Optimisation of an oak chips-grape mix maceration process. Influence of chip dose and maceration time

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A R T I C L E   I N F O
Article history:
Received 1 December 2015
Received in revised form 12 February 2016
Accepted 13 March 2016
Available online 14 March 2016

Keywords:
Oak chips-grape maceration
American oak chips
Syrah red wine
Warm climate
Colour stability

A B S T R A C T
Oak chips-related phenolics are able to modify the composition of red wine and modulate the colour stability. In this study, the effect of two maceration techniques, traditional and oak chips-grape mix process, on the phenolic composition and colour of Syrah red wines from warm climate was studied. Two doses of oak chips (3 and 6 g/L) at two maceration times (5 and 10 days) during fermentation was considered. Changes on phenolic composition (HPLC–DAD–MS), copigmentation/polymerisation (spectrophotometry), and colour (Tristimulus and Differential Colorimetry) were assessed by multivariate statistical techniques. The addition of oak chips at shorter maceration times enhanced phenolic extraction, colour and its stabilisation in comparison to the traditional maceration. On contrast, increasing chip dose in extended maceration time resulted in wines with lighter and less stable colour. Results open the possibility of optimise alternative technological applications to traditional grape maceration for avoiding the common loss of colour of wines from warm climate.

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1. Introduction

Phenolic compounds are the main chemical substances responsible for the sensory characteristics of wines such as colour, astringency and bitterness (Monagas, Bartolomé, & Gómez-Cordovés, 2005). Among them, colour is one of the most important attribute defining the quality of wines since it is the first characteristic perceived, and therefore, it influences the acceptability by consumers.

In traditional winemaking, anthocyanins and other phenolic compounds are extracted from grapes and diffused into the must and wine along the fermentative maceration process (Busse-Valverde, Gómez-Plaza, López-Roca, Gil-Munoz, & Bautista-Ortíz, 2011). While anthocyanins are the pigment accounting directly for the colour of red wine, colourless phenolics such as benzoic and hydroxycinnamic acids, flavanols or flavonols are involved in the stabilization of anthocyanins through copigmentation and polymerisation reactions so, they plays also a key role in the colour stability over time (Boulton, 2001; Escrivano-Bailón & Santos-Buelga, 2012). Given the importance of phenolic compounds for wine colour, studying and controlling the processing factors that influence their extraction and content during the maceration and fermentation of grapes is one of the main objectives to produce quality wines, especially in terms of full body-structure and stable colour (Sacchi, Bisson, & Adams, 2005). In these regard, different alternative maceration techniques have been developed to enhance the extraction of grape components responsible for the colour, resulting in wines with a different phenolic composition from those produced by traditional methods (Añón et al., 2014; Canals, Llauyd, Canals, & Zamora, 2008; Darias-Martín, Carrillo, Díaz, & Boulton, 2001; González-Neves, Gil, Barreiro, & Favre, 2010; Gordillo et al., 2014; Ivanova et al., 2011; Pérez-Lamela et al., 2007; Soto Vázquez, Rio Segade, & Orriols Fernández, 2010).

In particular, the use of oak chips fragments during winemaking is an approved oenological practice (OIV, 2012) increasingly applied by oenologists worldwide. Oak chips fragments obtained from barrels are a natural source of phenolic compounds like benzoic and cinnamic acids, and ellagitanins (among others) that are able to modify the wine composition and its sensory perception (Tao, García, & Sun, 2014). In most cases, oak chips fragments are applied after the fermentative stage of winemaking to accelerate the aging process artificially and to obtain wines with more complex structure in a short aging period (Del Barrio-Galán, ...
pared with a traditional macerated red wine. American oak (density of 1.100 g/mL, total acidity of 6.7 g/L and a pH of 3.65) harvested in 2014 vintage at optimum technological maturity southwest of Spain (warm climate). About 2700 kg of grapes were grown in “Condado de Huelva” Designation of Origin (DO), in the traditionally macerated red wine.

pared the phenolic composition and colour characteristics with a maceration of young Syrah wine from warm climate, and compared the phenolic composition and colour characteristics with a traditionally macerated red wine.

