlated with the higher values of color intensity and
534 total anthocyanins obtained in Prieto Picudo sparkling wines.
535 Figure 5 shows the sparkling wines and attribute average space obtained from GPA, as
536 determined by their olfactory, gustatory and foam quality perceptions. Sparkling wines
537 were properly located in the vectorial dimension defined by the first two factors, which
538 accounted for 56.19% of the total variance in the olfactory GPA space, for 58.17% in
539 the gustatory GPA space and for 61.45% in the foam quality GPA space. In the
540 olfactory space (Figure 5a), the consensus plot showed a clear different distribution of
541 the samples between rosé and white sparkling wines, thus indicating a marked
542 difference among them. In this sense, it is interesting to point out that both Garnacha
543 wines were perceived by the tasters as being very similar wines, as well as Godello,
544 Viura and Malvasía; Albarín and Verdejo were also located quite close in the GPA
545 space. Prieto Picudo was characterized by a higher olfactory intensity, dominated by
546 fruity aromas, than both Garnachas wines. All white sparkling wines were characterized
547 by citrus, exotic fruit and floral aromas. Moreover, Albarín and Verdejo showed higher
548 olfactory intensity and a higher correlation with yeasty and fruity aromas. With regards
549 to the gustatory space (Figure 5b), there was not a clear distribution between white and
550 rosé sparkling wines. Albarín and Godello were characterized by an overall good
551 equilibrium, full-body and persistence. On the other hand, Prieto Picudo and Verdejo
552 were clearly associated with bitterness and freshness sensations while neither the
553 Garnachas nor the Malvasía highlighted in any gustatory sensation. Finally, a clear

554 differentiation among samples was observed in the foam quality space (Figure 5c).
555 Although all the sparkling wines obtained good punctuations in the foam attributes (data
556 not shown), Verdejo, Malvasía and Prieto Picudo showed a higher correlation with
557 initial foam, foam collar, foam area and bubble size.

558 **4. CONCLUSIONS**

559 The early harvest for the winemaking of sparkling wines produced that all the base
560 wines showed low alcohol content, high acidity and low pH. This fact also explained
561 the low content of anthocyanins in rosé base wines, although Prieto Picudo wines
562 showed significantly higher values of color intensity and hue than both Garnachas. The
563 composition and content of hydroxycinnamic acids, flavonols and proanthocyanidins in
564 base wines was found to be independent on whether the grapes used were red or white
565 and on the winemaking technology employed for the elaboration of rosé or white wines
566 (with or without prefermentative maceration). Thus, Garnacha rosé wines contained
567 largely the highest content of these compounds while Prieto Picudo rosé base wines
568 showed values in the range of the white wines.

569 The stabilization-clarification of the base wines before the *sparkle* stage and the first six
570 months of ageing sparkling wine in contact with lees produced a drastic reduction in the
571 content of all forms of polyphenols in all wines. Decreases during clarification-
572 stabilization were attributed to the cold treatment and to the adsorption of phenolic
573 material by the bentonite and PVPP; decreases during aging were attributed to the
574 adsorption of these compounds to the cell walls of lees or to their transformation or
575 combination with other wine compounds. The stabilization-clarification produced a
576 great decrease in the content of catechin and proanthocyanidins but also in the
577 concentration of biogenic amines. The decrease in anthocyanins was more accused in

578 Garnacha wines than in Prieto Picudo, which was more stable in terms of colour. All
579 these losses were somehow compensated as the slow autolytic process of yeast
580 produced that part of the phenolics adsorbed in the first stages of aging were released
581 during the last months. The release of anthocyanins and hydroxycinnamic acids was
582 only observed in wines that showed high quantities of these compounds.
583 In final sparkling wines after 9 months of aging, Garnacha rosé wines and Albarín white
584 wines showed the highest values of total polyphenols. With the exception of Garnachas,
585 which showed a similar content of hydroxycinnamic acids and proanthocyanidins, the
586 latter represented more than 65% of total polyphenols in all the wines. The content of
587 biogenic amines was low in all the final sparkling wines. In the sensory analysis,
588 although all wines obtained good punctuations, Prieto Picudo, Verdejo and Albarín
589 were the most outstanding. Prieto Picudo showed higher visual color intensity, red
590 tones, olfactory intensity and foam quality than both Garnachas. All white sparkling
591 wines were characterized by citrus, exotic fruit and floral aromas, but Albarín and
592 Verdejo showed higher visual color intensity and olfactory intensity, and Verdejo and
593 Malvasía better foam quality.
594 The results obtained in the present study indicated that all the studied varieties were
595 suitable for the manufacturing of high quality sparkling wines, although Prieto Picudo,
596 Albarín and Verdejo seemed to be the most promising.

597 **4. CONCLUSIONES**

598 La vendimia precoz realizada en la elaboración de los vinos espumosos ocasionó que
599 todos los vinos base tuvieran bajo grado alcohólico, elevada acidez y bajo pH. Este
600 hecho también explicó el pobre contenido de antocianos en los vinos base rosados,

601 aunque el elaborado con la variedad Prieto Picudo mostró valores superiores de
602 intensidad de color y tonalidad que los vinos elaborados con la variedad Garnacha.
603 La composición y el contenido de ácidos hidroxicinámicos, flavonoles y
604 proantocianidinas en los vinos base fue independiente del color de la variedad de uva y
605 del proceso de vinificación seguido para la elaboración de los vinos rosados o blancos
606 (con o sin maceración prefermentativa). Así, los vinos base rosados elaborados con la
607 variedad Garnacha mostraron las cantidades más altas de estos compuestos, mientras
608 que los rosados de Prieto Picudo tuvieron valores similares a los obtenidos en los vinos
609 base blancos.
610 La estabilización-clarificación de los vinos base antes de la fase de tiraje y los primeros
611 seis meses de envejecimiento del vino sobre lías produjeron una reducción en el
612 contenido fenólico en todos los vinos. Los descensos durante los procesos de
613 clarificación-estabilización de los vinos base fueron debidos al tratamiento por frío y a
614 fenómenos de adsorción del material fenólico por la bentonita y el PVPP; las
615 disminuciones durante el envejecimiento fueron atribuidas a la adsorción de estos
616 compuestos por las lías de las levaduras o a su transformación o combinación con otros
617 componentes del vino. Los procesos de clarificación-estabilización produjeron un gran
618 descenso en el contenido de catequina y proantocianidinas así como en la cantidad de
619 aminas biógenas. La disminución en antocianos fue más acusada en los vinos
620 elaborados con la variedad Garnacha que en los obtenidos con la variedad Prieto
621 Picudo, siendo estos últimos más estables en términos de color. Todas estas pérdidas
622 fueron en parte compensadas por el lento proceso de autolisis de las levaduras, que
623 ocasionó que parte de los fenoles adsorbidos en las primeras etapas del envejecimiento
624 fueran liberados al medio durante los últimos meses. La liberación de antocianos y

