Effect of addition of overripe seeds from white grape by-products during red wine fermentation on wine colour and phenolic composition

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Abstract

The effect of the fermentative addition of overripe seeds by-product (Pedro Ximénez white grapes, 3g/L) on the phenolic composition and colour of red wines was studied by rapid resolution liquid chromatography (RRLC-MS) and Differential Colorimetry. Overripe seeds summited to postharvest dehydration by sun drying directly demonstrated were rich in phenolics such as gallic acid, epicatechin, and procyanidin B2 3-*O*-gallate, and hence a source of copigments capable to stabilize wine anthocyanins. The fermentative addition of overripe seeds led to Syrah wines with significant higher content of anthocyanins and procyanidins than traditional macerated wines, which had a positive effect on colour quality and stability. With respect to the colour quality, visually perceptible colour differences were found respect to the traditional macerated wines ($\Delta E^*_{ab} > 3$), getting overripe seeds macerated wines with bluish hues and higher chroma values. Moreover, Differential Colorimetry demonstrated that the addition of overripe seeds induces lower colour modification in wines during stabilization and, in consequence, higher colour stability

Keywords: Postharvest overripeness; copigmentation; phenolics; Differential Colorimetry; warm climate

1. Introduction

Some places in Spain, especially the Southern areas (Andalusia), the high temperatures reached during grape maturation induce inadequate ripeness of red grapes. Climatological conditions cause time discrepancy between the technological ripeness (sugars/acids ratio) and the phenolic ripeness of grapes, leading to unbalanced ripening (Webb, Whetton, & Barlow, 2011) .

The climate change is increasing this effect and causing problems for the winemakers to elaborate high-quality wines. In particular, unbalanced quantities of phenols in red grapes coming from seeds and skins make the colour stabilization of red wines by copigmentation more difficult (Boulton, 2001) .

In red wine, copigmentation occurs from the first steps of vinification through of noncovalent associations between anthocyanins and colourless compounds (copigments), which protect the coloured forms of anthocyanins and ensure an adequate pigment polymerization during wine ageing (De Freitas & Mateus, 2010; Trouillas et al., 2016). Thus, increasing the concentration of copigments or modulating the pigment/copigments ratio is an interesting technological strategy to improve the colour stabilization of red wines, especially in warm climate regions.

According to Jara-Palacios et al., (2014a), by-products from white grapes (grape pomace or its individual components) still contain a great variety of phenolics that could improve the colour of anthocyanins. This study demonstrated that copigmentation effects vary depending on the type of by-product used as copigments source. Consequently, different effectiveness on colour stabilization can be achieved by means of multiple copigmentation processes.

Interestingly, technological applications for the red wine industry based on reusing white grape by-products is possible in warm climate winemaking regions because red

and white grape varieties have similar ripening periods due to climatological conditions (Gordillo et al., 2014). Thus, large amounts of skins and seeds from white grape are available to be used at the time of red winemaking. As white wines are elaborated by applying short maceration time, phenolics remain in skins and seeds, which increases their industrial value as agricultural by-product (Pedroza, Carmona, Alonso, Salinas, & Zalacain, 2013).

Therefore, the maceration of white-winemaking by-products during red wine vinification is being increasingly used in the areas that intend to elaborate red wines having stable colour over time. Thereby, Gordillo et al. (2014) evaluated how the maceration of white grape pomace with red grapes enhanced the phenolic potential and colour of young red wine. More recently, Cejudo-Bastante et al. (2016) showed the possibility of using an enzymatic hydrolysate of grape seed during Syrah wine fermentation to compensating both colour and phenolic degradation. In this case, it was confirmed that seed phenolics are natural components of grapes having interesting copigmentation properties, as found previously (González-Manzano, Mateus, de Freitas, & Santos-Buelga, 2008).

Due to the promising results, the assessment of other white wine by-products has great interest, although other related research is still required to state their technological applications and optimize the potential benefits on red wine colour. This is the case of by-products from the elaboration of sweet sherry wines in southwestern of Spain. These wines are traditionally made with overripe grapes from white cultivars such as Pedro Ximénez (PX), which are summited to postharvest dehydration. This process relies on exposing off-vine grape bunches during 7 to 10 days to direct sun drying on the *pasera* site (Dumitriu, Peinado, Peinado, & de Lerma, 2015). Thus, grape composition is strongly affected because the sunlight and the water stress induce physicochemical

changes in plant metabolism including the concentration of sugars and the biosynthesis and polymerization of phenolics (Dumitriu et al., 2015).