However, other authors have shown inconsistent effects of oak chips-related compounds among vineyards or even controversial depending on the conditions applied including the chip dose, oak chips origin, toasting degree or maceration time without improving the phenolic potential or sensorial characteristics of wines (González-Sáiz et al., 2014; Soto Vázquez et al., 2010; Zimman, Joslin, Lyon, Meier, & Waterhouse, 2002). On the other hand, studies focused on the optimisation of the oak chips-grape maceration processes by modifying the maceration conditions are still scarce. Thus, the main objective of this work is to evaluate the impact of applying two proportion of oak chips (3 and 6 g/L) at two maceration times (5 and 10 days) during the fermentative maceration of young Syrah wine from warm climate, and compared the phenolic composition and colour characteristics with a traditionally macerated red wine.

2. Material and methods

2.1. Winemaking

Red wines were made from grapes Vitis vinifera var. Syrah grown in “Condado de Huelva” Designation of Origin (DO), in the southwest of Spain (warm climate). About 2700 kg of grapes were harvested in 2014 vintage at optimum technological maturity (density of 1.100 g/mL, total acidity of 6.7 g/L and a pH of 3.65) and in good sanitary conditions.

Grapes were destemmed and crushed and the must was distributed in stainless steel tanks of 220 L. Wines were made under different maceration conditions by applying two proportion of chips (3 and 6 g/L) and two maceration times (5 and 10 days), compared with a traditional macerated red wine. American oak (Quercus alba) low-toasted chips of 1 cm² average size (Tonelería Martín y Vázquez, Logroño, Spain) were used. All maceration treatments were made in triplicate as follows:

2.1.1. Traditional maceration

3 tanks were submitted to traditional grape maceration (without oak chips) for 5 maceration days (C5 wines); and 3 tanks for 10 maceration days (C10 wines). Both C5 and C10 wines were considered as control (C) wines.

2.1.2. Oak chips-grape maceration at 3 g/L of oak chips

3 tanks were submitted to the addition of 3 g/L of oak chips to the fermentation mash for 5 maceration days (S5 wines); and 3 tanks were submitted to the addition of 3 g/L of oak chips to the fermentation mash for 10 maceration days (S10 wines). Both S5 and S10 were considered as wines made with simple amount of oak chips (S, 3 g/L) into the fermentation mash.

2.1.3. Oak chips-grape maceration at 6 g/L of oak chips

3 tanks were submitted to the addition of 6 g/L of oak chips to the fermentation mash for 5 maceration days (D5 wines); and 3 tanks were submitted to the addition of 6 g/L of oak chips to the fermentation mash for 10 maceration days (D10 wines). Both D5 and D10 were considered as wines made with double amount of oak chips (D, 6 g/L) into the fermentation mash.

An identical red winemaking procedure was used for all assays. Oenological treatments were adjusted at the same levels for all of the assays: 60 mg/L total sulphur dioxide and 7 g/L of total titratable acidity by adding tartaric acid. For all wines, alcoholic fermentation was spontaneously developed. Fermentation caps were punched down once a day during the maceration period. After this, the mash was drawn off to remove the skins and other solid parts, and the free run musts were left to finish the fermentation under the same conditions. Subsequently, the malolactic fermentation was induced by inoculation of Oenococcus oeni lactic acid bacteria (>10¹⁰ CFU O. oeni/mL, VINIFERM Oe 104, Agrovin, Spain) at the rate of 14 mL/L at the end of alcoholic fermentation. When fermentative processes were finished, the wines were racked in 50 L stainless steel tanks and stored at 10–15 °C for a stabilisation period of 6 months.

Must and wine samples (100 mL) were taken at the initial point or grape crushing (1 day), at the middle of the fermentative alcoholic maceration (3 days), just after the skin removal (5 and 10 days), and 3 and 6 months during stabilisation period. A total of 108 samples were analysed in triplicate.

