IMPACT OF A POST-FERMENTATIVE MACERATION WITH OVERRIPE SEEDS ON THE COLOR STABILITY OF RED WINES.

Francisco J. Rivero, M. José Jara-Palacios, Belén Gordillo, Francisco J. Heredia and M. Lourdes González-Miret*

Food Colour and Quality Laboratory, Facultad de Farmacia, Universidad de Sevilla, 41012 Sevilla, Spain

Francisco J. Rivero: frivero@us.es
M. José Jara-Palacios: mjara@us.es
Belén Gordillo: bgordillo@us.es
Francisco J. Heredia: heredia@us.es
M. Lourdes González-Miret: miret@us.es

**** Corresponding author:***

M. Lourdes González-Miret
Food Colour & Quality Lab., Área de Nutrición y Bromatología. Facultad de Farmacia.
Universidad de Sevilla. 41012-Sevilla, Spain
Tel.: +34 955420938
e-mail: miret@us.es

Abstract

With the purpose of modulating the copigmentation equilibria of red wines, an environmentally sustainable process was performed based on post-fermentative addition of overripe seeds (OS). Simple (SW) and double (DW) addition were performed to produce different enrichment of phenolics from seeds, hence different copigmentation/polymerization ratios.

The determination of the phenolic composition showed different global increases in OS-macerates wines (catechin, epicatechin, gallic acid and procyanidins B1 and B2). The double post-maceration (DW) was more effective than the simple post-maceration addition to improve the phenolic structure of wines.

The application of Differential Tristimulus Colorimetry could assess the effects of this practice on the color characteristics and stability of wines. Results highlighted that both simple and double assays underwent colorimetric improvements against the control wines (CW, no seeds addition). DW led to the highest chromatic stability, showing lower lightness, higher chroma values and bluish hues than CW. This color difference was visually detectable.

Keywords: Overripe grapes; copigmentation; phenolic composition; red wine color; post-fermentative maceration

1. INTRODUCTION

Fermentative maceration is one of the more important steps of winemaking process since the must remains in contact with the raw materials (grapes seeds and skins), and hence phenolics, polysaccharides, nitrogen compounds, minerals and volatile compounds can be extracted. Thus, the wines achieve the aromatic, tasteful and visual structure. Later, the wine develops important sensory characteristics along the stabilization and ageing steps.

The chemical compounds pass from grapes to the must/wine differently during the maceration process, depending on their nature and extractability characteristics. Long macerations lead to a depletion of the grapes (Kocabey, Yilmaztekin & Hayaloglu, 2016) subsequently, the solids constitute wastes once finished the extraction stage.

Phenolic compounds belong to one of the more important chemical groups related to the winemaking process, having crucial influence on the organoleptic structure of the wines (Santos-Buelga & de Freitas, 2009). Differences on extractability exist among the different families within this group. On the one hand, the anthocyanins (located in the skin) and the low-molecular weight flavanols (located largely in the seeds) are extracted in aqueous medium, mainly during the first steps of maceration.

On the other hand, high-weight flavanols (also located mainly in the seeds), flavonols (in the skins) and phenolic acids (in both skins and seeds) are more soluble in alcoholic solutions (Jara-Palacios, Gordillo, González-Miret, Hernanz, Escudero-Gilete & Heredia, 2014a; González-Neves, Gil, Favre, Baldi, Hernández & Traverso, 2013; Gambuti, Capuano, Lecce, Fragasso & Moio, 2009).

Some phenolics, such as gallic acid and protocatechuic acid, need long macerations periods to achieve high extraction levels (Liu, Zhang, He, Duan & Shi, 2016; Zou,

47 Kilmartin, Inglis, & Frost, 2002). Therefore, the proper extraction of phenolics depends
48 greatly on both time and type of medium (hydroalcoholic) (Gambuti et al., 2009).
49 Nevertheless, extended macerations could involve sensory problems, mainly for
50 providing taste sensations of bitterness and astringency to the wine (Casassa et al.,
51 2013). The bitterness of wine is primarily triggered by flavan-3-ols. This taste could be
52 also caused by some flavonols, hydroxycinnamates and benzoic acid derivatives. On
53 the other hand, polymeric procyanidins (or tannins) are the main responsible for the
54 astringency. Both bitterness and astringency, which have high importance on the
55 gustative evolution of the wines, become mellower along the time (Ma, Guo, Zhang,
56 Wang, Liu & Li, 2014; Chira, Jourdes & Teissedre, 2012).
57 In addition, the phenolics are crucial to stabilize the anthocyanins along the time
58 through copigmentation phenomenon, protecting the sensible coloured forms of
59 monomeric anthocyanins and avoiding their degradation (Gordillo et al., 2014). It is
60 crucial to reach the adequate pigment/copigment ratio in order to achieve good
61 copigmentation capacity to prevent the degradation of the monomeric anthocyanins.
62 Furthermore, it is important to consider the adequate types of copigments because
63 some of them have a more suitable structure for the pigment protection (Jara-Palacios
64 et al., 2014a; Berké & de Freitas, 2007).
65 Previous studies carried out in traditional winemaking (Bimpilas, Panagopoulou,
66 Tsimogiannis & Oreopoulou, 2016; Rivero, Gordillo, Jara-Palacios, González-Miret &
67 Heredia, 2017) showed that the percentage of copigmentation decrease about 20%
68 (from 30% to 10%) during the early 6 months; being approximately 15% the
69 degradation of monomeric anthocyanins during the early 3 months and this rate
70 increases in subsequent months. On the other hand, Rivero et al. (2017) found that

71 wines elaborated with the addition of copigments during the fermentative maceration
72 process had lower anthocyanin degradation rate during the stabilization. Therefore,
73 this could indicate that adding copigments at the beginning of stabilization stage could
74 be an alternative practice to favour the copigmentation phenomenon, and hence to
75 attenuate the later degradation of monomeric anthocyanin.
76 Several macerations techniques (cold pre-fermentative maceration, post-fermentative
77 heating, carbonic maceration, *délestage* or enzymatic maceration) have been
78 developed as alternatives in red winemaking to improve the phenolic extractions from
79 red grapes, skins and seeds (Lukić, Budić-Leto, Bubola, Damijanić & Staver, 2017;
80 Gustavo González-Neves, Favre, Piccardo & Gil, 2016). This increment achieves the
81 subsequent formation of new and more stable pigments, which provides more stable
82 color of wines (Cejudo-Bastante, Gordillo, Hernanz, Escudero-Gilete, González-Miret &
83 Heredia, 2014)
84 Oak chips, enzymatic hydrolysate extracts or white grape pomace have been assayed
85 as exogenous sources for the addition of phenolics during the fermentative stage (Soto
86 Vázquez, Río Segade & Orriols Fernández, 2010; Baca-Bocanegra, Nogales-Bueno,
87 Hernández-Hierro & Heredia, 2018; Cejudo-Bastante, Rodríguez-Morgado, Jara-
88 Palacios, Rivas-Gonzalo, Parrado & Heredia, 2016; Gordillo et al., 2014). Recently, the
89 addition of an extra amount of seeds from overripe white grapes during the alcoholic-
90 fermentative maceration has obtained encouraging results (Rivero et al., 2017),
91 enhancing the bluish hues of wines as well as the stability of anthocyanins.
92 Nevertheless, this method showed some operational limits coming from the difference
93 on the harvesting time between white and red grapes, and hence overripe seeds from
94 white grapes are not available when red wine fermentation occurs. Therefore, we

need to have OS from previous vintage, which have had to be stored, usually frozen to avoid spoilages. On the other hand, the length of OS-wine contact is determined by the run-off time (red skin and seeds removal).