Due to the particular overripening process, the seeds from PX grape pomace are different in composition with respect to seeds from grapes harvested at technological ripeness (not overripe). For this reason, a new study of vinification based on using PX overripe seeds as an alternative source of copigments has been developed. The aim of this work is to study the effect of adding quantities of PX overripe seeds during red wine fermentation and assessing whether they could be a powerful source of copigments to improve the colour of the red wines made in warm climates zones. In addition of the chromatic effect in the wine, and therefore the consumer acceptance, the use of this agricultural waste should affect the economic and environmental profit for the wine industry.

2. Material and methods

2.1. Winemaking protocols and samples

Overripe seeds (OS) were used in the vinification experiments during the fermentation process with Syrah grapes. About 1350 g of OS were manually separated from Pedro **Ximénez** grape pomace (D.O. Montilla-Moriles, Southwest Spain, 2014 vintage). OS were stored frozen (-20 ºC) until used in the 2015 vintage vinification, when wines were made using *V. vinifera* cv. Syrah grapes grown in D.O. Condado de Huelva (Southwestern Spain). About 900 kg of grapes were harvested at optimum technological maturity.

Grapes were destemmed and crushed, and the fermentation mash was distributed in six 220 L stainless steel tanks to perform two types of experimental vinification:

(a) OSW (3 tanks) wines made by adding 3 g /L of OS: a total addition of 450 g of OS to 150 kg of grape mash per tank.

(b) CW (3 tanks) wines made by traditional winemaking (without OS addition), as control wine.

For all wines, alcoholic fermentation spontaneously occurred and skin maceration was developed manually punching down each tank once a day during 7 days. After this, the mash was drawn off to remove the solid parts, and the free run musts were left to finish the fermentation under the same conditions in 50 L stainless steel tanks. Selected *Oenococcus oeni* lactic acid bacteria (VINIFERM Oe 104, Agrovin, Ciudad Real, Spain) were inoculated (14 mL/hL) at the end of alcoholic fermentation. When the fermentative processes finished, the wines were again racked in 50 L stainless steel tank maintained for a stabilization period of 5 months. Then the wines were bottled and stored during 5 months.

Must and wine samples were taken at the beginning of fermentative maceration period (day 1), at the middle of the alcoholic fermentation (day 4), just after skin removal (day 7), and at different moments along the stabilization and bottling process (20, 135, 225, and 315 days).

2.2. Phenolic extraction from overripe seeds

OS were treated with methanol:water (750 /250 mL/mL) according to a modification of the methodology described by Jara-Palacios et al. (2013) to extract and assess the phenolic composition of this by-product. The extraction procedure was made in triplicate as follows: An amount of 50 g OS was homogenized in 250 mL of solvent, shaking for 1 h in a shaking apparatus (VWR Incubating minishaker, Barcelona, Spain) , and further centrifuged at 4190 g for 15 min; the supernatant was collected and the residue submitted to the same process twice. The supernatants were combined and the methanolic extract was concentrated to dryness and freeze dried until the analyses.

143 *2.3. Colorimetric analysis*

144 The whole visible spectra (380-770 nm) of the samples was measured in triplicate at
145 constant intervals ($\Delta\lambda$=2 nm) with an Agilent 8453 UV-Vis spectrophotometer (Palo
146 Alto, USA), using 2 mm path length glass cells and distilled water as white reference.
147 The CIELAB colour parameters (L*, a*, b*, C^*_{ab} and h_{ab}) were calculated for from
148 transmittance spectra by using the original software CromaLab® (Heredia, Alvarez,
149 González-Miret & Ramírez, 2004), following the recommendations of the Commission
150 Internationale de l'Eclairage (CIE, 2004): 10º Standard Observer and D65 Standard
151 Illuminant as references. Colour differences (ΔE^*_{ab}) were calculate by the Euclidean
152 distance between two points in the three-dimensional space: $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 +$
153 $(\Delta b^*)^2]^{1/2}$.

154 *2.4. Copigmentation determination*

155 The contribution of copigmented anthocyanins (% copigmentation) to the total wine
156 colour at pH 3.6 were determined in triplicate following the method proposed by
157 Boulton (1996). Wine samples were first adjusted to pH 3.6.