2.2. HPLC–DAD–ESI/MS analysis of phenolic compounds

HPLC separation, identification and quantification of anthocyanin and flavonol is performed in an Agilent 1200 chromatographic system equipped with a quaternary pump, an UV–vis diode-array detector, an automatic injector, and ChemStation software (Palo Alto, CA, USA). Prior direct injection, the samples were filtered through a 0.45 lm Nylon filter (E0034, Análisis Vínicos, Spain). All analyses were performed in triplicate. The anthocyanin and flavonol identification was carried out following the method proposed by Gordillo, Cejudo-Bastante, Rodríguez-Pulido, Lourdes González-Miret, and Heredia (2013). Phenolic compounds were separated using a Zorbax C18 column (250 μm 4.6 mm, 5 μm particle size) maintained at 38 °C. Acetonitrile–formic acid–water (3:10:87) as solvent A and acetonitrile–formic acid–water (50:10:40) as solvent B were used. The elution profile was as follows: 0–10 min 94%A; 10–15 min 70%A; 15–25 min 60%A; 25–35 min 55%A; 35–40 min 50%A; 40–42 min 40%A; 42–43 min 94%A. The flow rate was 0.8 mL/min and the injection volume was 50 μL. UV–vis spectra were recorded from 200 to 800 nm with a bandwidth of 2.0 nm. The quantification was made at 525 and 360 nm (anthocyanin and flavonols, respectively) using the calibration curves obtained in the same chromatographic conditions for malvidin 3-glucoside and queretin standards. The concentration of phenolic compounds was expressed as mg/L.

For flavan-3-ol and phenolic acid analysis, samples were fractionated prior to chromatographic analysis previously described by González-Manzano, Santos-Buelga, Pérez-Alonso, Rivas-Gonzalo, and Escrivano-Bailón (2006). Briefly, Oasis® MCH (Waters Corporation Milford, MA, USA) cartridges were used for the separation of flavan-3-ols and phenolic acids. 1 mL of each wine was diluted (1:1) with 0.1 M HCl and eluted through previously conditioned cartridges. Anthocyanins and flavonols were retained in the eluates while flavan-3-ols and phenolic acids were eluted with 8 mL of methanol. A small volume of water was added to the eluates and concentrated under vacuum at lower than 30 °C until complete elimination of methanol. The volume of the aqueous residue was adjusted to 0.5 mL with ultrapure water, filtered (0.45 μm) and the concentration of phenolic compounds was expressed as mg/L.

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analysed by HPLC–DAD–MS as previously described. The above-mentioned HPLC system was coupled to a hybrid triple quadrupole/linear ion trap (QqLIT) mass spectrometer API 3200 QTrap (Applied Biosystems, Foster City, CA, USA) equipped with a Turbo V ionisation source and controlled by Analyst software (version 1.5; Applied Biosystems) via the DAD cell outlet. Phenolic acids and flavan-3-ol chromatographic separation was performed on a reversed-phase column Spherisorb ODS-2 (150 × 4.6 mm, 3 μm) from Waters (Milford, MA, USA) maintained at 25 °C. 0.25% acetic acid in water (A) and acetonitrile (B) were used as the mobile phases. The following linear gradient was used to achieve the chromatographic separation: from 0% to 10% B in 5 min, from 10% to 14.5% B in 35 min, from 14.5% to 19% B in 5 min, from 19% to 25% B in 5 min, hold at 55% B for 5 min, from 55% to 80% B in 5 min, hold at 80% B for 3 min, from 80% to 0% B in 2 min and hold at 0% B for 5 min. The flow rate was set at 0.5 mL min⁻¹ and the injection volume was 100 μL. UV–vis spectra were recorded from 200 to 600 nm, while acquiring at the selected wavelengths of 280 nm for flavan-3-ols quantification and 330 nm for phenolic acids.

The mass spectrometer was operated in the negative electrospray ionisation (ESI) mode under the following specific conditions: IS: –4500 V; source temperature (TEM), 400 °C; CUR: 20 ps; GS1: 40 ps; GS2: 30 ps; DP: –40 V; EP: –7 V; and CE: –20 eV. Nitrogen (>99.98%) was employed as curtain, ion source and collision gas. The detection was accomplished in the enhanced MS (EMS) full-scan mode, from m/z 100 to 1700, and in the enhanced product ion (EPI) mode, to obtain the corresponding full-scan MS/MS spectra.