625 ácidos hidroxicinámicos sólo se observó en aquellos vinos que contenían elevadas
626 cantidades de estos compuestos.
627 Entre los vinos espumosos finales después de 9 meses de envejecimiento, los vinos
628 rosados elaborados con la variedad Garnacha y los blancos obtenidos con la variedad
629 Albarín mostraron el mayor contenido en polifenoles totales. Con excepción de los
630 vinos espumosos elaborados con la variedad Garnacha, que mostraron concentraciones
631 similares de ácidos hidroxicinámicos y proantocianidinas, en el resto de vinos las
632 proantocianidinas representaron más del 65% de los compuestos fenólicos. El contenido
633 de aminas biógenas fue bajo en todos los vinos espumosos finales. En el análisis
634 sensorial, aunque todos los vinos obtuvieron buenas puntuaciones, los elaborados con
635 Prieto Picudo, Verdejo y Albarín fueron los más destacados. Los vinos obtenidos con
636 Prieto Picudo mostraron mayores puntuaciones en intensidad de color visual, tonos
637 rojos, intensidad olfativa y calidad de la espuma que los elaborados con la variedad
638 Garnacha. Todos los vinos blancos se caracterizaron por aromas cítricos, de frutas
639 exóticas y florales, sin embargo, los elaborados con Albarín y Verdejo mostraron mayor
640 intensidad de color visual y aromática, y los elaborados con Verdejo y Malvasía mejor
641 calidad de la espuma.
642 Los resultados obtenidos en este estudio indicaron que todas las variedades estudiadas
643 eran apropiadas para la elaboración de vinos espumosos de calidad, aunque Prieto
644 Picudo, Albarín y Verdejo parecían ser las más prometedoras.

645 **ACKNOWLEDGEMENTS**
646 The authors would like to thank the *Instituto Nacional de Investigación y Tecnología*
647 *Agraria y Alimentaria* (INIA) for the funding provided for this study through the project

RTA2009-00029-C02-01 (with FEDER funds). Leticia Martínez thanks the *Consejería de Educación del Gobierno de La Rioja* for the FPI-CAR grant.

REFERENCES

[1] M.A. Pozo-Bayón, A.J. Martínez-Rodríguez, E. Pueyo, M.V. Moreno-Arribas. Chemical and biochemical features involved in sparkling wine production: from a traditional to an improved winemaking technology. Trends Food Sci. Technol. 20 (2009) 289-299.

[2] M.A. Pozo-Bayón, M.T. Hernández, P.J. Martín-Álvarez, M.C. Polo. Study of low molecular weight phenolic compounds during the aging of sparkling wines manufactured with red and white grape varieties. J. Agric. Food Chem. 51 (2003) 2089-2095.

[3] M.A. Pozo-Bayón, M. Monagas, Polo, M.C. C. Gómez-Cordovés. Occurrence of pyranoanthocyanins in sparkling wines manufactured with red grape varieties. J. Agric. Food Chem. 52 (2004) 1300-1306.

[4] M. Ortega-Heras, S. Pérez-Magariño, E. Cano-Mozo, C. González-Huerta, P. Herrera. Caracterización de los vinos elaborados a partir de la variedad Prieto Picudo de Castilla y León (España). Proceedings of the XXXth OIV World Congress, Budapest, 2007.

[5] C. Andrés-Lacueva, R.M. Lamuela-Raventós, S. Buxaderas, M.C de la Torre-Boronat. Influence of variety and aging on foaming properties of cava (sparkling wine). 2. J. Agric. Food Chem. 45 (1997) 2520-2525.

[6] M. Puig-Deu, E. López-Tamames, S. Buxaderas, M.C. Torre-Boronat. Quality of base and sparkling wines as influenced by the type of fining agent added pre-fermentation. Food Chem. 66 (1999) 35-42.

[7] M. Ibern-Gómez, C. Andrés-Lacueva, R.M. Lamuela-Raventós, S. Buxaderas, V.L. Singleton, M.C. de la Torre-Boronat. Browning of cava (sparkling wine) during aging in contact with lees due to the phenolic composition. Am. J. Enol. Vitic. 51 (2000) 29-36.

[8] V. Moreno-Arribas, E. Pueyo, F.J. Nieto, P.J. Martín-Álvarez, M.C. Polo. Influence of the polysaccharides and the nitrogen compounds on foaming properties of sparkling wines. Food Chem. 70 (2000) 309-317.

[9] C. Andres-Lacueva, M. Ibern-Gomez, R.M. Lamuela-Raventos, S. Buxaderas, M.C. de la Torre-Boronat. Cinnamates and resveratrol content for sparkling wine characterization. Am. J. Enol. Vitic. 32 (2002) 5-13.

[10] G. Vanrell, R. Canals, M. Esteruelas, F. Fort, J.M. Canals, F. Zamora. Influence of the use of bentonite as a riddling agent on foam quality and protein fraction of sparkling wines (Cava). Food Chem. 104 (2007) 148-155.

[11] C.A. Stefenon, M. Colombo, C. de M. Bonesi, V. Marzarotto, R. Vanderlinde, M. Salvador, J.A.P. Henriques. Antioxidant activity of sparkling wines produced by champenoise and charmat methods. Food Chem. 119 (2010) 12-18.

[12] M. Vilanova. Sensory descriptive analysis and consumer acceptability of Godello wines from Valdeorras Apellation Origen Controlée (Northwest Spain). J. Sens. Stud. 21 (2006) 362-372.

[13] A. Masa, M. Vilanova. Flavonoid and aromatic characterisation of cv. Albarín blanco (*Vitis vinifera* L.). Food Chem. 107 (2008) 273-281.

[14] E. Sánchez-Palomo, E. Gómez García-Carpintero, R. Alonso-Villegas, M.A. González-Viñas. Characterization of aroma compounds of Verdejo white wines from the La Mancha region by odour activity values. Flavour Fragr. J. 25 (2010) 456−462.

[15] M. Chamkha, B. Cathala, V. Cheynier, R. Douillard. Phenolic composition of Champagnes from Chardonnay and Pinot Noir vintages. J. Agric. Food Chem. 51 (2003) 3179-3184.

[16] P. Jeandet, D. Chaudruc, B. Robillard, F. Peters, D. Tusseau, A. Conreux. Determination of the *trans*-resveratrol content of Champagne wines by reversed-phase HPLC. J. Int. Sci. Vigne Vin. 40 (2006) 117-119.

[17] A.M. Jordão, F.J. Goncalves, A.C. Correia, J. Cantão, M.D. Rivero-Pérez, M.L. González-SanJosé. Proanthocyanidin content, antioxidant capacity and scavenger activity of Portuguese sparkling wines (Bairrada Appellation of Origin). J. Sci. Food Agric. 90 (2010) 2144–2152.

[18] M. López-Barajas, A. Viu-Marco, E. López-Tamames, S. Buxaderas, M.C. de la Torre-Boronat. Foaming in grape juices of white varieties. J. Agric. Food Chem. 45 (1997) 2526-2529.

[19] M. López-Barajas, E. López-Tamames, S. Buxaderas, M.C. de la Torre-Boronat. Effect of vinification and variety on foam capacity of wine. Am. J. Enol. Vitic. 49 (1998) 397-402.

[20] S. Bodmer, C. Imark, M. Kneubühl. Biogenic amines in foods: Histamine and food processing. Inflamm. Res. 48 (1999) 296–300.

[21] M.V. Moreno-Arribas, M.C. Polo. Occurrence of lactic acid bacteria and biogenic amines in biologically aged wines. Food Microbiol. 25 (2008) 875–881.

[22] Office International de la Vigne et du Vin (OIV). Recueil des Méthodes Internationales d´Analyse des Vins et des mouts. OIV, 1990.

[23] Y. Glories. La couleur des vins rouges. 2éme partie. Mesure, origine et interprétation. Conn. Vigne Vin. 18 (1984) 253–271.