158 *2.5. HPLC-DAD analysis of phenolic compounds*

159 The determination of monomeric anthocyanins and flavonols of the samples measured
160 in triplicate was made according to the method reported by Cejudo-Bastante et al.
161 (2016); which performs identification based on the retention times and HPLC-DAD-
162 ESI-MS^n. An Agilent 1200 chromatographic system, equipped with quaternary pump,
163 UV-VIS diode-array detector, automatic injector, and ChemStation software (Palo Alto,
164 USA) was used to perform the HPLC separation and quantification. Prior to direct
165 injection, the samples were filtered through a 0.45 μm Nylon filter. A volume of 50 μL
166 of sample was injected onto a Zorbax C_{18} column (250 x 4.6 mm, 5 μm particle size),
167 setting temperature at 40 ºC and flow rate of 0.63 mL/min. Acetonitrile, formic acid,

and water (3 mL:10 mL:87mL solvent A, and 50 mL:10 mL:40 mL, solvent B) were used, with the following elution profile: 0-10 min with 6% B; 10-15 min with 11% B; 15-20 min with 20% B; 20-25 min with 23% B; 25-30 min with 26% B; 30-35 min with 40% B; 35-38 min with 50% B; 38-46 min with 60% B; and 46-47 min with 6% B. All UV-vis spectra were recorded from 200 to 800 nm with a bandwidth of 2.0 nm. The external calibration method was used for the quantification of anthocyanins (520 nm) and flavonols (360 nm) by comparing the areas with the standards malvidin 3-*O*-glucoside and quercetin, respectively. The concentration of phenolic compounds was expressed as mg/L for wine samples.

The analyses of flavan-3-ols (monomeric and procyanidins), as well as the hydroxycinnamic and benzoic acids of each sample were performed in triplicate according to Jara-Palacios et al. (2014b) using RRLC after filtration through a 0.45 μm Nylon filter. The chromatographic system was an Agilent 1290, equipped with quaternary pump, UV-VIS diode-array detector, automatic injector, and ChemStation software (Palo Alto, USA). A C_{18} Poroshell 120 column (2.7 μm, 5 cm x 4.6 mm), with an injection volume of 0.5 μL, was used. The solvents were formic acid:water (1 mL /999 mL) as solvent A, and acetonitrile as solvent B at the following gradients: 0-5 min of 5% B linear; 5-20 min of 50% B linear; and 20-25 min of washing, which was followed by re-equilibration of the column. The flow-rate was 1.5 mL/min, and the column temperature was set to 25 ºC. Identification of colourless phenolics was performed according to the retention times of the standards (when available), UV-vis spectra and mass spectra, as described by JaraPalacios et al. (2014b). The quantification was made at 280 nm (flavan-3-ols, procyanidins and benzoic acids) and 320 nm (hydroxycinnamic acid acids) by external calibration comparing the areas with the gallic

192 acid, *p*-coumaric acid, and catechin standards. The concentration was expressed as mg/L
193 for wine samples and mg/100 g of dry seeds for PXOS samples.
194 In addition, the total anthocyanin, flavonol, benzoic acid, hydroxycinnamic acid
195 derivatives, monomeric flavan-3-ol and procyanidin contents were calculated as the sum
196 of individual phenolic compounds identified by HPLC.
197 The Total phenolic content of each sample was determined in triplicate using the Folin-
198 Ciocalteau method (Singleton & Rossi, 1965) using an Agilent 8453 UV-Vis
199 spectrophotometer.
200 *2.6. Statistical analysis*
201 Statistica version 8.0 software (Statistica, 2007) was used for statistical analysis.
202 Univariate analysis of variance (ANOVA) was applied using the general linear model
203 program (Tukey test, $p<0.05$) to establish significant differences among wines and for
204 each variable.
205 **3. Results and discussion**
206 *3.1. Phenolic composition of overripe seed (Pedro Ximénez white grapes)*
207 The phenolic composition of OS is summarized in Table 1. Quantitatively, the mean
208 value of total phenolic content was 5535 mg/100 g dry extract, which agrees the results
209 reported in other not overripe seeds (Cejudo-Bastante et al., 2016; Jara-Palacios et al.,
210 2014b) .
211 Regarding the qualitative profile, 20 compounds belonging to different phenolic
212 families were identified. The major corresponded to monomeric flavanols (40%
213 including catechin, epicatechin and EC-gallate) procyanidins (38% including B2,B3, B4
214 and B7 procyanidins as well as galloylated dimmers, trimers and tetramers), and
215 benzoic acids (20% including gallic and protocatechuic acids).