Phenolic compounds were identified by comparison of their retention time, UV–vis spectra and mass spectra features with data reported in the literature and the previously recorded in our laboratory. The phenolic acids quantification was made at 330 nm using external calibration curves of purchased standards, using gallic acid for gallic acid quantification and p-coumaric acid for the rest of identified phenolic acids. Flavan-3-ols were quantified using external calibration curves recorded at 280 nm of its corresponding purchased standard. Phenolic compounds concentration was expressed as mg/L.

### 2.3. Colorimetric analysis

The absorption spectra (380–770 nm) of wine samples were recorded at constant intervals (Δλ = 2 nm) with a Hewlett-Packard UV–vis HP8453 spectrophotometer (Palo Alto, CA), using 2 mm path length glass cells and distilled water as a reference. The CIELAB parameters were calculated by Tristimulus Colorimetry from the absorption spectra by using the original software CromaLab© (Heredia, Álvarez, González-Miret, & Ramírez, 2004) and following the recommendations of the Commission International de L’Eclairage: the CIE 1964 10° Standard Observer and the Standard Illuminant D65. CIELAB parameters were calculated: L* (the correlate of lightness; ranging from 0, black, to 100, white), and two colour coordinates, a* (which takes positive values for reddish colours and negative values for greenish ones) and b* (positive for yellowish colours and negative for the bluish ones) from these coordinates, other colour parameters are defined: the hue angle (h), the correlate of chromaticity or tone, and the chroma (C), the correlate of saturation or intensity of colour. L*, C, and h can be distinguished as quantitative or qualitative parameters as they indicate quantitative (L* and C), or qualitative (h) attributes of colour.

The colour changes of wines during maceration and their colour stability over the storage period were evaluated by Differential Colorimetry according to the methodology described in Gordillo et al. (2015), which is based on the application of various different colour-difference formulas in the CIELAB space. The colour difference between pairs of samples was computed as the Euclidean distance between two points in the three-dimensional CIELAB space by means of the CIE76 colour difference formulae: \[ \Delta E^{ab} = \sqrt{\Delta L^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \]. Moreover, the trend of the changes in each individual colour attribute between pairs of samples was evaluated by means of the absolute lightness, chroma, and hue differences (ΔL*, ΔC*, Δh*). Specifically, Δh* is the difference between two hues, in sexagesimal degrees.

### 2.4. Copigmented and polymerised anthocyanin determination

The contribution of copigmented anthocyanins to the total wine colour at pH 3.6 (% Copigmentation) and the degree of anthocyanin polymerisation (% Polymerisation) were determined following the method proposed by Boulté (1996). The pH values of the wine sample were previously adjusted to pH 3.6 using 1 M NaOH or HCl.

Total wine colour is assumed to be \( A^{\text{ace}} \), the measure of absorbance at 520 nm after the elimination of \( SO_2 \), effect by means of the addition of 20 μl of 10% acetaldehyde to 2 ml of wine sample, and kept for 45 min. The colour due to polymeric pigments is \( A^{\text{pol}} \), the absorbance measured at 520 nm after the addition of 160 μl 5% \( SO_2 \) solution to 2 ml of wine sample. The wine colour without the copigmented anthocyanins effect is \( A^{\text{co}} \), the absorbance measured at 520 nm of the wine sample diluted 1:20 with a buffer solution (24 ml pure ethanol is added to 176 ml distilled water, dissolve 0.5 g of potassium bitartrate into the solution. The solution pH is adjusted to 3.6 with HCl or NaOH as needed). The reading is corrected for the dilution by multiplying by 20. That dilution leads to the dissociation of the copigment complex while the contributions of the free anthocyanins and the polymeric pigments remain. All absorbance readings are converted to 10 mm pathlength. The following data were calculated:

\[
\% \text{ Copigmentation} = \left( \frac{A^{\text{ace}} - A^{\text{co}}}{A^{\text{ace}}} \right) \times 100
\]

\[
\% \text{ Polymerisation} = \left( \frac{A^{\text{pol}}}{A^{\text{ace}}} \right) \times 100
\]

### 2.5. Statistical analysis

Statistical analysis was carried out by using Statistica version 8.0 software (Statistica, 2007). In order to study significant differences between the different types of wines in terms of phenolic composition and colour characteristics, a multifactorial analysis of variance was carried out using the general linear model procedure (GLM). Tukey test was used to evaluate the significance of the analysis.