[24] S. Gómez-Alonso, E. García-Romero, I. Hermosín-Gutiérrez. HPLC analysis of diverse grape and wine phenolics using direct injection and multidetection by DAD and fluorescence. J. Food Compos. Anal. 20 (2007) 618–626.

[25] Z. Guadalupe, A. Soldevilla, M.P. Sáenz-Navajas, B. Ayestarán. Analysis of polymeric phenolics in red wines using different techniques combined with gel permeation chromatography fractionation. J. Chromatogr. A. 1112 (2006) 112–120.

[26] J.A. Kennedy, G.P. Jones. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. J. Agric. Food Chem. 49 (2001) 1740-1746.

[27] S. Gómez-Alonso, I. Hermosín-Gutiérrez, E. García Romero. Simultaneous HPLC analysis of biogenic amines, amino acids, and ammonium ion as aminoenone derivatives in wine and beer samples. J. Agric. Food Chem. 55 (2007) 608-613.

[28] M.L. González-San José, M. Ortega-Heras, S. Pérez-Magariño. Micro-oxygenation treatment and sensory properties of young red wines. Food Sci. Technol. Int. 14 (2008) 123-130.

[29] M. Gallart, X. Tomás, G. Suberbiola, E. López-Tamames, S. Buxaderas. Relationship between foam parameters obtained by gas-sparging method and sensory evaluation of sparkling wines. J. Sci. Food. Agric. 84 (2004) 127-133.

[30] H. Kelebek, A. Canbas, S. Selli. HPLC-DAD–MS Analysis of anthocyanins in rose wine made from cv. Öküzgözü grapes, and effect of maceration time on anthocyanin content. Chromatographia. 66 (2007) 207-212.

[31] T. Girbau-Sola, M. López-Barajas, E. López-Tamames, S. Buxaderas. Foam aptitude of Trepat and Monastrell red varieties in cava elaboration. 2. Second fermentation and aging. J. Agric. Food Chem. 50 (2002) 5600-5604.

[32] Y. Vasserot, S. Caillet, A. Maujean. Study of anthocyanin adsorption by yeast lees. Effect of some physico-chemical parameters. Am. J. Enol. Vitic. 48 (1997) 433–437.

[33] A. Morata, M.C. Gómez-Cordobés, J. Suberviola, B. Bartolomé, B. Colomo, J.A. Suárez. Adsorption of anthocyanins by yeast cell walls during the fermentation of red wines. J. Agric. Food Chem. 51 (2003) 4084-4088.

[34] J.P. Mazauric, J.M. Salmon. Interactions between yeast lees and wine polyphenols during simulation of wine aging: II. Analysis of desorbed polyphenol compounds from yeast lees. J. Agric. Food Chem. 54 (2006) 3876-3881.

[35] C. Benabdeljalil, V. Cheynier, H. Fulcrand, A. Hakiki, M. Mosaddak, M. Moutounet. Mise en évidence de nouveaux pigments formés par réaction des anthocyanes avec des métabolites de levures. Sci. Aliments. 20 (2000) 203-220.

[36] H. Alexandre, M. Guilloux-Benatier. Yeast autolysis in sparkling wine – a review. Aust. J. Grape Wine R. 12 (2006) 119–127.

[37] A. Reynolds. Managing wine quality: Oenology and wine quality (Volume 2), first ed., Woodhead Publishing, Cambridge, 2010.

[38] M.S. García-Falcón, C. Pérez-Lamela, E. Martínez-Carballo, J. Simal-Gándara. Determination of phenolic compounds in wines: influence of bottle storage of young red wines on their evolution. Food Chem. 105 (2007) 248–259.

[39] B.W. Zoecklein, K.C. Fugelsang, B.H. Gump, F.S. Nury. Análisis y producción de vino, first ed., Acribia, Zaragoza, 2000.

[40] P. Ribéreau-Gayon, Y. Glories, A. Maujean, D. Dubourdieu. Tratado de enología: Microbiología del vino-vinificaciones y química del vino-estabilización y tratamientos, first ed., Hemisferio Sur, Buenos Aires, 2003.

[41] S. Vidal, L. Francis, S. Guyot, N. Marnet, M. Kwiatkowski, R. Gawel, V. Cheynier, E. Waters. The mouth-fell properties of grape and apple proanthocyanidins in wine-like medium. J. Sci. Food Agr. 83 (2003) 564-573.

[42] M.O. Downey, J.S. Harvey, S.P. Robinson. Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. Aust. J. Grape Wine Res. 9 (2003) 15-27.

[43] M. Monagas, C. Gómez-Cordovés, B. Bartolomé, O. Laureano, J.M. Ricardo da Silva. Monomeric, oligomeric and polymeric flavan-3-ol composition of wines and grapes from *Vitis vinifera* L. cv. Graciano, Tempranillo, and Cabernet Sauvignon. J. Agric. Food Chem. 51 (2003) 6475-6481.

[44] S. Gagne, C. Saucier, L. Geny. Composition and cellular localization of tannins in Cabernet Sauvignon skins during growth. J. Agric. Food Chem. 54 (2006) 9465-9471.

[45] A. Vernhet, K. Dupre, L. Boulange-Petermann, V. Cheynier, P. Pellerin, M. Moutounet. Composition of tartrate precipitates deposited on stainless steel tanks during the cold stabilization of wines. Part II. Red wines. Am. J. Enol. Vitic. 50 (1999) 398-403.

[46] I. Revilla, M.L. González-SanJosé. Compositional changes during the storage of red wines treated with pectolytic enzymes: Low molecular-weight phenols and flavan-3-ol derivative levels. Food Chem. 80 (2003) 205–214.

[47] J.P. Mazauric, J.M. Salmon. Interactions between yeast lees and wine polyphenols during simulation of wine ageing: I. Analysis of remnant polyphenolic compounds in the resulting wines. J. Agric. Food Chem. 53 (2005) 5647–5653.

[48] K. Fernández, J.A. Kennedy, E. Agosin. Characterization of *Vitis vinifera* L. cv. Carménère grape and wine proanthocyanidins. J. Agric. Food Chem. 55 (2007) 3675–3680.

[49] E. Maxa, W. Brandes. Biogene amine in fruchtsaften. Mitt. Klosterneuburg. 43 (1993) 101–106.

[50] D. Torrea-Goñi, C. Ancín-Azpilicueta. Influence of yeast strain on biogenic amine content in wines: Relationship with the utilization of amino acids during fermentation. Am. J. Enol. Vitic. 52 (2001) 185–190.

[51] M. Kallay, M. Body-Szalkai. Ammine biogene nei vini ungheresi. Riv. Vitic. Enol. 3 (1996) 29-38.

[52] U. Yücel, A. Üren. The effect of harvest time and bentonite treatment on histamine and tyramine contents of wines. Gida. 33 (2008) 11-18.

[53] V. Moreno-Arribas, E. Pueyo, M.C. Polo, P.J. Martin-Alvarez. Changes in the amino acid composition of the different nitrogenous fractions during the aging of wine with yeasts. J. Agric. Food Chem. 46 (1998) 4042–4051.

[54] E.H. Soufleros, E. Bouloumpasi, A. Zotou, Z. Loukou. Determination of biogenic amines in Greek wines by HPLC and ultraviolet detection after dansylation and examination of factors affecting their presence and concentration. Food Chem. 101 (2007) 704-716.