In comparison to the composition reported for not overripe seeds, some differences for the most aforementioned compounds were observed in relation to the type of grape, variety, or extraction conditions. OS stated for having higher amount of procyanidin B2 and the tetramer 2 with respect to not overripe seeds from Zalema, grown in warm climate, as reported by Jara-Palacios et al. (2014b) . Moreover, OS was also richer in gallic acid, protocatechuic acid and catechin.

Considering the phenolic composition of the enzymatic hydrolysate of the seeds described by Cejudo-Bastante et al. (2016), differences were found in the content of all the compounds being higher in OS, except for procyanidin B1. These differences could be due to the technological conditions of the enzymatic hydrolysis applied. Regarding the results reported for red grapes seeds (González-Manzano et al., 2008), the qualitative profile of OS (proportions of monomeric flavanols and procyaninds) is similar to the Tempranillo variety seeds. This is interesting from a chemical point of view because some of the phenolics identified are present in high quantities in OS, among them, gallic acid (52 mg/100 g), catechin (97 mg/100 g), epicatechin (45.8 mg/100 g), EC gallate (20.4 mg/100 g), procyanidin B2 (29.4 mg/100 g) and procyanidin B2-3-*O*-gallate (40 mg/100 g). These compounds have been described to act as effective copigments due to their structural complexity (Teixeira et al., 2013; Berké & de Freitas, 2007; Liu, Zhang, He, Duan, & Shi, 2016)

3.2. Phenolic composition of wines

Figure 1 shows the evolution of the total levels of anthocyanins (1a) and, procyanidins (1b) during the whole process of vinification. Results indicate that the dose of OS applied (3 g/L) during fermentative maceration cause an important effect on the phenolic stabilization of Syrah wines.

240 Quantitatively, the wine with overripe seeds (OSW) had a high total amount of
241 anthocyanins and procyanidins than traditional macerated wine (CW) at the end of the
242 maceration stage (12% and 50% higher, respectively).
243 These results confirm that the fermentative addition of OS positively influenced the
244 extraction of pigments and some copigments too. On the contrary, the extraction of
245 other types of copigments such as benzoic and hydroxycinnamic acids was similar for
246 the CW and OSW.
247 In general, regarding the stabilization and bottling period (135 and 315 days,
248 respectively), the degradation of anthocyanin pigments was slightly higher in CW than
249 in OSW (36% *vs* 33% of total anthocyanin loss, respectively), being increasingly lower
250 these differences during the bottle aging. Nevertheless, wines with additional amounts
251 of OS had higher amount of anthocyanin at the end of aging stage.
252 This behaviour is in accordance with the greater quantity of the total procyanidins
253 (Figure 1b) in OSW wines during most of the stabilization period. Therefore, these
254 copigments could act preventing higher pigment losses in wines, consistently with
255 previous studies (Cejudo-Bastante et al., 2016). On the other hand, a reduction of
256 procyanidins is observed at the end of bottling stage (from 225 to 315 days). This could
257 be due to the different reactions that simultaneously occur at the advanced stages of
258 vinification. Among others, the formation of tannin-anthocyanin or tannin-tannin
259 condensation products as has been reported by Monagas, Gómez-Cordovés, &
260 Bartolomé (2006). This fact could explain the lower difference of anthocyanins contents
261 between CW and OSW during the bottle aging.
262 With the aim of assessing the significant differences of the individual pigments and
263 copigments between CW and OSW wines, the mean value of all parameters was
264 compared during the stabilization and bottling stages (mean±SD, n=12 for each