The overripe seeds are obtained from high-maturate grapes processed in *pasera* sites, places where grapes are submitted to postharvest direct-sun dehydration, in order to increase the sugar content for the elaboration of typical sweet wines from Andalucía (south of Spain). The long maturation time, the high temperatures and the long sun periods needed to produce the dehydration and the sugar concentration lead to the synthesis and the polymerization of phenolics (Dumitriu, Peinado, Peinado & de Lerma, 2015). Thus, these seeds are important source of phenolics and copigments such as epicatechin, gallic acid and procyanidin B2-3-O-gallate.

The aim of this work is to assess the overripe seeds by-products as a source of phenolics when adding in a subsequent post-fermentative seed-maceration. In this way, it is possible to strengthen an eventual low copigment/pigment ratio, and thus, to avoid the anthocyanin degradation. Moreover, this post-fermentative seed-maceration could be longer than the fermentative maceration (not conditioned by the run off), and therefore, could produce a higher extraction of phenols from the overripe seeds, which have low bitterness due to their high polymerization grade (Liu, Pan, Yan, He & Duan, 2010). In addition, overripe seeds can be obtained within the same vintage, so it does not need the storage.

Thus, post-fermentative seed-maceration involves important operational, economic and environmental benefits to the industry of wine. The sustainability of process is becoming a mandatory standard, and it makes relevant the oenological proposals based on reusing agro-industrial wastes.

2. MATERIAL AND METHODS

2.1. Winemaking protocols and samples

The overripe seeds (OS) were obtained from *Vitis vinifera* L. cv. Pedro Ximénez (PX) high-maturate grapes (D.O. Montilla-Moriles, Southwest Spain, 2016 vintage, 24 °Bé of sugar content). The seeds were manually separated from grape pomace. Around 5400 g of these seeds (average total phenolics: 5535 mg/100 g of dry seeds) were used for the elaboration red wines from *V. vinifera* cv. Syrah grapes (900 kg harvested at optimum technological maturity) grown in D.O. Condado de Huelva (Southwestern Spain).

The fermentation mash (destemmed and crushed grapes) was distributed in six stainless steel tanks of 220 L capacity to perform the alcoholic fermentation by adding 25 g/hL of selected *Saccharomyces cerevisiae* yeast (Viniferm BY, Agrovin, Ciudad Real, Spain). Skin maceration was developed manually punching down each tank once a day during 6 days. After this, the mash was drawn off to remove the solid parts, and the free run wines were racked to nine 50 L stainless steel tanks. Based on results from previous studies (Rivero et al., 2017) three types of experimental post-fermentative treatment were performed:

- SW (3 tanks) single post-fermentative maceration: addition of 600 g of overripe seeds per tank, macerated during 30 days (12 g/L seeds, 30 days)
- DW (3 tanks) double post-fermentative maceration: addition of 600 g of overripe seeds per tank, macerated during 30 days, and a further second addition of 600 g OS, macerated 30 days more (12 g/L seeds, 30 days, and a second addition of 12 g/L seeds, 30 days).

– CW (3 tanks) wines made by traditional winemaking (without post-fermentative addition of overripe seeds), as control wine.

Simultaneously to the OS addition (without OS in control wines), selected *Oenococcus oeni* lactic acid bacteria (VINIFERM Oe 104, 14 mL/hL, Agrovin, Ciudad Real, Spain) were inoculated to develop the malolactic fermentation.

At the end of malolactic fermentation, sulfur dioxide levels were adjusted (total sulfur dioxide about 100 mg/L and free sulfur dioxide about 60 mg/L in all wines). The wines were kept in the stainless steel tanks during 150 days until the end of the stabilization process.

The winemaking was carefully followed by analysing wine samples under oenological point of view. For the study, samples (50 mL) were taken at three points:

- initial point: 0 days, addition of overripe seeds
- seeds removal: after 30 days for SW and 60 days for DW
- final point: 150 days after overripe seeds addition (end of stabilization)

2.2. Oenological parameters

The conventional analysis of oenological parameters (pH, total and volatile acidity, free and total SO_2, malic and lactic acids and reducing sugars) (Table 1) were performed according to the Official Methods established by European Union.

2.3. Copigmented and Polymerized Anthocyanin Determination

The contribution of copigmented anthocyanins to the total wine color at pH 3.6 (% copigmented anthocyanins) and the degree of anthocyanin polymerization (% polymeric pigments) were determined following the method proposed by Boulton (1996). The pH values of wine samples were first adjusted to 3.6 using 1M NaOH or HCl.

166 *2.4. Colorimetric analysis*

167 The visible spectra (380-770 nm) was measured in *triplicate* at constant intervals ($\Delta\lambda$=2
168 nm) with an Agilent 8453 UV-Vis spectrophotometer (Palo Alto, USA), using 2 mm path
169 length glass cells and distilled water as white reference. The CIELAB colour parameters
170 (L*, a*, b*, C^*_{ab} and h_{ab}) were calculated from the transmittance spectra by using the
171 original software CromaLab® (Heredia, Alvarez, González-Miret & Ramírez, 2004),
172 following the recommendations of the Commission Internationale de l'Eclairage (CIE,
173 2004); 10° Standard Observer and D65 Standard Illuminant were used as references.
174 Colour differences (ΔE^*_{ab}) were calculate as the Euclidean distance between two
175 points in the three-dimensional space defined by L*, a*, and b*: $\Delta E^*_{ab} = [(\Delta L^*)^2 +$
176 $(\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

177 *2.5. HPLC-DAD analysis of phenolic compounds*

178 The monomeric anthocyanins and flavonols were determined in triplicate according to
179 the method reported by Cejudo-Bastante et al., (2016), which performs identification
180 based on the retention times and HPLC-DAD-ESI-MS^n.

181 The HPLC separation and quantification were performed in an Agilent 1200
182 chromatographic system, equipped with quaternary pump, UV-VIS diode-array
183 detector, automatic injector, and ChemStation software (Agilent Technologies, Palo
184 Alto, USA). The samples were filtered through a 0.45 μm Nylon filter prior to direct
185 injection; then, a volume of 50 μL was injected onto a Zorbax C_{18} column (250 x 4.6
186 mm, 5 μm particle size). Acetonitrile, formic acid and water were used as solvents,
187 being 3:10:87 solvent A and 50:10:40 solvent B (mL:mL:mL). The elution profile was: 0-
188 10 min with 6% B; 10-15 min with 11% B; 15-20 min with 20% B; 20-25 min with 23% B;
189 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. The temperature was set at 40 °C and 0.63 mL/min flow rate. All UV-Vis spectra were recorded from 200 to 800 nm with a bandwidth of 2.0 nm, using the external calibration method 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 phenolics was expressed as mg/L.

The analyses of flavan-3-ols (monomeric and procyanidins), as well as the hydroxycinnamic and benzoic acids were performed, in triplicate, according to Jara-Palacios, Hernanz, González-Manzano, Santos-Buelga, Escudero-Gilete & Heredia., (2014b) using RRLC. After filtration through a 0.45 μm Nylon filter, samples were injected (0.5 μL injection volume) in an Agilent 1290 chromatographic system, equipped with quaternary pump, UV-VIS diode-array detector, automatic injector, and ChemStation software (Agilent Technologies, Palo Alto, USA). A C_{18} Poroshell 120 column (2.7 μm, 5 cm x 4.6 mm) was used. The solvents were formic acid and water (1:999 mL: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 °C. Identification of phenolics was performed according to the retention times of the standards (when available), UV-vis spectra and mass spectra, as described by Jara-Palacios 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 acid, *p*-coumaric acid and catechin standards. The concentration was expressed as mg/L for wine samples. In addition, the total anthocyanin, flavonol, benzoic acid,

hydroxycinnamic acid derivatives, monomeric flavan-3-ol and procyanidin contents were calculated as the sum of individual phenolic compounds identified by HPLC. The Total phenolic content of each sample was determined in triplicate by the Folin-Ciocalteau method (Singleton & Rossi., 1965) using an Agilent 8453 UV-Vis spectrophotometer.