FIGURE CAPTIONS

Figure 1. Concentration (mg L^{-1}) of total anthocyanins (T-A) and non-acylated anthocyanins (A-Gluc) during the different stages of the sparkling wine elaboration: base wines (BW), base wines after clarification and stabilization (CBW), sparkling wines after 3 months (T3M), 6 months (T6M) and 9 months (T9M) of aging on yeast lees. Data not shown are below the quantification limit (1.9 mg L^{-1}).

Figure 2. Concentration (mg L^{-1}) of total hydroxycinnamic acids, hydroxycinnamates, and catechin in wines during the different stages of the sparkling wine elaboration: base wines (BW), base wines after clarification and cold stabilization (CBW), and sparkling wines after 3 months (T3M), 6 months (T6M) and 9 months (T9M) of aging on yeast lees. Values are means ± S.D. (n = 3).

Figure 3. Concentration (mg L^{-1}) of total phenolics in wines during the different stages of the sparkling wine elaboration: base wines (BW), base wines after clarification and cold stabilization (CBW), and sparkling wines after 3 months (T3M), 6 months (T6M) and 9 months (T9M) of aging on yeast lees. Values are means ± S.D. (n = 3).

Figure 4. Effect of clarification and cold stabilization of base wines on biogenic amines: a) biogenic amines (mg L^{-1}) in base wines obtained previous clarification and cold stabilization; b) biogenic amines (mg L^{-1}) in base wines after clarification and cold stabilization. Values are means ± S.D. (n = 3). Data not shown are below the quantification limit (0.08 mg L^{-1}).

Figure 5. GPA on the mean ratings for a) olfactory, b) gustatory and c) quality foam attributes in the final sparkling wines (T9M).

Table 1. Oenological parameters of base wines before clarification and stabilization (BW) and final sparkling wines after 9 months of aging on yeast lees (T9M)

	Albarín	Viura	Godello	Malvasía	Verdejo	Garnacha	Garnacha*	P. Picudo
BW								
pH	2.79	2.95	2.84	2.99	2.93	3.03	2.98	3.08
TA[1]	9.5	8.2	7.6	8.0	8.2	7.5	8.7	8.5
SO_2T[2]	56	53	64	65	56	63	58	30
Alcohol[3]	11.1	10.7	11.7	10.6	10.3	11.8	11.1	11.5
VA[4]	0.28	0.16	0.31	0.27	0.21	0.18	0.14	0.17
A420[5]	0.0515	0.0453	0.0503	0.0535	0.0535	-	-	-
CI[6]	-	-	-	-	-	0.59	0.53	0.68
Hue[7]	-	-	-	-	-	0.520	0.517	0.668
T9M								
pH	2.76	2.89	2.82	3.10	2.94	2.85	2.94	3.02
TA[1]	7.2	7.4	7.2	7.4	7.4	6.9	7.4	7.1
SO_2T[2]	35	43	43	31	33	44	41	21
Alcohol[3]	12.2	11.6	12.2	11.6	11.6	12.3	11.9	12.5
VA[4]	0.39	0.24	0.32	0.28	0.30	0.25	0.20	0.29
A420[5]	0.0297	0.0230	0.0207	0.0260	0.0355	-	-	-
CI[6]	-	-	-	-	-	0.14	0.11	0.24
Hue[7]	-	-	-	-	-	0.974	0.880	1.042

[1]TA: titratable acidity as g of tartaric acid per liter; [2]SO_2T: total sulfur dioxide as mg of total sulfur dioxide per liter; [3]Alcohol: mL ethanol for 100 mL of wine at 20 ºC; [4]VA: volatile acidity as g of acetic acid per liter; [5]A420: absorbance at 420 nm; [6]CI: color intensity as sum of absorbances at 420, 520 and 620 nm; [7]Hue: A420/A520.

Table 2. Proanthocyanidin concentration (mg L^{-1}), % catechin, % epicatechin, % epicatechin-gallate terminal subunits and mean degree of polymerization (mDP) in wines during the different stages of the sparkling wine elaboration: base wines (BW), base wines after clarification and stabilization (CBW), sparkling wines after 3 months (T3M), 6 months (T6M) and 9 months (T9M) of aging on yeast lees