### 3. Results and discussion

#### 3.1. Changes in phenolic composition

The extraction of phenolic compounds under different conditions of maceration time (5 and 10 days) and chip dose (0, 3 and 6 g/L) was studied to establish which of these factors have a greater impact in the quality of Syrah wines during winemaking when an oak chips-grape mix maceration process is applied respect to traditional maceration. In the qualitative analysis of phenolic composition, 27 compounds belonging to diverse phenolic families were identified by HPLC-MS: 10 anthocyanins, 6 phenolic acids, 4 flavan-3-ols, and 6 flavonols. Table 1 shows the mean concentration (mg/L ± SD, n = 3) of compounds identified in the wine samples at the end of the fermentative maceration period (skin
<table>
<thead>
<tr>
<th>Phenolic compounds</th>
<th>C5</th>
<th>S5</th>
<th>D5</th>
<th>C10</th>
<th>S10</th>
<th>D10</th>
<th>Effect</th>
<th>Maceration time</th>
<th>Chips dose</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of anthocyanins</td>
<td>1429.5 ± 3.6a</td>
<td>1636.0 ± 0.3b</td>
<td>1500.2 ± 27.7ab</td>
<td>1239.3 ± 42.6c</td>
<td>1170.9 ± 37.4cd</td>
<td>1081.6 ± 89.4def</td>
<td>***</td>
<td>*</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Sum of glucosides</td>
<td>952.8 ± 3.2a</td>
<td>1094.3 ± 0.9b</td>
<td>1013.2 ± 13.7ab</td>
<td>828.0 ± 28.8c</td>
<td>775.6 ± 19.9cd</td>
<td>710.0 ± 54.8def</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Sum of acetates</td>
<td>325.5 ± 4.2a</td>
<td>365.4 ± 0.3b</td>
<td>311.2 ± 5.9a</td>
<td>279.1 ± 6.8c</td>
<td>270.5 ± 8.8c</td>
<td>254.2 ± 17.8c</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Sum of coumaroylated</td>
<td>151.2 ± 0.2ab</td>
<td>176.3 ± 0.6a</td>
<td>155.7 ± 7.7ab</td>
<td>132.2 ± 7.2bc</td>
<td>124.8 ± 9.1c</td>
<td>117.4 ± 17.6c</td>
<td>***</td>
<td>ns</td>
<td>ns</td>
<td>**</td>
</tr>
<tr>
<td>Sum of phenolic acids</td>
<td>181.1 ± 2.0a</td>
<td>181.2 ± 3.8a</td>
<td>184.8 ± 5.2a</td>
<td>107.3 ± 9.2b</td>
<td>123.2 ± 0.3b</td>
<td>112.7 ± 0.8b</td>
<td>***</td>
<td>ns</td>
<td>ns</td>
<td>**</td>
</tr>
<tr>
<td>Sum of flavan-3-ols</td>
<td>1277.2 ± 2.3a</td>
<td>1487.1 ± 1.4b</td>
<td>135.5 ± 5.3ab</td>
<td>148.8 ± 0.5ab</td>
<td>184.9 ± 4.8c</td>
<td>169.9 ± 0.1c</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Sum of benzoic acids</td>
<td>73.2 ± 0.2a</td>
<td>73.4 ± 0.5a</td>
<td>78.2 ± 0.1b</td>
<td>33.0 ± 0.5c</td>
<td>48.1 ± 0.3e</td>
<td>44.1 ± 0.1e</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Sum of hydroxycinamic acids</td>
<td>107.9 ± 2.1a</td>
<td>107.8 ± 4.3a</td>
<td>106.6 ± 5.1a</td>
<td>74.3 ± 9.2b</td>
<td>75.2 ± 0.8b</td>
<td>68.1 ± 0.9b</td>
<td>***</td>
<td>ns</td>
<td>ns</td>
<td>**</td>
</tr>
<tr>
<td>Sum of flavonols</td>
<td>34.5 ± 2.1a</td>
<td>34.8 ± 3.6a</td>
<td>33.1 ± 1.4a</td>
<td>36.1 ± 7.8a</td>
<td>30.3 ± 2.2a</td>
<td>29.1 ± 5.9a</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