2.6. Statistical analysis

Statistical analysis was carried out by using Statistica® version 8.0 software (Stat Soft). Univariate analysis of variance (ANOVA) was applied using the general linear model program (Tukey test, $p<0.05$) to establish whether the mean values of the sample data differed significantly from each other.

3. RESULTS AND DISCUSSION

3.1. Phenolic implications in wines

The phenolic composition of the wines (SW, DW and CW) was determined at 0, 30/60 and 150 days from the OS addition, in order to state the impact of the different OS additions on the stabilization of final wines.

Table 2 shows the individual compounds grouped: 10 anthocyanins, 6 phenolic acids, 2 flavan-3-ols, 4 procyanidins and 7 flavonols, as well as the sum of individual phenolics for each group (mg/L ± SD, n=3) and the total phenolic content expressed as gallic acid (mg GAE/L). The Table 2 also includes the percentages of copigmentation and polymerization of wines at the the end of the stabilization stage (150 days from the OS addition).

Statistical differences ($p<0.05$) regarding the phenolic composition were found between CW and the two other wines (SW and DW) at the end of their corresponding

238 post-fermentative macerations (30 and 60 days from the OS addition, respectively).
239 Moreover, all wines were statistically different (p<0.05) on phenolic composition at the
240 end of the stabilization process (CW *vs* SW, CW *vs* DW and SW *vs* DW).
241 Pedro Ximénez OS are rich in flavan-3-ols, procyanidins and benzoic acids (Rivero et al.,
242 2017) but poor in flavonols and hydroxycinnamic acids (Jara-Palacios et al., 2014a).
243 Accordingly, results demonstrated that the wines submitted to OS post-maceration
244 (SW and DW) achieved higher amounts of flavan-3-ols, benzoic acids and, although in a
245 lesser extent, procyanidins comparing to the control (CW) at the OS removal (30 days
246 for SW and 60 days for DW). Also, significant (p<0.05) differences were found between
247 the wines with adding seeds (SW and DW) at 60 days, showing DW higher levels of
248 flavan-3-ols and procyanidins. Interestingly, DW showed higher values than SW at the
249 end of the study (150 days), which support the major effectiveness of adding double
250 amount of seeds at longer post-maceration time (12 g/L seeds 30 days, and a second
251 addition of 12 g/L seeds 30 days) to improve the phenolic structure of wines.
252 This fact resulted in different global increases of phenolics in DW and SW respect to
253 the traditional maceration (CW). In particular, benzoic acids increased by 5% and 2%,
254 flavanols by 22% and 8%, and procyanidins by 10% and 8% in DW and SW, respectively.
255 On contrast, the contributions of overripe seeds on the flavonols and hydroxycinnamic
256 acids contents were almost negligible due to their scarce presence.
257 The differences observed among the groups of phenolics were also found for the
258 major individual compounds (epicatechin, catechin, and gallic acid). Their contents
259 were comparatively higher in DW and SW than in CW. The greatest effects was found
260 in the double addition assay, being DW wines richer in all the aforementioned
261 compounds than CW. In the case of SW, the differences respect to CW were only

262 significant for catechin (76.7 mg/L versus 59.2 mg/L, respectively). These results are in
263 accordance with other studies that used higher amounts of seeds (60 g/L) in pre-
264 fermentative stages (Kovac, Alonso & Revilla, 1995).
265 Regarding procyanidins, SW and DW were generally richer in procyanidins B1 and B2
266 than CW, in particular DW also in procyanidin B2 3-O-gallate. In this sense, adding
267 single quantity of overripe seeds at shorter post-maceration time or double quantity at
268 longer post-maceration time seems to have almost similar effect on the minor
269 copigments.
270 The differences observed among the maceration treatments on the major and minor
271 phenolics of wines could be also due to the particular kinetic of extraction of each
272 compound and to the solid structure of seeds that can differently limit their
273 extractability. Flavanols need longer times of extraction (20 days) but not as extensive
274 as in the case of gallic acid, which increases mainly in late stages of maceration (Tian
275 et al., 2009; Rivero et al., 2017). Thus, flavanols could have a complete extraction, and
276 the benzoic acids (gallic acid) incomplete.
277 Therefore, adding single quantity of overripe seeds could be insufficient to improve
278 the global phenolic structure of red wine compared to adding double amount.
279 Notwithstanding, in both cases, the maceration treatment applied demonstrated
280 positive effects respect to traditional maceration.
281 Moreover, Table 2 shows the quantitative effect of the compounds extracted during
282 the post-fermentative maceration on the monomeric anthocyanins and its subsequent
283 quantitative effect along a 3-months stabilization period. At the end of the different
284 post-fermentative macerations (at 30 and 60 days), DW and SW had lower contents on
285 pigments than CW (7 % and 13 %, respectively). This could be due to the formation of

286 polymeric anthocyanins (condensation reactions) during the early stages, between
287 anthocyanin and the phenolics extracted from overripe seeds, such as flavan-3-ols
288 (Gordillo et al., 2014). Other effects such as the adsorption produced by the addition of
289 external sources of phenolics (grape pomace, chips or seeds) could involve the
290 decrease of monomeric anthocyanins (Gordillo et al., 2014; 2016). In this study, the
291 adsorption could depend on both the amount of added seeds and the time of the post-
292 fermentative maceration, which can lead to differences on the final anthocyanic
293 content of the wines.
294 During the stabilization stage, the monomeric anthocyanin content decreased in the
295 different wines (Table 2). This typically occurs due to reactions (oxidation, hydration,
296 adsorption and polymerization) that lead to the loss of monomeric anthocyanins
297 (Cejudo-Bastante, Rivero-Granados & Heredia, 2017). At this respect, at the end of the
298 stabilization period (150 days from the beginning of post-fermentative maceration),
299 DW and SW reached higher degree of polymerization than CW (46 % in SW and 46% in
300 DW versus 40 % in CW).
301 This indicates higher proportions of more stable forms of anthocyanins in wines
302 macerated with overripe seeds (higher chemical stability) and could also explain the
303 higher decreases in monomeric anthocyanins (20.6 mg/L and 36.3 mg/L in SW and DW
304 versus 57.4 mg/L in CW at the end of stabilization stage).
305 Regarding colourless phenolics, flavan-3-ols, flavonols and procyanidins were the most
306 affected during stabilisation stage, probably due to their higher implication in
307 polymerization reactions with anthocyanins (He et al., 2012; Rentzsch, Schwarz,
308 Winterhalter & Hermosín-Gutiérrez, 2007; Stavridou, Soufleros, Bouloumpasi & Dagkli,
309 2016).

The amount of some phenolics can also decrease through polymerization between compounds of the same nature, as the case of flavanols and procyanidins (Guadalupe & Ayestarán, 2008)

On the other hand, during stabilisation stage the concentration of benzoic acids remained stable or even increased slightly as well as the gallic acid, as reported in previous studies (Gris et al., 2013).

3.2. Color characteristics and changes

The different maceration treatments applied led to different characteristics, evolution and stability of wine color. Table 3 shows the average values and standard deviation (n=3) of the CIELAB parameters (L*, a*, b*, C^*_{ab}, and h_{ab}) for the different wines at three sampling moments: initial (0 days), end of the post-fermentative maceration processes (30/60 days), and end of the stabilization stage (150 days). Moreover, significant differences between the wines at the three different vinification steps are included.