		Albarín	Viura	Godello	Malvasía	Verdejo	Garnacha	Garnacha*	P. Picudo
BW	PA[1]	91.02±10.00^{b}	61.01±3.70^{a}	70.86±9.46^{a,b}	48.31±1.96^{a}	53.01±1.64^{a}	89.48±10.33^{b}	90.31±10.33^{b}	51.92±13.90^{a}
	% Cat[2]	8.96±2.51^{a,b}	10.77±2.46^{a,b}	10.13±3.14^{a,b}	5.52±2.57^{a}	12.45±0.89^{b}	7.29±0.87^{a,b}	7.01±0.65^{a,b}	9.69±3.97^{a,b}
	% Epi[3]	3.87±0.95^{a}	6.06±0.90^{a}	5.22±1.45^{a}	7.57±0.79^{a}	6.68±0.45^{a}	3.18±1.93^{a}	3.22±1.45^{a}	7.53±5.05^{a}
	% Epigal[4]	3.61±0.82^{a,b}	n.d.[5]	1.43±1.70^{a}	n.d.	7.38±5.68^{a,b}	3.82±1.17^{a,b}	3.93±1.36^{a,b}	9.50±3.99^{b}
	mDP	3.75±2.48^{a}	9.41±1.84^{a}	4.91±3.42^{a}	7.04±1.74^{a}	4.31±1.32^{a}	5.51±1.61^{a}	5.82±1.47^{a}	5.24±2.36^{a}
CBW	PA	65.46±0.75^{f}	40.47±0.28^{c}	43.84±0.88^{a}	46.12±0.11^{b}	43.07±0.21^{a}	48.77±0.70^{d}	57.13±0.91^{e}	46.35±0.79^{b}
	% Cat	3.56±0.08^{c}	4.23±0.12^{a}	4.26±0.26^{a}	3.79±0.07^{c,d}	4.88±0.04^{e}	2.35±0.14^{b}	2.37±0.04^{b}	3.94±0.13^{a,d}
	% Epi	4.33±0.26^{a}	7.83±0.15^{f}	6.75±0.26^{e}	4.97±0.07^{b,c}	6.48±0.13^{d,e}	5.29±0.25^{c}	4.63±0.18^{a,b}	6.09±0.13^{d}
	% Epigal	9.77±0.46^{b,c}	11.39±0.50^{d}	8.53±0.48^{a}	13.54±0.05^{e}	8.97±0.27^{a,b}	8.97±0.42	8.47±0.28^{a}	10.65±0.44^{c,d}
	mDP	5.26±0.25^{a}	5.22±0.15^{a}	6.50±0.26^{b,c}	5.47±0.03^{a}	5.21±0.11^{a}	6.22±0.25^{b,c}	6.69±0.19^{c}	6.04±0.18^{b}
T3M	PA	42.88±1.02^{b}	38.26±1.01^{c}	43.04±0.94^{b}	48.67±0.61^{a}	48.64±1.21^{a}	48.17±1.13^{a}	42.08±1.01^{b}	46.16±1.16^{a}
	% Cat	3.58±0.18^{b}	5.78±0.75^{d}	3.65±0.22^{b}	2.74±0.12^{a,c}	3.33±0.30^{a,b}	2.07±0.15^{c}	2.66±0.28^{a,c}	2.94±0.15^{a,b}
	% Epi	1.84±0.27^{a}	2.63±0.29^{b}	1.56±0.04^{a}	2.59±0.26^{b}	3.47±0.38^{c}	1.70±0.18^{a}	1.84±0.05^{a}	2.64±0.11^{b}
	% Epigal	12.02±1.08^{a,c}	9.49±0.95^{a,b}	14.56±1.19^{c}	9.58±0.40^{a,b}	4.67±0.53^{d}	12.06±1.65^{a,c}	10.93±1.07^{a}	7.67±0.79^{b}
	mDP	6.70±0.49^{a,c}	6.89±0.52^{a,b,c}	5.74±0.40^{c}	8.14±0.29^{b,d}	11.92±0.86^{e}	7.23±0.83^{a,b}	7.50±0.60^{a,b}	9.44±0.70^{d}
T6M	PA	29.19±1.82^{a}	41.01±4.37^{c}	30.83±1.78^{a,b}	28.17±1.85^{a}	39.12±1.53^{b,c}	27.14±0.90^{a}	28.92±1.12^{a}	27.54±2.23^{a}
	% Cat	3.94±0.54^{a}	14.34±6.33^{b}	3.71±1.06^{a}	7.41±1.18^{a}	4.98±1.53^{a}	4.33±0.40^{a}	4.33±1.10^{a}	4.05±0.87^{a}
	% Epi	6.55±4.51^{a}	9.10±3.79^{a,b}	12.52±0.78^{b,c}	7.06±0.68^{a}	6.94±0.66^{a}	11.46±0.55^{a,b,c}	16.45±1.20^{c}	6.55±1.02^{a}
	% Epigal	18.98±3.38^{b}	25.85±6.34^{c}	4.10±0.88^{a}	19.99±4.75^{b}	19.14±1.14^{b}	6.01±0.52^{a}	6.55±0.28^{a}	6.60±0.68^{a}
	mDP	4.02±0.71^{a}	2.32±0.48^{c}	7.07±0.68^{b}	3.44±0.60^{a,c}	3.88±0.25^{a}	6.18±0.34^{b,d}	4.89±0.32^{a,d}	7.67±0.80^{b}
T9M	PA	53.13±3.44^{c}	43.04±1.38^{a,d}	35.21±1.52^{b}	35.60±1.30^{b}	47.40±1.68^{a,c}	37.19±1.50^{b,d}	45.97±2.12^{a}	46.84±2.27^{a,c}
	% Cat	4.94±0.86^{b}	11.95±0.67^{c}	8.93±2.10^{a}	8.53±1.01^{a}	4.78±0.27^{b}	8.11±0.39^{a}	7.08±1.53^{a,b}	6.91±0.42^{a,b}
	% Epi	5.23±0.47^{a}	6.03±0.34^{a}	13.33±0.81^{c}	5.21±0.21^{a}	4.42±0.58^{a}	10.38±1.73^{b}	3.82±0.60^{a}	4.15±0.63^{a}
	% Epigal	13.17±1.21^{a,b}	17.84±0.46^{e}	10.59±0.57^{c}	14.90±2.20^{b,d}	12.58±1.60^{a,b,c}	11.05±0.87^{a,c}	15.71±0.90^{d,e}	13.57±0.74^{a,b,d}
	mDP	5.36±0.49^{c,d}	3.43±0.12^{e}	3.97±0.29^{a,e}	4.25±0.44^{a}	5.73±0.53^{d}	4.41±0.32^{a,b}	4.60±0.33^{a,b,c}	5.05±0.31^{b,c,d}

[1]PA: total proanthocyanidins content (mg L^{-1}); [2] %Cat: % of catechin terminal subunits; [3] %Epi: % of epicatechin terminal subunits; [4] %Epigal: % of epicatechin-gallate terminal subunits. [5]n.d.: undetected. Values are means ± S.D. (n = 3). Different letters in the same row indicate that means significantly differ at $p < 0.05$.

Table 3. Biogenic amine concentration (mg L^{-1}) in sparkling wines during aging on lees: sparkling wines after 3 months (T3M), 6 months (T6M) and 9 months (T9M) of aging on yeast lees

		Albarín	Viura	Godello	Malvasía	Verdejo	Garnacha	Garnacha*	P. Picudo
T3M	Putrescine	2.60±0.01^{h}	0.48±0.00^{b}	0.57±0.00^{c}	0.32±0.00^{a}	1.79±0.03^{f}	1.01±0.01^{e}	0.95±0.01^{d}	2.09±0.05^{g}
	Histamine	0.25±0.02^{c}	n.c.[1]	n.c.	n.c.	0.18±0.01^{a}	n.c.	n.c.	0.21±0.01^{b}
	Agmatine	0.18±0.02^{a}	0.22±0.00^{a,b}	0.22±0.01^{a,b}	0.19±0.00^{a}	0.21±0.02^{a,b}	0.24±0.01^{b}	0.20±0.00^{a}	0.29±0.05^{c}
	Espermidine	0.14±0.01^{a}	n.c.	n.c.	n.c.	0.13±0.01^{a}	n.c.	n.c.	0.10±0.02^{b}
	Tiramine	0.08±0.01^{b}	n.c.	n.c.	0.13±0.00^{a}	0.12±0.00^{a}	0.17±0.01^{c}	n.c.	n.c.
	Triptamine	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	0.09±0.00^{a}
	Cadaverine	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
	Phenylethylamine	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	0.09±0.00^{a}
	Isoamylamine	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
	Total amines	3.25±0.03^{g}	0.70±0.06^{a,b}	0.79±0.01^{b}	0.63±0.00^{a}	2.52±0.02^{e}	1.42±0.01^{d}	1.15±0.01^{c}	2.87±0.07^{f}
T6M	Putrescine	3.41±0.03^{g}	0.62±0.01^{a}	0.68±0.01^{a}	0.47±0.01^{b}	1.64±0.09^{e}	1.11±0.03^{d}	0.96±0.04^{c}	2.56±0.00^{f}
	Histamine	0.11±0.02^{c}	n.c.	0.16±0.02^{a}	0.23±0.05^{c}	0.20±0.02^{a,c}	n.c.	0.09±0.00^{b}	0.18±0.00^{a}
	Agmatine	0.32±0.06^{c}	0.38±0.05^{c}	0.15±0.02^{a,b}	0.20±0.01^{a}	0.21±0.04^{a}	0.70±0.07^{d}	0.70±0.02^{d}	0.10±0.01^{b}
	Espermidine	0.10±0.01^{a}	n.c.	0.10±0.01^{a}	n.c.	0.30±0.01^{c}	0.25±0.01^{b}	n.c.	n.c.
	Tiramine	0.24±0.00^{e}	0.16±0.00^{c}	0.10±0.00^{a,b}	0.13±0.02^{d}	0.10±0.02^{b}	0.17±0.01^{c}	0.08±0.01^{a}	n.c.
	Triptamine	0.12±0.02^{a}	0.12±0.00^{a}	0.23±0.01^{b}	0.23±0.01^{b}	0.23±0.01^{b}	0.29±0.02^{c}	0.09±0.01^{a}	0.10±0.00^{a}
	Cadaverine	0.10±0.0^{b}	n.c.	0.10±0.01^{a,b}	0.10±0.01^{a,b}	0.14±0.01^{c}	0.15±0.00^{c}	n.c.	0.09±0.01^{a}
	Phenylethylamine	0.14±0.03^{b,c}	0.09±0.01^{a}	0.09±0.00^{a}	0.11±0.03^{a,c}	0.14±0.00^{b,c}	0.16±0.01^{b}	0.08±0.01^{a}	0.16±0.00^{b}
	Isoamylamine	0.09±0.01^{a}	n.c.	0.10±0.01^{b}	0.08±0.00^{a}	0.11±0.00^{b}	0.13±0.01^{c}	n.c.	n.c.
	Total amines	4.62±0.08^{g}	1.37±0.05^{c}	1.72±0.03^{e}	1.55±0.07^{a}	3.07±0.08^{a,b}	2.97±0.08^{a}	2.00±0.05^{f}	3.17±0.02^{b}
T9M	Putrescine	3.28±0.03^{g}	0.52±0.02^{a}	0.60±0.01^{a}	0.43±0.00^{b}	1.73±0.10^{e}	1.05±0.01^{d}	0.87±0.00^{c}	2.04±0.02^{f}
	Histamine	0.29±0.03^{d}	n.c.	n.c.	n.c.	0.14±0.00^{a}	0.19±0.01^{b}	0.16±0.03^{a}	0.26±0.00^{c}
	Agmatine	0.24±0.03^{a,c}	0.14±0.00^{b}	0.22±0.01^{a}	0.26±0.02^{c,d}	0.22±0.02^{a}	0.22±0.03^{a}	0.16±0.00^{b}	0.28±0.01^{d}
	Espermidine	0.14±0.02^{a}	n.c.	n.c.	0.11±0.01^{a,b}	0.20±0.03^{c}	0.14±0.01^{a}	0.10±0.01^{b}	0.30±0.04^{d}
	Tiramine	0.16±0.02^{b}	0.20±0.02^{c}	n.c.	0.12±0.01^{a}	0.15±0.01^{a,b}	0.19±0.03^{c}	n.c.	0.28±0.02^{d}
	Triptamine	0.09±0.00^{a}	n.c.	0.08±0.02^{a}	0.08±0.00^{a}	n.c.	n.c.	n.c.	0.14±0.01^{b}
	Cadaverine	0.16±0.00^{a}	n.c.	0.09±0.01^{b,c}	0.14±0.01^{a}	0.15±0.02^{a}	0.08±0.00^{b}	n.c.	0.10±0.00^{c}
	Phenylethylamine	0.13±0.00^{b}	n.c.	n.c.	0.09±0.00^{b}	0.11±0.00^{a,b}	n.c.	n.c.	0.13±0.02^{a}
	Isoamylamine	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
	Total amines	4.49±0.06^{f}	0.87±0.03^{a}	0.99±0.03^{a}	1.24±0.02^{b}	2.71±0.11^{d}	1.88±0.05^{c}	1.29±0.03^{b}	3.53±0.06^{e}