265 maceration treatment; Tukey test, $p<0.05$). The results as well as the percentage of
266 copigmentation (%) and the total phenolics (mg GAE/L) are shown in Table 2.
267 According to the Tukey test ($p<0.05$), significant global differences were found among
268 the maceration treatments for some phenolic families. In particular, higher contents of
269 Total Anthocyanins (OSW=171 mg/L *vs* CW=140 mg/L) and Total Procyanidins
270 (OSW=55 mg/L *vs* CW=47 mg/L) characterized the OSW. Moreover, the amount of
271 total flavan-3-ols was also higher in OSW, although these differences were not
272 significant (OSW=106 mg/L *vs* CW=93 mg/L).
273 Regarding the individual phenolics, 25 of the 30 identified compounds were found in
274 higher contents in OSW wines, which represent the 83% of the phenolic composition of
275 wines. Among them, the differences for malvidin 3-glucoside, malvidin 3-acetyl-
276 glucoside and all the coumaroylated derivatives were significant, as well as for
277 epicatechin and procyanidin B2 3-*O*-gallate. Important quantities of such colourless
278 phenolics, described as good copigments, were found in OS (Table 1), so the results
279 confirm their effective diffusion to the wines (Mirabel, Saucier, Guerra, & Glories,
280 1999).
281 Similar behaviour was observed for other individual phenolics that represent major
282 compounds of OS (catechin, procyanidin B2 and procyanidin B7), although the
283 differences were not significant between CW and OSW wines. Previous studies
284 reported that the extraction of monomeric flavan-3-ols and procyanidins mainly occurs
285 during the first stages of fermentative maceration (Busse-Valverde, Gómez-Plaza,
286 López-Roca, Gil-Muñoz, & Bautista-Ortín, 2011) which could explains the results.
287 However, the content of some phenolic compounds being in high quantities in OS were
288 quite similar in CW and OSW: gallic acid (CW=36 mg/L *vs* OSW=35 mg/L) and
289 protocatechuic acid (CW=18.3 mg/L *vs* OSW=18.7 mg/L). Probably, their lower kinetic

290 of extraction determined that they could not be adequately extracted because of the
291 short maceration time (Zou, Kilmartin, Inglis, & Frost, 2002). Thus, increasing the
292 maceration time or the dose of overripe seeds could be interesting practices to optimize
293 the effects of this kind of grape by-product in red wine vinifications.

294 *3.3. Colour evolution during vinification*

295 The evolution of the CIELAB colour parameters, lightness (L*) chroma (C^*_{ab}) and hue
296 (h_{ab}), of control wine (CW) and wine with overripe seeds (OSW) during the vinification
297 is shown in Figure 2.

298 The trend of both wines along the fermentative maceration and stabilization stages was
299 quite similar. At the beginning, L* decreased around 20% during the fermentative
300 maceration due to the extraction of pigments, and later remains stable over time (Figure
301 2a). In the case of chroma, C^*_{ab} values increased during fermentative maceration, from
302 17 to 37 CIELAB units, and later decreased approximately 15-20% during the
303 stabilization and bottling stages (Figure 2b). However, the behaviour of hue angle was
304 slightly different for CW and OSW wines (Figure 2c): values remained balanced during
305 the fermentative maceration in OSW while increased for CW. From that moment h_{ab}
306 values progressively increased in both wines, achieving less bluish hues. This is mainly
307 due to the gradual transformation of monomeric anthocyanins into polymeric pigments
308 (Gutierrez, Lorenzo, & Espinosa, 2005). This process could be also responsible for the
309 loss of C^*_{ab} values observed along the final steps.

310 In general, during the stabilization and bottle aging stages, OSW showed higher C^*_{ab}
311 values and lower increase of h_{ab} than CW; therefore, OSW maintained more bluish and
312 vivid colours than CW. These differences could be due to the higher effect of
313 copigmentation existing in OSW wine from the first stage of vinification with respect to

CW (Table 2), which indicates a better pigment/copigment ratio in wines macerated with OS.

Along the most part of winemaking process OSW wines showed slightly lower L* values than CW, although quite constant for both wines, being around 65 and 70 CIELAB units. The highest L* values were reached in the last steps of vinification (Heras-Roger et al., 2014). At final stage of bottling, L* values maintained stable in OSW while increased in CW, which can be due to the achievement of higher stabilization level by copigmentation. In the same way, at the end of bottling, the more copigmented wine OSW wines had higher chroma values than CW, as previously indicated (González-Manzano et al., 2008)

The bottling stage was the most interesting period. Important differences started in this step, and trends in the CIELAB colour parameters due to the phenolic composition were observed. The most important differences between wines were regarding the hue angle, being high the increases in CW while OSW remained quite stable.