In general, increases in L* and decreases in C^*_{ab} values were produced during the OS-maceration. At the end of this stage (30 days for SW and 60 days for DW), the control wines (CW) showed lower L* and higher C^*_{ab} values compared to SW and DW, which lead to color difference values of 4.1 units and 14.9 units for CW30-SW30 and CW60-DW60, respectively. According to Gordillo et al. (2014), the color difference between these pairs of wines can be visually perceived.

These results are in accordance with the phenolic composition (summarized in Table 2). SW and DW showed lower monomeric anthocyanin concentration than CW, which could promote the higher increases in L* and decreases in C^*_{ab}. Nevertheless, lightness and chroma underwent the contrary effect along the stabilization phase, that is,

decrease and increase, respectively. In such a way, the global process led to slightly darker and more vivid those wines submitted to double OS maceration (DW), being wines with lower L* values and higher C^*_{ab} than CW (L* = 72.31 vs 74.55 units; C^*_{ab} = 25.66 vs 24.23 units, respectively). However, C^*_{ab} and L*values were similar in SW and CW (L*=73.69 vs 74.55 units and C^*_{ab}=23.46 vs 24.23 units, respectively). Regarding the hue, after an initial increase towards positive values (less bluish-red) during the first step (mainly the OS macerated wines, SW and DW), the wines finally were close to pure-red hues (close to 0°), being the lowest values for SW. Hue angle (h_{ab}) showed significant differences (p<0.05) between DW and CW at the end of the stabilization stage. The wines with double addition of PX overripe seeds were more bluish than the CW (h_{ab}=3.7° vs 4.9°). This could be due to a higher copigmentation of monomeric anthocyanins in DW caused for the larger copigment/pigment ratio (Sum Copigments/Sum Total Anthocyanin= 10.4 vs 6.4, DW and CW, respectively, measured by HPLC and showed in Table 2), which can result in a higher bathochromic effect or positive shift toward higher wavelength within the visible range (Boulton, 2001).

Considering these parameters, the color differences among the wines at the end of the stabilization stage were 1.52 units for CW-SW, 2.70 units for CW-DW and 2.64 units for SW-DW. That is to say, the double assay (DW) lead to color changes that can be perceptible (Gordillo et al, 2014), being the single (SW) similar in color to the control wine.

Figure 1 shows the location of the initial (0 days) and final (150 days) wines (CW, SW and DW) on the CIELAB (a*b*)-diagram, where can be seen the chroma (the distance from the origin of coordinates to the color point) and the hue (the angle formed with the semiaxis +a*) for every color point. As observed, the different OS maceration led to

differences in wine color at the end of the stabilization stage, and the color points of SW, DW and CW appear separated within the a*b*-plane.

Considering the global process, the determination of the color differences (ΔE^*_{ab}) occurring from the OS addition to the end of the stabilization period allows evaluating the color stability of each wine. Therefore, in order to assess these observations, the CIELAB color difference (ΔE^*_{ab}) and the differences of the color parameters (L*, C^*_{ab} and h_{ab}) were calculated for every wine (CW, SW and DW). The assessment was made considering the end of the maceration process (30/60 days) as well as the end of the winemaking process (150 days), regarding the initial point (0 days) (Table 4). SW and DW showed higher color changes (color difference ΔE^*_{ab} and individual color parameters ΔL^*, ΔC^*_{ab}, Δh_{ab}) along the OS-maceration time than their corresponding control wine (CW 30 days for SW and CW 60 days for DW). In the case of hue, the wines submitted to OS-maceration showed higher increases (less bluish-red). This could be due to a combined effect of the higher synthesis of polymeric anthocyanins (yellowish) with the copigments extracted from seeds during the post-fermentative maceration (Burtch, Mansfield & Manns., 2017), together with the light yellowish colors provided directly by copigments such as flavan-3-ols and procyanidins (Ashraf-Khorassani & Taylor, 2004) supplied by the overripe seeds. This colorimetric behavior reverted during the stabilization stage (from seeds removal), in both quantitative (C^*_{ab} and L*) and qualitative (h_{ab}) terms.

Along the global process (from initial to end of stabilization) the lowest color difference (ΔE^*_{ab}) was found for DW. All wines underwent losses of chroma (ΔC^*_{ab}); DW showed the lowest change followed by SW and CW, being significantly ($p<0.05$) different among them. With respect to lightness (ΔL^*), CW and SW showed few

increases (towards clearer color) and few decrease in DW (towards darker color), but the differences were not significant. In the case of hue (Δh_{ab}), similar increases occurred in CW and DW while SW showed a significant (p<0.05) lower change (5.8°, 5.1°, 4.6°, respectively).

The comparison of the final wines (at 150 days) allows assessing the effect caused by the OS-maceration. With this purpose, the color differences (ΔE^*_{ab}) between the OS-macerated wines and their corresponding control wines (SW_{150} *vs* CW_{150} and DW_{150} *vs* $CW_{150)}$ were determined. ΔE^*_{ab} was higher in the double overripe seed addition than simple addition (3.25 and 1.58 units, respectively). This could be due to copigments from Pedro Ximénez overripe seeds (larger amount in DW) such as benzoic acids or procyanidins, which are involved in the increment of polymeric anthocyanins during the stabilization stage (Liu et al., 2016; Berké & de Freitas, 2007; Rivero et al., 2017). Thus, the polymerization and copigmentation processes led to the highest color difference (ΔE^*_{ab}) between CW and DW, which only in DW were visually perceptible by the human eye (Gordillo et al., 2014).

Regarding the contribution of the individual colorimetric variables to the color differences (lightness %ΔL, chroma %ΔC and hue %ΔH), it was found that the double addition of overripe seeds induced greater change on lightness followed by chroma and hue (%ΔL*= 77%, $\%\Delta C^*_{ab}$= 20% and %ΔH= 3%), which is in accordance with previous works (Rivero et al., 2017).

4. CONCLUSIONS

The addition of overripe seeds to red wine elaboration could represent an alternative technique to strengthen the eventual low copigment/pigment ratio of wines by increasing the content of some compounds, mainly flavanols, benzoic acids and

406 procyanidins. Moreover, adding OS during post-fermentative steps allows performing
407 long macerations periods. This addition improves the copigment proportion, avoiding
408 the anthocyanin degradation (by copigmentation and polymerization processes), and
409 leads to more suitable wines for the aging process. In this study, double addition of
410 overripe seeds led to better results.
411 This process exerted positive effects on the wine color, giving wines with lower
412 lightness and higher chroma values (darker and more vivid colors) and more bluish
413 hues than the wines traditionally produced. Results proved the effectiveness of the
414 post-fermentative maceration with overripe seeds, mainly the double addition, to
415 stabilize the color of wines and to provoke lower color modifications along the time,
416 producing wines chromatically more stable for a better ageing.
417 This study obtained interesting and promising results for wines with unbalanced
418 copigment/pigment proportion that is the case of winemaking in warm-climates. In
419 addition, it involves different benefits: environmental, achieved though the reuse of
420 overripe seeds (a waste of the wine industry), operational and economical due to the
421 possibility of using overripe seeds within the same vintage, avoiding the storage.
422

423 **ACKNOWLEDGMENTS**
424 Authors thank the Ministerio de Economía y Competitividad, Gobierno de España
425 (project AGL2014-58486-C2-2-R) and V Plan Propio de Investigación de la Universidad
426 de Sevilla (Acción II.3) for the financial support; also thank the assistance of the
427 technical staff of Biology Service (SGI, Universidad de Sevilla).
428 **Note**
429 The authors declare no competing financial interest.