[1] n.c.: unquantifiable. Values are means ± S.D. (n = 3). Different letters in the same row indicate that means significantly differ at $p < 0.05$.

Figure 1

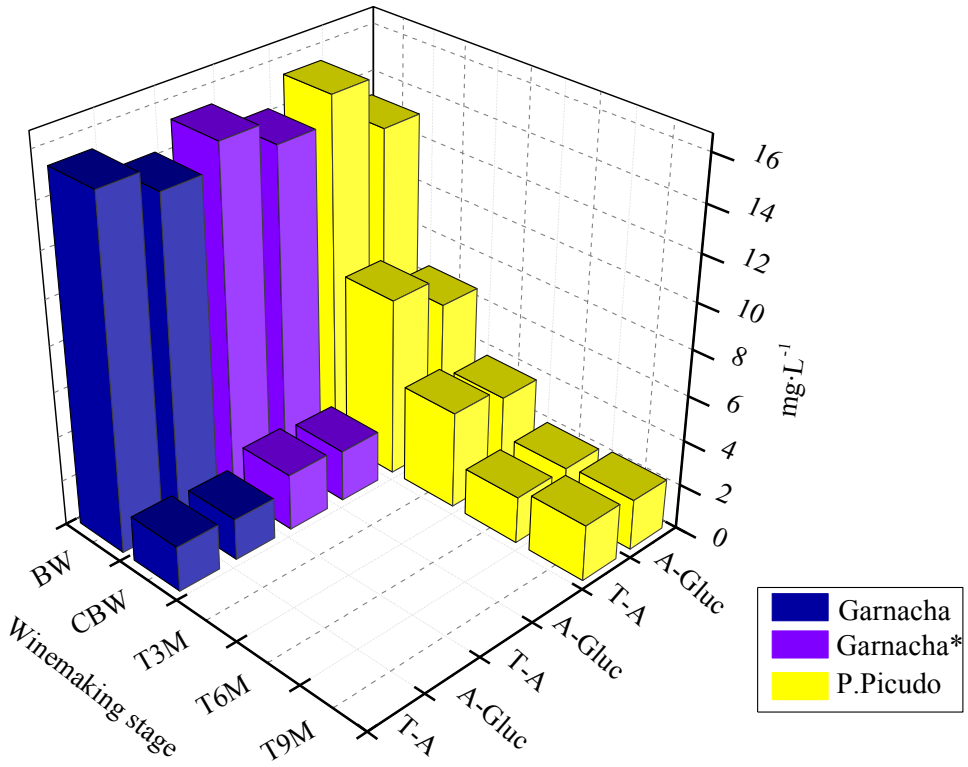

16
14
12
10
8
6
4
2
0
mg·L-1
BW
CBW
T3M
T6M
T9M
Winemaking stage
T-A
A-Gluc
T-A
A-Gluc
T-A
A-Gluc
Garnacha
Garnacha*
P.Picudo