The colour differences (ΔE^*_{ab}) were calculated at the end of each vinification stage between CW and OSW. These differences tended to be bigger at the last steps of vinification. ΔE^*_{ab} was around 2 CIELAB units at the end of fermentation, slightly similar to the final of stabilization, while at the final of bottling stage this difference increased to 3.5 CIELAB units approximately. The OSW and CW wines could be considered visually different, mainly at the bottle aging stage since ΔE^*_{ab} was higher than 2.7 CIELAB units, which involves chromatic changes that can be perceived by the human eye (Martínez, Melgosa, Pérez, Hita, & Negueruela, 2001).

In order to evaluate the colour attribute that most influence the change of colour the relative contributions of lightness (%ΔL), chroma (%ΔC) and hue (%ΔH) at the different vinification stages were compared. Considering the step with the highest

339 perceptible colour changes (bottle stage), it can be observed that the addition of overripe
340 seeds induced a greater change on lightness. Thus, L* was the colour parameter most
341 affected by the OS addition, followed by chroma and hue (%ΔL=50, %ΔC=33 and
342 %ΔH=17).
343 Table 3 shows the colour differences (ΔE^*_{ab}) between the skin removal (day 7) and the
344 end of bottle aging stage (day 315) for CW and OSW, to assess the colour stability of
345 both wines along the time. Therefore, it is possible to know whether the addition of
346 overripe seeds at the beginning of the vinification is able to stabilize the colour of
347 wines. Results show the lowest colour difference for overripe seed wines (ΔE^*_{ab}=10.1
348 for OSW and 11.3 for CW). These values indicate that the addition of OS induces lower
349 colour modification and, in consequence, higher colour stability.
350 The chromatic modifications were less intense in OSW wines (ΔC^*_{ab}=−6 vs −7 u.;
351 ΔL*=+2 vs +3.1 in OSW and CW, respectively). Nevertheless, the hue showed the
352 highest chromatic modification, with significant differences (p<0.05) (Δh_{ab}=+16.0° *vs*
353 +180°, in OSW and CW, respectively). This is in accordance with Figure 2c, where it is
354 observed that OSW had lower increase of h_{ab} than CW during the stabilization and
355 bottle aging stages. The lower increase of h_{ab} values in OSW indicates slightly bluish
356 hues that can be due to the presence of higher amounts of bluish forms of anthocyanins
357 (3-acetyl-glucosides and glucosides) (Heredia, Francia-Aricha, Rivas-Gonzalo, Vicario,
358 & Santos-Buelga, 1998). Moreover, bluish tones are considered indicative of
359 intermolecular copigmentation (Casassa, Keirsey, Mireles, & Harbertson, 2012). Table
360 2 shown the results, with significant differences (p<0.05) between CW and OSW
361 regarding these anthocyanins.

4. Conclusions

Overripe seeds from white grapes were a rich natural source of copigments with large quantities of gallic acid, epicatechin and procyanidin B2 3-*O*-gallate. Macerating OS during the fermentative stage of vinification at 3 g/L led to Syrah wines with better phenolic composition than traditional macerated wines. The improvement of phenolic composition included higher pigment extraction and effectiveness of copigments diffusion from OS to wine, from the first stage of vinification, and a higher chemical stabilization during the last steps of the winemaking process. This stabilization had a positive effect on the wine colour, mostly reflected on the chroma and hue. Thus, darker colours with more bluish tones were achieved in the whole process of vinification.

Therefore, it is possible to conclude that the use of OS by-product as natural source of copigments could be an alternative to overcome the lack of phenolics in red wines, besides having environmental beneficial repercussion on winemaking regions. Notwithstanding, further studies focused on the maceration time or OS dose applied are still necessary to optimize the potential benefits of this by-products in red wine attributes.

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6. References

Berké, B., & de Freitas, V. (2007). A colorimetric study of oenin copigmented by procyanidins. *Journal of the Science of Food and Agriculture*, *87*(2), 260–265.