430 **REFERENCES**

431 Ashraf-Khorassani, M. & Taylor, L. T. (2004). Sequential Fractionation of Grape Seeds
432 into Oils, Polyphenols, and Procyanidins via a Single System Employing CO_2 -
433 Based Fluids. *Journal of Agricultural and Food Chemistry*, *52*(9), 2440–2444.

434 Baca-Bocanegra, B., Nogales-Bueno, J., Hernández-Hierro, J.M. & Heredia, F.J. (2018).
435 Evaluation of extractable polyphenols released to wine from cooperage
436 byproduct by near infrared hyperspectral imaging, *Food Chemistry*, *244*, 206–
437 2012

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

440 Bimpilas, A., Panagopoulou, M., Tsimogiannis, D. & Oreopoulou, V. (2016).
441 Anthocyanin copigmentation and color of wine: The effect of naturally obtained
442 hydroxycinnamic acids as cofactors. *Food Chemistry*, *197*, 39–46.

443 Boulton, R. (1996). A method for the assessment of copigmentation in red wines.
444 In *In 47th annual meeting of the American society for enology and viticulture*.
445 Reno, NV .

446 Boulton, R. (2001). The copigmentation of anthocyanins and its role in the color of red
447 wine: A critical review. *American Journal of Enology and Viticulture, 52* (2), 67–
448 87

449 Burtch, C.E., Mansfield, A.K. & Manns, D.C. (2017). Reaction Kinetics of Monomeric
450 Anthocyanin Conversion to Polymeric Pigments and Their Significance to Color
451 in Interspecific Hybrid Wines. *Journal of Agricultural and Food Chemistry*,
452 *65(31),* 6379-6386.

453

454 Casassa, L. F., Larsen, R. C., Beaver, C. W., Mireles, M. S., Keller, M., Riley, W. R.,
455 Smithyman,R. & Harbertson, J. F. (2013). Sensory Impact of Extended
456 Maceration and Regulated Deficit Irrigation on Washington State Cabernet
457 Sauvignon Wines. *American Journal of Enology and Viticulture*, *64*(4), 505–514.
458 Cejudo-Bastante, M. J., Gordillo, B., Hernanz, D., Escudero-Gilete, M. L., González-
459 Miret, M. L. & Heredia, F. J. (2014). Effect of the time of cold maceration on the
460 evolution of phenolic compounds and colour of Syrah wines elaborated in
461 warm climate. *International Journal of Food Science & Technology*, *49*(8), 1886–
462 1892.
463 Cejudo-Bastante, M. J., Rivero-Granados, F. J. & Heredia, F. J. (2017). Improving the
464 color and aging aptitude of Syrah wines in warm climate by wood–grape mix
465 maceration. *European Food Research and Technology*, *243*(4), 575–582.
466 Cejudo-Bastante, M. J., Rodríguez-Morgado, B., Jara-Palacios, M. J., Rivas-Gonzalo, J.
467 C., Parrado, J. & Heredia, F. J. (2016). Pre-fermentative addition of an enzymatic
468 grape seed hydrolysate in warm climate winemaking. Effect on the differential
469 colorimetry, copigmentation and polyphenolic profiles. *Food Chemistry*, *209*,
470 348–357.
471 Chira, K., Jourdes, M., & Teissedre, P. L. (2012). Cabernet sauvignon red wine
472 astringency quality control by tannin characterization and polymerization
473 during storage. *European Food Research and Technology, 234,* 253-261.
474 CIE 15. (2004). Technical Report: Colorimetry (3rd ed.). Vienna, Austria: Commission
475 Internationale de l'Eclairage Central Bureau.Dumitriu, D., Peinado, R. A.,
476 Peinado, J. & de Lerma, N. L. (2015). Grape pomace extract improves the in

vitro and in vivo antioxidant properties of wines from sun light dried Pedro Ximénez grapes. *Journal of Functional Foods*, *17*, 380–387.

Dumitriu, D., Peinado, R. A., Peinado, J. & de Lerma, N. L. (2015). Grape pomace extract improves the in vitro and in vivo antioxidant properties of wines from sun light dried Pedro Ximénez grapes. *Journal of Functional Foods*, *17*, 380–387.

Gambuti, A., Capuano, R., Lecce, L., Fragasso, M. G. & Moio, L. (2009). Extraction of phenolic compounds from 'Aglianico'and 'Uva di Troia'grape skins and seeds in model solutions: Influence of ethanol and maceration time. *Vitis*, *48*(4), 193–200.

González-Neves, G., Favre, G., Piccardo, D. & Gil, G. (2016). Anthocyanin profile of young red wines of Tannat, Syrah and Merlot made using maceration enzymes and cold soak. *International Journal of Food Science & Technology*, *51*(1), 260–267.

González-Neves, G., Gil, G., Favre, G., Baldi, C., Hernández, N. & Traverso, S. (2013). Influence of winemaking procedure and grape variety on the colour and composition of young red wines. *South African Journal of Enology and Viticulture*, *34*(1), 138–146.

Gordillo, B., Baca-Bocanegra, B., Rodriguez-Pulído, F. J., González-Miret, M. L., García Estévez, I., Quijada-Morín, N., Heredia, F. J. & Escribano-Bailón, M. T. (2016). Optimisation of an oak chips-grape mix maceration process. Influence of chip dose and maceration time. *Food Chemistry*, *206*, 249–259.

Gordillo, B., Cejudo-Bastante, M. J., Rodríguez-Pulido, F. J., Jara-Palacios, M. J., Ramírez-Pérez, P., González-Miret, M. L. & Heredia, F. J. (2014). Impact of Adding White Pomace to Red Grapes on the Phenolic Composition and Color

501 Stability of Syrah Wines from a Warm Climate. *Journal of Agricultural and Food*
502 *Chemistry*, *62*(12), 2663–2671.
503 Gris, E. F., Mattivi, F., Ferreira, E. A., Vrhovsek, U., Filho, D. W., Pedrosa, R. C. &
504 Bordignon-Luiz, M. T. (2013). Phenolic profile and effect of regular
505 consumption of Brazilian red wines on in vivo antioxidant activity. *Journal of*
506 *Food Composition and Analysis*, *31*(1), 31–40.
507 Guadalupe, Z. & Ayestarán, B. (2008). Changes in the color components and phenolic
508 content of red wines from Vitis vinifera L. cv. "Tempranillo" during vinification
509 and aging. *European Food Research and Technology*, *228*(1), 29–38.
510 He, F., Liang, N.N., Mu, L., Pan, Q.H., Wang, J., Reeves, M. J. & Duan, C.Q. (2012).
511 Anthocyanins and Their Variation in Red Wines II. Anthocyanin Derived
512 Pigments and Their Color Evolution. *Molecules*, *17*(12), 1483–1519.
513 Heredia, F. J., Álvarez, C., González-Miret, M. L. & Ramírez, A. (2004). CromaLab,
514 Análisis de color. Sevilla. España. Registro General de la Propiedad Intelectual.
515 SE-1052-04
516 Jara-Palacios, M. J., Gordillo, B., González-Miret, M. L., Hernanz, D., Escudero-Gilete,
517 M. L. & Heredia, F. J. (2014a). Comparative Study of the Enological Potential of
518 Different Winemaking Byproducts: Implications in the Antioxidant Activity and
519 Color Expression of Red Wine Anthocyanins in a Model Solution. *Journal of*
520 *Agricultural and Food Chemistry*, *62*(29), 6975–6983.
521 Jara-Palacios, M. J., Hernanz, D., González-Manzano, S., Santos-Buelga, C., Escudero-
522 Gilete, M. L. & Heredia, F. J. (2014b). Detailed phenolic composition of white
523 grape by-products by RRLC/MS and measurement of the antioxidant activity.
524 *Talanta*, *125*, 51–57.