Figure 2

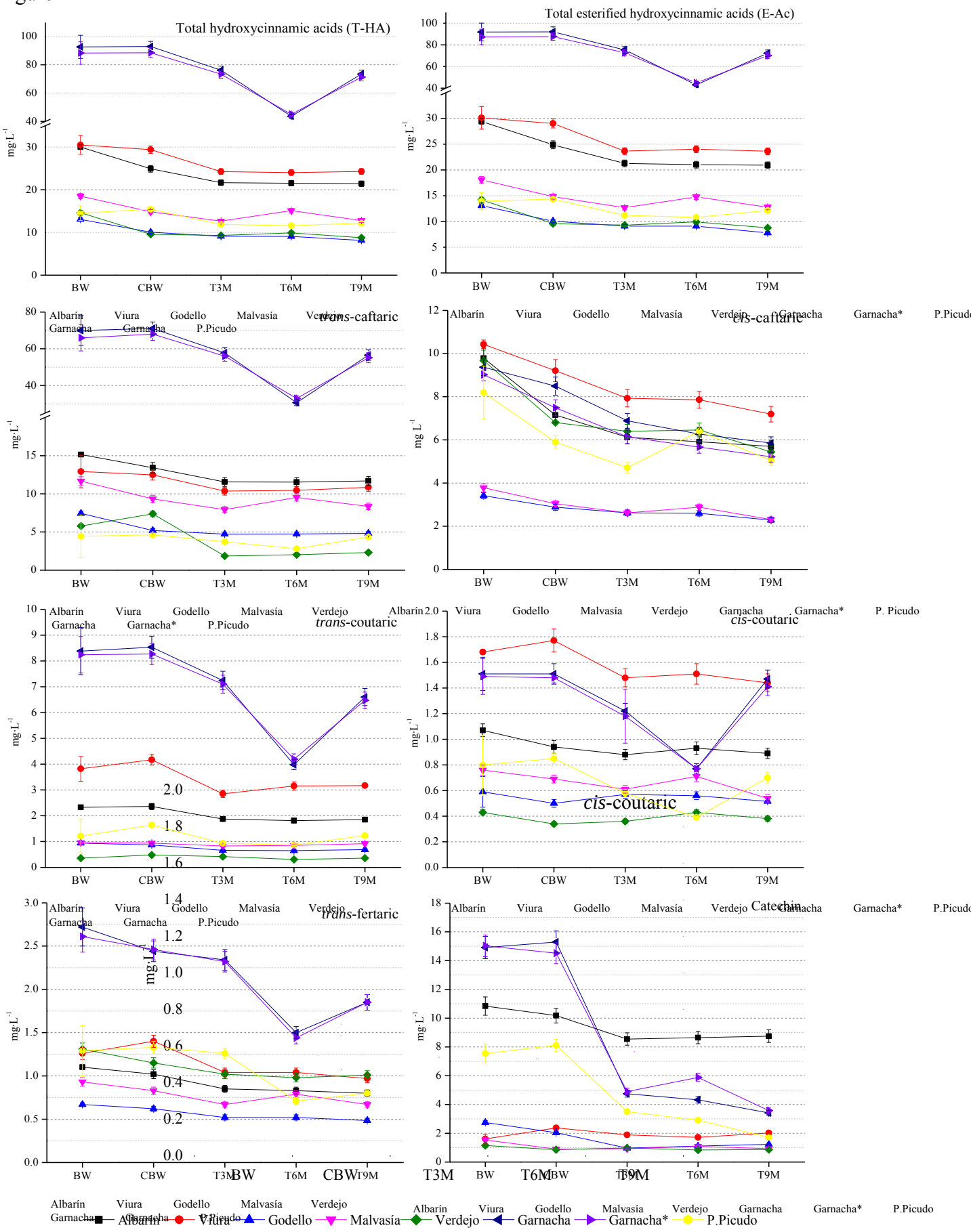

Total hydroxycinnamic acids (T-HA)
Total esterified hydroxycinnamic acids (E-Ac)
trans-caftaric
cis-caftaric
trans-coutaric
cis-coutaric
trans-fertaric
Catechin
mg·L-1
BW
CBW
T3M
T6M
T9M
Albarín
Garnacha
Viura
Garnacha*
Godello
P.Picudo
Malvasía
Verdejo

Figure 3

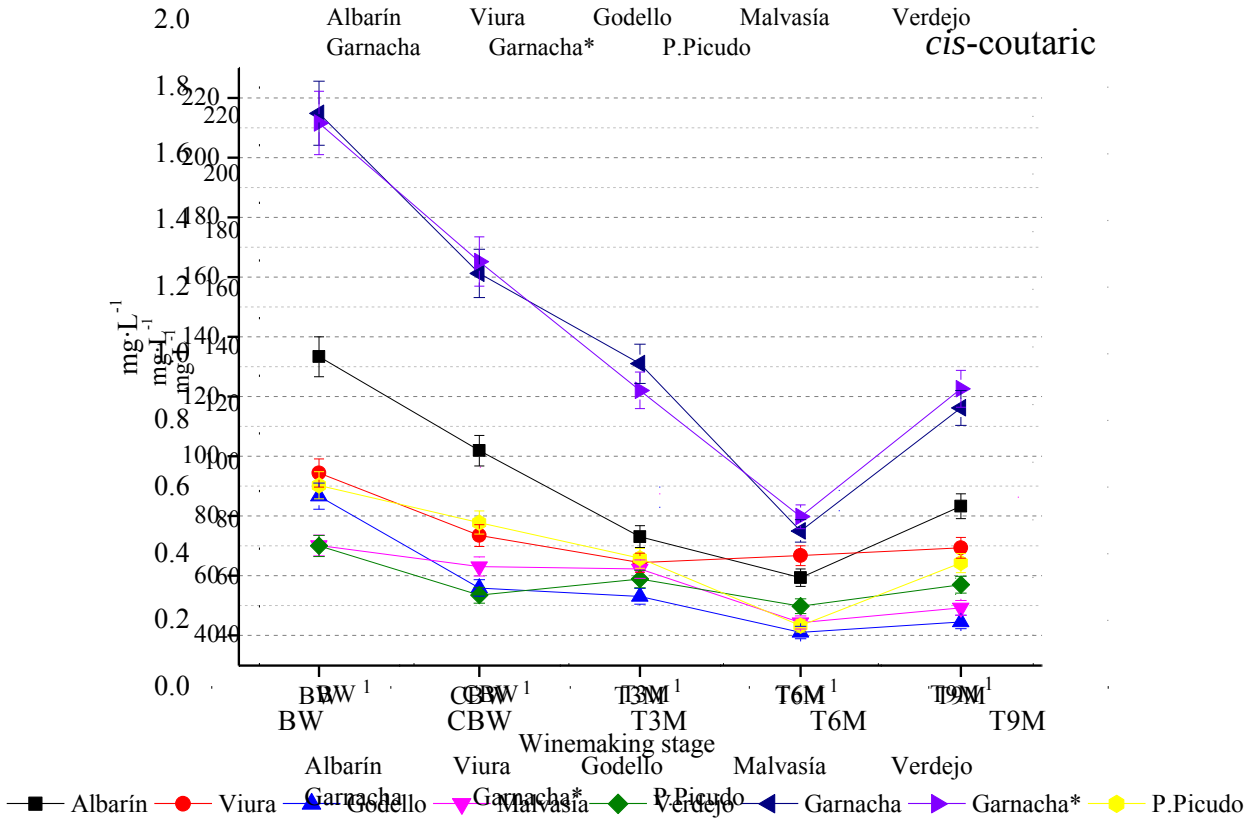

Albarín
Viura
Godello
Malvasía
Verdejo
Garnacha
Garnacha*
P.Picudo
cis-coutaric
mg·L-1
BW
CBW
T3M
T6M
T9M
Winemaking stage

Figure 4.