386 Boulton, R. (1996). A method for the assessment of copigmentation in red wines. In
387 47th Annual meeting of the American Society for Enology and Viticulture,
388 Reno, NV.
389 Boulton, R. (2001). The Copigmentation of Anthocyanins and Its Role in the Color of
390 Red Wine: A Critical Review. *American Journal of Enology and Viticulture*,
391 *52*(2), 67–87.
392 Busse-Valverde, N., Gómez-Plaza, E., López-Roca, J. M., Gil-Muñoz, R., & Bautista-
393 Ortín, A. B. (2011). The Extraction of Anthocyanins and Proanthocyanidins
394 from Grapes to Wine during Fermentative Maceration Is Affected by the
395 Enological Technique. *Journal of Agricultural and Food Chemistry*, *59*(10),
396 5450–5455.
397 Casassa, L. F., Keirsey, L. S., Mireles, M. S., & Harbertson, J. F. (2012).
398 Cofermentation of Syrah with Viognier: Evolution of Color and Phenolics
399 during Winemaking and Bottle Aging. *American Journal of Enology and*
400 *Viticulture*, *63*(4), 538–543.
401 Cejudo-Bastante, M. J., Rodríguez-Morgado, B., Jara-Palacios, M. J., Rivas-Gonzalo, J.
402 C., Parrado, J., & Heredia, F. J. (2016). Pre-fermentative addition of an
403 enzymatic grape seed hydrolysate in warm climate winemaking. Effect on the
404 differential colorimetry, copigmentation and polyphenolic profiles. *Food*
405 *Chemistry*, *209*, 348–357.
406 CIE 15: Technical Report: Colorimetry, 3rd edition (2004). Commission Internationale
407 de l'Eclairage Central Bureau: Vienna, Austria
408 De Freitas, V. A. P., & Mateus, N. (2010). Updating Wine Pigments. In C. Santos-
409 Buelga, M.T. Escribano-Bailon, & V. Lattanzio (Eds.), *Recent Advances in*
410 *Polyphenol Research, Vol. 2* (pp. 59–80). Wiley-Blackwell. Oxford, UK .

411 Dumitriu, D., Peinado, R. A., Peinado, J., & de Lerma, N. L. (2015). Grape pomace
412 extract improves the in vitro and in vivo antioxidant properties of wines from
413 sun light dried Pedro Ximénez grapes. *Journal of Functional Foods*, *17*, 380–
414 387.
415 González-Manzano, S., Mateus, N., de Freitas, V., & Santos-Buelga, C. (2008).
416 Influence of the degree of polymerisation in the ability of catechins to act as
417 anthocyanin copigments. *European Food Research and Technology*, *227*(1), 83–
418 92.
419 Gordillo, B., Cejudo-Bastante, M. J., Rodríguez-Pulido, F. J., Jara-Palacios, M. J.,
420 Ramírez-Pérez, P., González-Miret, M. L., & Heredia, F. J. (2014). Impact of
421 adding white pomace to red grapes on the phenolic composition and color
422 stability of Syrah wines from a warm climate. *Journal of Agricultural and Food*
423 *Chemistry*, *62*(12), 2663–2671.
424 Gutierrez, I., Lorenzo, E., & Espinosa, A. (2005). Phenolic composition and magnitude
425 of copigmentation in young and shortly aged red wines made from the cultivars,
426 Cabernet Sauvignon, Cencibel, and Syrah. *Food Chemistry*, *92*(2), 269–283.
427 Heras-Roger, J., Pomposo-Medina, M., Díaz-Romero, C., & Darias-Martín, J. (2014).
428 Copigmentation, colour and antioxidant activity of single-cultivar red wines.
429 *European Food Research and Technology*, *239*(1), 13–19.
430 Heredia, F. J., Francia-Aricha, E. M., Rivas-Gonzalo, J. C., Vicario, I. M., & Santos-
431 Buelga, C. (1998). Chromatic characterization of anthocyanins from red grapes-
432 I. pH effect. *Food Chemistry*, *63*(4), 491-498.
433 Heredia, F. J., Álvarez, C., González-Miret, M. L., & Ramírez, A. (2004). CromaLab®.
434 Análisis de color. Sevilla, España: Registro General de la Propiedad Intelectual
435 SE-1052-04.