525 Kocabey, N., Yilmaztekin, M. & Hayaloglu, A. A. (2016). Effect of maceration duration
526 on physicochemical characteristics, organic acid, phenolic compounds and
527 antioxidant activity of red wine from Vitis vinifera L. Karaoglan. *Journal of Food*
528 *Science and Technology*, *53*(9), 3557–3565.
529 Kovac, V., Alonso, E. & Revilla, E. (1995). The effect of adding supplementary quantities
530 of seeds during fermentation on the phenolic composition of wines. *American*
531 *Journal of Enology and Viticulture*, *46*(3), 363–367.
532 Liu, Y., Zhang, B., He, F., Duan, C.Q. & Shi, Y. (2016). The Influence of Prefermentative
533 Addition of Gallic Acid on the Phenolic Composition and Chromatic
534 Characteristics of Cabernet Sauvignon Wines. *Journal of Food Science*, *81*(7),
535 C1669–C1678.
536 Liu, Y.X., Pan, Q.H., Yan, G.L., He, J.J. & Duan, C.Q. (2010). Changes of Flavan-3-ols with
537 Different Degrees of Polymerization in Seeds of 'Shiraz', 'Cabernet Sauvignon'
538 and 'Marselan' Grapes after Veraison. *Molecules*, *15*(11), 7763–7774.
539 Lukić, I., Budić-Leto, I., Bubola, M., Damijanić, K. & Staver, M. (2017). Pre-fermentative
540 cold maceration, saignée, and various thermal treatments as options for
541 modulating volatile aroma and phenol profiles of red wine. *Food Chemistry*,
542 *224*, 251–261.
543 Ma, W., Guo, A., Zhang, Y., Wang, H., Liu, Y., & Li, H. (2014). A review on astringency
544 and bitterness perception of tannins in wine. *Trends in Food Science &*
545 *Technology, 40,* 6-19.
546 Rentzsch, M., Schwarz, M., Winterhalter, P. & Hermosín-Gutiérrez, I. (2007). Formation
547 of Hydroxyphenyl-pyranoanthocyanins in Grenache Wines: Precursor Levels

548 and Evolution during Aging. *Journal of Agricultural and Food Chemistry*, *55*(12),
549 4883–4888.
550 Rivero, F. J., Gordillo, B., Jara-Palacios, M. J., González-Miret, M. L. & Heredia, F. J.
551 (2017). Effect of addition of overripe seeds from white grape by-products
552 during red wine fermentation on wine colour and phenolic composition. *LWT -*
553 *Food Science and Technology*, *84*, 544–550.
554 Santos-Buelga,C. & de Freitas, V. (2009). Influence of Phenolics on Wine Organoleptic
555 Properties. In M.V. Moreno-Arribas, M.C. Polo (Eds.), *Wine Chemistry and*
556 *Biochemistry,* (pp. 529-570). Science+Business Media
557 Singleton, V. L. & Rossi, J. A. (1965). Colorimetry of total phenolics with
558 phosphomolybdic−phosphotungstic acid reagents. *Am. J. Enol. Vitic. 16*, 144−158.
559 Soto Vázquez, E., Río Segade, S. & Orriols Fernández, I. (2010). Effect of the
560 winemaking technique on phenolic composition and chromatic characteristics
561 in young red wines. *European Food Research and Technology*, *231*(5), 789–802.
562 Stavridou, K., Soufleros, E. H., Bouloumpasi, E. & Dagkli, V. (2016). The Phenolic
563 Potential of Wines from French Grape Varieties Cabernet Sauvignon, Merlot
564 and Syrah Cultivated in the Region of Thessaloniki (Northern Greece) and Its
565 Evolution during Aging. *Food and Nutrition Sciences*, *07*(02), 122–137.
566 Tian, R.R., Pan, Q.H., Zhan, J.C., Li, J.M., Wan, S.B., Zhang, Q.H. & Huang, W.D. (2009).
567 Comparison of Phenolic Acids and Flavan-3-ols During Wine Fermentation of
568 Grapes with Different Harvest Times. *Molecules*, *14*(2), 827–838.
569 Zou, H., Kilmartin, P. A., Inglis, M. J., & Frost, A. (2002). Extraction of phenolic
570 compounds during vinification of Pinot Noir wine examined by HPLC and cyclic
571 voltammetry. *Australian Journal of Grape and Wine Research*, 8(3), 163-174.

572 **FIGURE CAPTIONS**

573

574 Figure 1. Location of the initial (0 days) and final (150 days) wines on the CIELAB

575 (a*b*)-diagram

Table 1

Range (minimum and maximum value) of Conventional Analytical Data of wines.

	CW	**SW**	**DW**
pH	3.74 - 3.88	3.75 - 3.85	3.75 - 3.87
Total acidity (g/L as tartaric acid)	4.90 - 5.75	4.90 - 5.55	4.95 - 5.70
Volatile acidity (g/L as acetic acid)	0.47 - 0.71	0.46 - 0.82	0.46 - 0.77
Free SO_2 (mg/L)	65 - 20	68 - 17	79 - 15
Total SO_2 (mg/L)	88 - 95	89 - 97	97 - 101
Reducing sugars (g/L)	1.17	1.12	1.10
Malic acid (g/L)	1.47 - ≤0.01	1.52 - ≤0.01	1.45 - ≤0.01
Lactic acid (g/L)	0.29 - 1.67	0.27 - 1.60	0.30 - 1.63

Table 2

Mean values and standard deviations (n = 3) of the phenolic compounds concentration (mg/L), total phenols (mg GAE/L) and percentages of copigmentation and polymerization of wines at seeds addition (0 day), seeds removal (30/60 days after OS addition) and at the end of stabilization stage (150 days after OS addition).