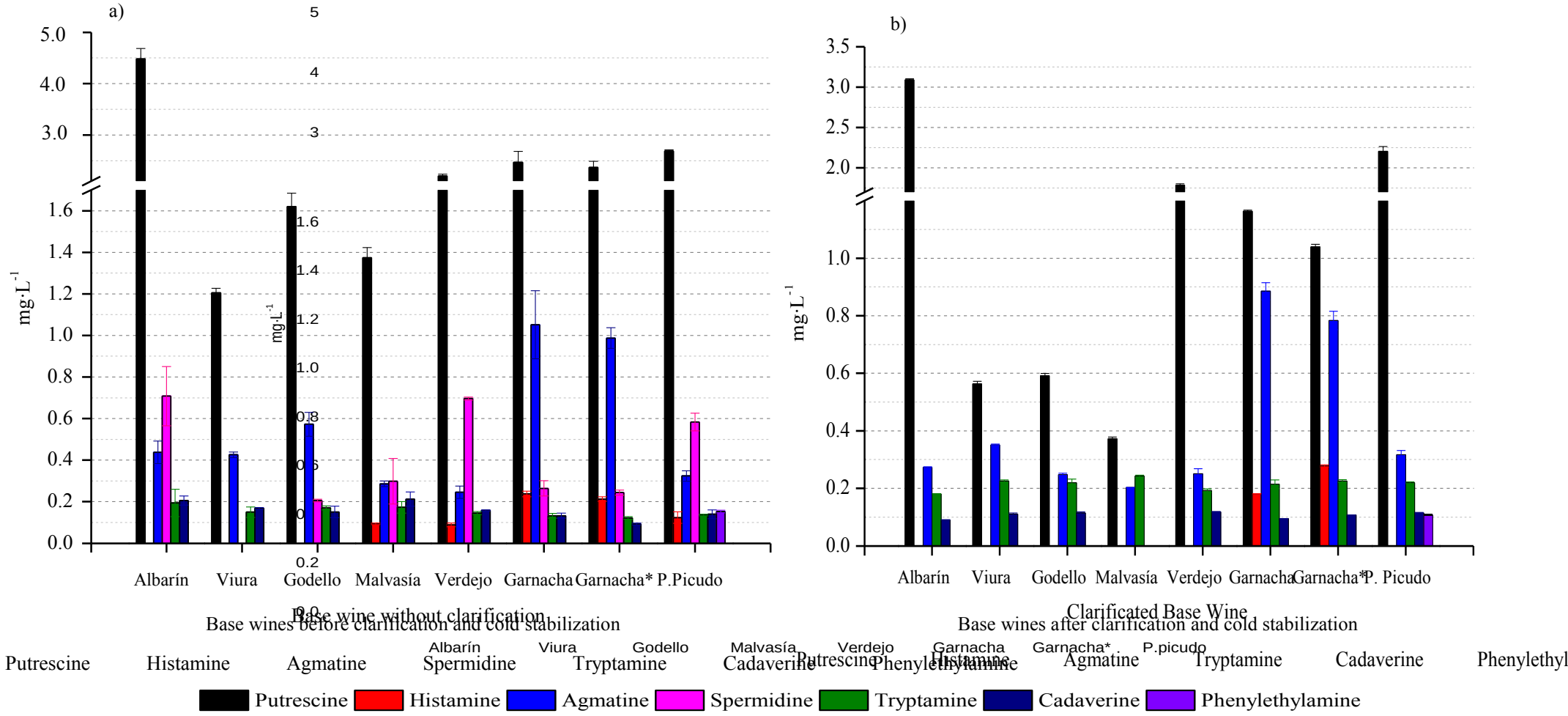
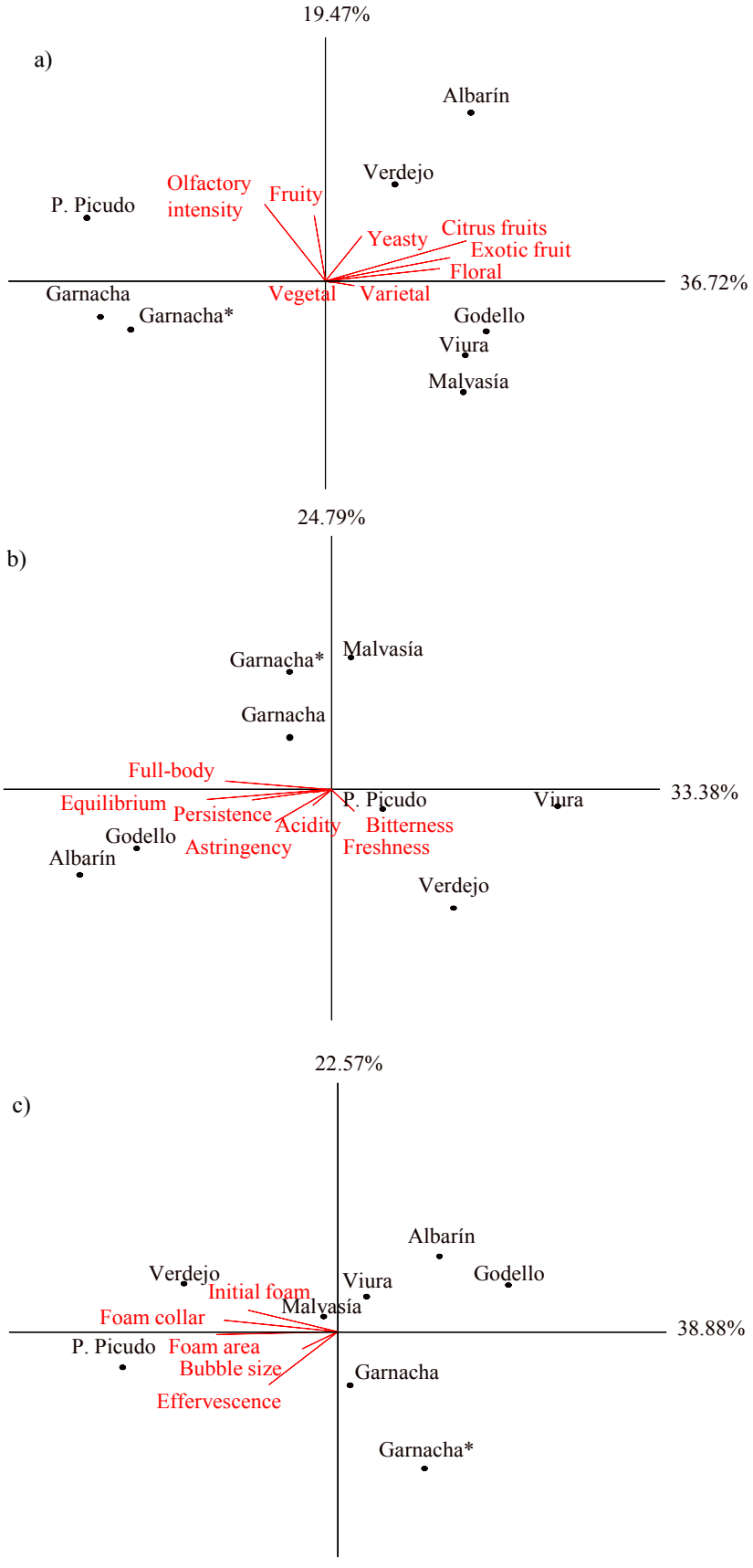

a)
b)
mg·L-1
Albarín
Viura
Godello
Malvasía
Verdejo
Garnacha
Garnacha*
P.Picudo
Base wine without clarification
Base wines before clarification and cold stabilization
Clarificated Base Wine
Base wines after clarification and cold stabilization
Putrescine
Histamine
Agmatine
Spermidine
Tryptamine
Cadaverine
Phenylethylamine

Figure 5


a)
19.47%
Albarín
Verdejo
Olfactory intensity
Fruity
P. Picudo
Yeasty
Citrus fruits
Exotic fruit
Floral
Garnacha
Vegetal
Varietal
36.72%
Garnacha*
Godello
Viura
Malvasía
b)
24.79%
Garnacha*
Malvasía
Garnacha
Full-body
Equilibrium
Persistence
Acidity
Bitterness
P. Picudo
Viura
33.38%
Godello
Astringency
Freshness
Albarín
Verdejo
c)
22.57%
Albarín
Verdejo
Viura
Godello
Initial foam
Malvasía
Foam collar
38.88%
P. Picudo
Foam area
Bubble size
Garnacha
Effervescence
Garnacha*