436 Jara-Palacios, M. J., González-Manzano, S., Escudero-Gilete, M. L., Hernanz, D.,
437 Dueñas, M., González-Paramás, A. M., Santos-Buelga, C. (2013). Study of
438 Zalema Grape Pomace: Phenolic Composition and Biological Effects in
439 *Caenorhabditis elegans*. *Journal of Agricultural and Food Chemistry*, *61*(21),
440 5114–5121.
441 Jara-Palacios, M. J., Gordillo, B., González-Miret, M. L., Hernanz, D., Escudero-Gilete,
442 M. L., & Heredia, F. J. (2014a). Comparative Study of the Enological Potential
443 of Different Winemaking Byproducts: Implications in the Antioxidant Activity
444 and Color Expression of Red Wine Anthocyanins in a Model Solution. *Journal
445 of Agricultural and Food Chemistry*, *62*(29), 6975–6983.
446 Jara-Palacios, M. J., Hernanz, D., González-Manzano, S., Santos-Buelga, C., Escudero-
447 Gilete, M. L., & Heredia, F. J. (2014b). Detailed phenolic composition of white
448 grape by-products by RRLC/MS and measurement of the antioxidant activity.
449 *Talanta*, *125*, 51–57.
450 Liu, Y., Zhang, B., He, F., Duan, C. Q., & Shi, Y. (2016). The Influence of
451 Prefermentative Addition of Gallic Acid on the Phenolic Composition and
452 Chromatic Characteristics of Cabernet Sauvignon Wines. *Journal of Food
453 Science*, *81*(7), C1669–C1678.
454 Martínez, J. A., Melgosa, M., Pérez, M. M., Hita, E., & Negueruela, A. I. (2001). Note.
455 Visual and Instrumental Color Evaluation in Red Wines. *Food Science and
456 Technology International*, *7*(5), 439–444.
457 Mirabel, M., Saucier, C., Guerra, C., & Glories, Y. (1999). Copigmentation in model
458 wine solutions: Occurrence and relation to wine aging. *American Journal of
459 Enology and Viticulture*, *50*(2), 211–218.

460 Monagas, M., Gómez-Cordovés, C., & Bartolomé, B. (2006). Evolution of the phenolic
461 content of red wines from Vitis vinifera L. during ageing in bottle. *Food*
462 *Chemistry*, *95*(3), 405–412.
463 Pedroza, M. A., Carmona, M., Alonso, G. L., Salinas, M. R., & Zalacain, A. (2013).
464 Pre-bottling use of dehydrated waste grape skins to improve colour, phenolic
465 and aroma composition of red wines. *Food Chemistry*, *136*(1), 224–236.
466 Singleton, V. L. & Rossi, J. A. (1965). Colorimetry of total phenolics with
467 phosphomolybdic−phosphotungstic acid reagents. *American Journal of Enology*
468 *and Viticulture*, *16*, 144−158.
469 Teixeira, N., Cruz, L., Brás, N. F., Mateus, N., Ramos, M. J., & de Freitas, V. (2013).
470 Structural Features of Copigmentation of Oenin with Different Polyphenol
471 Copigments. *Journal of Agricultural and Food Chemistry*, *61*(28), 6942–6948.
472 Trouillas, P., Sancho-García, J. C., De Freitas, V., Gierschner, J., Otyepka, M., &
473 Dangles, O. (2016). Stabilizing and Modulating Color by Copigmentation:
474 Insights from Theory and Experiment. *Chemical Reviews*, *116*(9), 4937–4982.
475 Webb, L. B., Whetton, P. H., & Barlow, E. W. R. (2011). Observed trends in winegrape
476 maturity in Australia. *Global Change Biology*, *17*(8), 2707–2719.
477 Zou, H., Kilmartin, P. A., Inglis, M. J., & Frost, A. (2002). Extraction of phenolic
478 compounds during vinification of Pinot Noir wine examined by HPLC and
479 cyclic voltammetry. *Australian Journal of Grape and Wine Research*, *8*(3), 163–
480 174.

Figure captions

Fig. 1. Evolution of the main phenolic families in control wines and wines fermented with overripe seeds along the vinification process. (a) Total Anthocyanins, (b) Total Procyanidins. Results are presented as means (mg/L) ± standard deviations (n=3). Symbols: Control wine (♦); Overripe seed wine (◊)

Fig. 2. Changes in the CIELAB colour parameters for control wines and wines fermented with overripe seeds along the vinification process. (a) L*, lightness; (b) C^*_{ab}, chroma; (c) h_{ab}, hue angle. Results are presented as means ± standard deviations (n=3). Symbols: Control wine (♦); Overripe seed wine (◊)