	Post-maceration treatment*								
	CW0	**CW30**	**SW30**	**CW60**	**SW60**	**DW60**	**CW150**	**SW150**	**DW150**
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Total Anthocyanins	149.18 ± 1.81	133.94 a ± 1.85	123.99 b ± 1.26	144.18 a ± 6.25	121.71 b ± 8.56	125.16 b ± 3.49	57.43 a ± 11.98	20.56 b ± 0.22	36.34 b ± 4.89
Total glucoside derivatives	73.79 ± 1.83	64.67 a ± 0.76	59.92 b ± 0.82	67.64 a ± 3.50	59.43 b ± 1.07	59.61 b ± 1.35	27.94 a ± 8.05	9.51 b ± 0.58	18.59 ab ± 1.91
Total acetate derivatives	41.71 ± 0.70	39.62 a ± 0.50	37.10 b ± 0.37	45.16 a ± 0.14	37.42 b ± 4.19	40.09 b ± 0.88	19.77 a ± 2.18	7.83 b ± 0.52	12.38 b ± 2.31
Total *p*-coumaric derivatives	33.68 ± 1.01	29.65 a ± 0.64	26.97 b ± 0.19	31.38 a ± 2.83	24.85 b ± 3.32	25.46 b ± 1.32	9.72 a ± 1.75	3.22 b ± 0.28	5.36 ab ± 0.67
Total Hydroxycinnamic acids	57.23 ± 0.04	57.79 a ± 0.05	57.67 b ± 0.00	58.03 a ± 0.05	57.95 a ± 0.12	57.87 a ± 0.34	55.85 a ± 0.01	56.51 b ± 0.39	56.83 b ± 0.08
Total Benzoic acids	188.17 ± 1.04	189.33 a ± 0.26	192.96 b ± 1.02	191.35 a ± 0.95	196.30 a ± 1.21	202.30 b ± 2.21	190.41 a ± 0.12	200.89 b ± 4.45	205.74 b ± 1.29
Total Flavan-3-ols	127.25 ± 1.49	168.67 a ± 7.51	183.25 b ± 1.72	212.98 a ± 3.09	217.69 b ± 2.81	271.45 c ± 8.41	92.72 a ± 21.33	56.48 b ± 4.65	88.61 a ± 2.57
Total Procyandins	9.56 ± 0.15	9.97 a ± 0.14	10.83 b ± 0.26	12.71 a ± 0.27	11.85 b ± 0.33	14.09 c ± 0.25	8.65 a ± 1.07	7.79 a ± 0.76	9.28 a ± 0.27
Total Flavonols	37.76 ± 0.08	25.06 a ± 0.72	23.77 a ± 0.56	26.44 ab ± 3.12	26.71 a ± 0.75	23.29 b ± 1.41	17.22 a ± 0.66	14.10 b ± 0.36	17.34 a ± 0.25
Monomeric anthocyanins									
Delphinidin-3-glucoside	2.86 ± 0.09	1.91 a ± 0.05	1.74 b ± 0.03	2.48 a ± 0.11	2.12 b ± 0.13	1.95 b ± 0.15	0.98 a ± 0.28	0.50 b ± 0.01	0.77 ab ± 0.04
Petunidin-3-glucoside	6.13 ± 0.18	5.09 a ± 0.05	4.70 b ± 0.07	5.42 a ± 0.32	4.53 b ± 0.43	4.75 b ± 0.10	1.93 a ± 0.72	0.78 b ± 0.03	1.40 ab ± 0.13
Peonidin-3-glucoside	5.40 ± 0.30	4.48 a ± 0.19	3.99 b ± 0.11	4.84 a ± 0.21	3.98 b ± 0.49	4.16 b ± 0.06	2.87 a ± 0.21	1.05 b ± 0.04	1.83 c ± 0.11
Malvidin-3-glucoside	59.41 ± 1.29	53.19 a ± 0.63	49.49 b ± 0.61	54.90 a ± 2.89	48.80 b ± 0.38	48.74 b ± 1.17	21.85 a ± 6.82	6.87 b ± 0.49	15.13 ab ± 0.23
Petunidin-3-acetyl-glucoside	2.70 ± 0.35	2.24 a ± 0.01	2.12 b ± 0.05	2.50 a ± 0.13	2.27 ± 0.07	2.29 a ± 0.04	1.49 a ± 0.47	0.62 b ± 0.01	0.75 b ± 0.17
Peonidin-3-acetyl-glucoside	6.57 ± 1.53	5.07 a ± 0.05	4.72 b ± 0.02	7.35 a ± 0.15	6.81 ab ± 0.52	6.83 b ± 0.20	4.53 a ±0.97	1.93 b ± 0.13	2.33 b ± 0.60
Malvidin-3-acetyl-glucoside	32.44 ± 0.51	32.30 a ± 0.45	30.26 b ± 0.30	35.30 a ± 0.00	28.32 b ± 3.70	30.96 b ± 0.71	13.75 a ± 3.63	5.28 b ± 0.39	9.31 ab ± 1.88
Petunidin-3-*p*-coumaroyl-gluc.	2.39 ± 0.10	2.09 a ± 0.04	1.89 b ± 0.01	2.97 a ± 0.06	2.52 a ± 0.51	2.70 a ± 0.16	1.25 a ± 0.54	0.43 a ± 0.01	0.48 a ± 0.18
Peonidin-3-*p*-coumaroyl-gluc.	6.67 ± 0.21	5.18 a ± 0.09	4.63 b ± 0.01	6.04 a ± 0.60	4.73 b ± 0.55	4.70 b ± 0.22	1.90 a ± 0.09	0.70 b ± 0.06	1.01 c ± 0.07
Malvidin-3-*p*-coumaroyl-gluc.	24.62 ± 0.70	22.38 a ± 0.51	20.45 b ± 0.17	22.37 a ± 2.26	17.59 ab ± 2.25	18.06 b ± 0.93	6.57 a ± 2.20	2.09 b ± 0.23	3.87 ab ± 0.55
% Copigmented Anthocyanins							7.33 a ± 1.64	17.26 b ± 2.80	20.23 b ± 4.28
% Polymerized Anthocyanins							39.85 a ± 2.56	46.39 b ± 0.38	46.16 b ± 2.18

Hydroxycinnamic acids									
p-coumaric acid	27.12 ± 0.05	27.81 a ± 0.08	27.86 a ± 0.05	27.75 a ± 0.05	27.31 a ± 0.08	27.40 a ± 0.36	26.15 a ± 0.02	26.17 a ± 0.02	26.13 a ± 0.01
p-coumaric derivate	30.11 ± 0.01	29.98 a ± 0.09	29.81 a ± 0.05	30.28 a ± 0	30.63 a ± 0.05	30.47 a ± 0.13	29.70 a ± 0.01	30.34 b ± 0.39	30.70 b ± 0.08
Benzoic acids									
Gallic acid	56.62 ± 0.10	58.45 a ± 0.46	61.93 a ± 0.38	60.34 a ± 0.32	64.62 b ± 0.28	70.06 c ± 1.43	61.93 a ± 0.28	67.98 b ± 2.27	71.70 c ± 0.92
Protocatechuic acid	43.90 ± 0.33	44.03 a ± 0.03	43.57 a ± 0.32	44.54 a ± 0.11	44.46 a ±1.03	44.90 a ± 0.64	42.21 a ± 0.01	42.19 a ± 0.02	42.24 a ± 0.01
Vaillinic acid	44.08 ± 0.80	43.89 a ± 0.32	44.54 a ± 0.28	43.94 a ± 0.59	44.53 a ± 0.38	44.90 a ± 0.46	42.22 a ± 0.01	46.00 b ± 1.80	47.34 b ± 0.37
Syringic acid	43.55 ± 0.01	42.95 a ± 0.17	42.91 a ± 0.19	42.53 a ± 0.09	42.68 a ±0.07	42.45 a ± 0.03	44.06 a ± 0.16	44.72 a ± 0.38	44.54 a ± 0.21
Flavan-3-ols									
(+)-catechin	46.58 ± 0.01	59.21 a ± 1.34	76.66 b ± 4.70	89.64 a ± 1.48	95.09 b ± 0.04	125.81 c ± 6.18	34.64 a ± 7.43	23.33 b ± 0.38	36.62 a ± 2.27
(-)-epicatechin	80.72 ± 1.48	109.46 a ± 8.18	104.02 a ± 0.85	123.34 a ± 4.29	109.46 b ± 3.43	144.64 c ± 0.00	58.07 a ± 13.90	33.15 b ± 4.45	51.98 ab ± 0.85
Procyanidins									
Procyanidin B1	5.38 ± 0.11	5.35 a ± 0.10	6.02 b ± 0.26	5.95 a ± 0.09	5.63 b ± 0.04	6.33 c ± 0.04	3.17 a ± 0.74	2.53 a ± 0.63	3.20 a ± 0.04
Procyanidin B2	1.60 ± 0.10	1.83 a ± 0.02	2.01 b ± 0.02	2.92 a ± 0.03	3.00 a ± 0.19	3.51 b ± 0.28	2.50 a ± 0.29	2.66 a ± 0.14	3.26 b ± 0.25
Procyanidin B2 3-O-gallate	1.39 ± 0.09	1.44 a ± 0.05	1.44 a ± 0.04	1.96 a ± 0.15	1.84 a ± 0.09	2.25 b ± 0.09	1.27 a ± 0.01	1.19 a ± 0.10	1.23 a ± 0.06
Procyanidin B7	1.20 ± 0.01	1.36 a ± 0.05	1.37 a ± 0.00	1.88 a ± 0.19	1.94 a ± 0.09	2.00 a ± 0.09	1.71 a ± 0.07	1.41 b ± 0.02	1.59 ab ± 0.10
Flavonols									
Myricetin-3-glucuronide	7.80 ± 0.09	5.40 a ± 0.18	5.14 a ± 0.13	5.21 a ± 0.81	5.29 a ± 0.41	4.74 a ± 0.19	3.63 a ± 0.30	3.09 b ± 0.08	3.55 a ± 0.08
Quercetin-3-glucuronide	9.52 ± 0.18	6.15 a ± 0.20	5.94 a ± 0.14	7.22 a ± 0.50	6.64 b ±0.05	6.08 b ± 0.52	4.34 a ± 0.70	2.89 b ± 0.52	4.15 a ± 0.35
Quercetin-3-glucoside	11.30 ± 0.22	6.63 a ± 0.12	6.42 a ± 0.15	7.21 ab ± 1.14	7.68 a ± 0.24	6.88 b ± 0.34	4.72 a ± 0.37	4.08 b ± 0.09	4.93 a ± 0.11
Laricitrin-3-glucoside	3.03 ± 0.07	2.04 a ± 0.06	1.87 b ± 0.06	1.87 ab ± 0.34	1.88 a ± 0.04	1.57 b ± 0.09	1.26 a ± 0.10	1.03 b ± 0.02	1.15 ab ± 0.02
Kaempferol-3-glucoside	0.23 ± 0.03	0.17 a ± 0.08	0.07 a ± 0.07	0.07 a ± 0.09	0.15 b ± 0.07	0.05 a ± 0.04	n.d	n.d	n.d
Isorhamnetin-3-glucoside	4.71 ± 0.09	2.85 a ± 0.07	2.66 b ± 0.08	3.12 ab ± 0.50	3.34 a ± 0.32	2.67 b ± 0.14	1.95 a ± 0.27	1.51 b ± 0.02	1.88 ab ± 0.11
Syringetin-3-glucoside	1.16 ± 0.03	1.81 a ± 0.07	1.67 b ± 0.06	1.73 ab ± 0.29	1.72 a ± 0.06	1.40 b ± 0.08	1.31 a ± 0.55	1.20 a ± 0.08	1.52 a ± 0.14
Total phenols	1199.26 ± 85.40	1196.58 a ± 54.52	1361.95 b ± 61.88	1646.60 a ± 80.54	1831.44 b ± 26.15	1940.75 b ± 129.55	1763.46 a ± 86.68	1749.14 a ± 266.44	2095.01 a ± 171.60

* CW: wines made by traditional winemaking, without overripe seeds addition; SW: wines made by single seed post-fermentative maceration (12 g/L, 30 days); DW: wines made by double seed post-fermentative maceration (12 g/L, 30 days and a second addition of 12 g/L, 30 days). Different letters in the same row indicate significant differences ($p < 0.05$). n.d.: not detected.

Table 3

CIELAB color characteristics (L*, a*, b*, C^*_{ab}, and h_{ab}; mean ± SD, n=3) at the beginning (0 days), the end of the overripe seeds post-maceration processes (30/60 days), and the end of the stabilization stage (150 days), of CW, SW and DW wines.

Stage		Post-maceration treatment*		
		CW	**SW**	**DW**
		Mean ± SD	Mean ± SD	Mean ± SD
Initial 0 day	L*	73.24 a ± 0.03	72.86 b ± 0.17	72.72 b ± 0.07
	a*	29.87 a ± 0.27	29.65 a ± 0.17	29.19 b ± 0.12
	b*	-3.87 a ± 0.53	-4.62 a ± 0.42	-4.01 a ± 0.32
	C^*_{ab}	30.12 a ± 0.34	29.95 a ± 0.19	29.40 b ± 0.17
	h_{ab}	-7.37 a ± 0.94	-8.03 a ± 0.52	-7.30 a ± 0.74
Maceration 30 days	L*	80.74 a ± 0.37	83.13 b ± 0.20	
	a*	18.36 a ±0.46	15.23 b ± 0.07	
	b*	2.70 a ±0.04	3.67 b ± 0.12	
	C^*_{ab}	18.56 a ± 0.45	15.67 b ± 0.04	
	h_{ab}	8.37 a ± 0.31	13.56 b ± 0.47	
Maceration 60 days	L*	77.14 a ± 0.68		81.28 b ± 0.81
	a*	22.06 a ± 0.92		16.33 b ± 0.54
	b*	1.11 a ± 0.27		2.97 b ± 0.23
	C^*_{ab}	22.09 a ± 0.90		16.60 b ± 0.49
	h_{ab}	2.92 a ± 0.82		10.33 b ± 1.11
Stabilization 150 days	L*	74.55 a ± 1.45	73.69 a ± 0.03	72.31 a ± 0.64
	a*	24.14 a ± 0.71	23.44 a ± 0.26	25.61 b ± 0.59
	b*	2.06 a ± 0.24	1.01 b ± 0.14	1.64 c ± 0.05
	C^*_{ab}	24.23 a ± 0.69	23.46 a ± 0.25	25.66 b ± 0.60
	h_{ab}	5.25 a ± 0.35	2.48 b ± 0.36	3.66 c ± 0.04

* CW: wines made made by traditional winemaking without overripe seeds addition; SW: wines made by single seed post-fermentative maceration (12 g/L, 30 days); DW: wines made by double seed post-fermentative maceration (12 g/L, 30 days and a second addition of 12 g/L, 30 days). Different letters in the same row indicate significant differences ($p < 0.05$).

Table 4

Color difference (ΔE^*_{ab}) and differences on color parameters (ΔL^*, ΔC^*_{ab}, Δh_{ab}) due to the post-maceration process (from overripe seeds addition to overripe seeds removal) and to the global winemaking process (from overripe seeds addition to the end of the stabilization stage).

		Post-maceration treatment*		
		CW	**SW**	**DW**
Stage		Mean ± SD	Mean ± SD	Mean ± SD
Maceration 30 days	ΔE^*_{ab}	15.23 a ± 0 ,12	19.20 b ± 0.53	
	ΔL^*	7.50 a ± 0.40	9.89 b ± 0.19	
	ΔC_{ab}	-11.56 a ± 0.11	-14.45 b ± 0.36	
	Δh_{ab}	6.47 a ± 0.37	7.89 b ± 0.50	
Maceration 60 days	ΔE^*_{ab}	10.07 a ± 0.64		16.51 b ± 0.65
	ΔL^*	3.90 a ± 0.70		7.65 b ± 0.39
	ΔC_{ab}	-8.03 a ± 0.56		-13.11 b ± 0.41
	Δh_{ab}	4.62 a ± 0.17		6.50 b ± 0.36
Global	ΔE^*_{ab}	8.44 a ± 0.32	8.09 a ± 0.46	6.88 b ± 0.16
	ΔL^*	1.31 a ± 1.46	0.45 a ± 0.06	-0.65 a ± 0.46
	ΔC_{ab}	-5.89 a ± 0.34	-6.66 b ± 0.31	-4.59 c ± 0.12
	Δh_{ab}	5.77 a ± 0.22	4.56 b ± 0.41	5.06 a ± 0.28

* CW, wines made made by traditional winemaking without overripe seeds addition; SW, wines made by single seed post-fermentative maceration (12 g/L, 30 days); DW, wines made by double seed post-fermentative maceration (12 g/L, 30 days and after a second addition of 12 g/L, 30 days).Different letters in the same row indicate significant differences ($p < 0.05$).

Figure 1

Click here to download Figure(s): Rivero et al_ Postfermentation addition-Figure1.docx

FIGURE 1