**Anthocyanins**

- **Delphinidin 3-glucoside**: 55.9 ± 1.8a
- **Petunidin 3-glucoside**: 99.9 ± 1.9a
- **Peonidin 3-glucoside**: 78.6 ± 1.9a
- **Malvidin 3-glucoside**: 718.4 ± 11.4a
- **Petunidin 3-acetyl-glucoside**: 24.9 ± 1.1a
- **Peonidin 3-acetyl-glucoside**: 41.1 ± 1.1a
- **Malvidin 3-acetyl-glucoside**: 259.6 ± 2.1a
- **Petunidin 3-p-coumaroil-glucoside**: 16.7 ± 0.3a
- **Peonidin 3-p-coumaroil-glucoside**: 41.1 ± 0.8a
- **Malvidin 3-p-coumaroil-glucoside**: 93.3 ± 0.3a

**Benzoic acids**

- **Gallic acid**: 73.2 ± 0.1a
- **Elagic acid**: 0.7 ± 0.2a
- **Hydroxycinamic acids**
  - **t-Caffeic proto-tocatechuic acid**: 54.7 ± 0.1a
  - **c-Coumaric acid**: 3.5 ± 2.1a
  - **t-Coumaric acid**: 32.3 ± 0.1a
  - **Ferulic acid**: 8.2 ± 0.3a
  - **Coumaric hexose acid**: 4.7 ± 5.5a
  - **Caffeic acid**: 4.4 ± 2.1a

**Flavan-3-ols**

- **Gallocatechin(GC)**: 101.5 ± 0.7a
- **Procyanidin B3**: 11.1 ± 0.3a
- **(+)-Catechin**: 9.8 ± 0.6ab
- **(−)-Epicatechin**: 5.2 ± 0.4a

**Flavonols**

- **Myricetin 3-glucuronide**: 8.9 ± 0.6a
- **Quercetin 3-glucuronide**: 6.7 ± 0.6a
- **Quercetin 3-glucuronide**: 12.1 ± 0.4a
- **Kaempferol 3-glucoside**: 0.5 ± 0.1a
- **Isoflavonin 3-glucoside**: 3.9 ± 0.4a
- **Syringetin 3-glucoside**: 2.3 ± 0.2a

**Colour data**

- **L**: 58.9 ± 0.3a
- **a**: 50.1 ± 0.2a
- **b**: 53.8 ± 1.1a
- **C<sub>b</sub>**: 50.2 ± 0.3a
- **h<sub>ab</sub>**: 60.0 ± 0.1a

*tr: traces; different letters in the same row mean significant differences (ns: no significant; ***<p value <0.01; **<p value <0.05; *<p value <0.001).
flavan-3-ols were slightly decreased while the levels of benzoic
the chip dose was increased, the total levels of anthocyanins and

differently affected the content of each phenolic family. When

tional macerated wines (C5). However, the proportion of chips

removal, grouped according to their maceration time and chip
dose applied.

The data were subjected to a multifactor analysis of variance by
using the general linear model procedure (GLM, Tukey Test) for
testing the significance of the effects of the factors on the phenolic
composition. In addition, new dependent variables were calculated
as the sum of individual phenolic compounds identified for each
phenolic family (anthocyanins and their derivatives; phenolic, ben-
zoic and hydroxycinnamic acids; flavan-3-ols and flavonols). They
were included in the multifactorial analysis to know whether a
combination of phenolics grouped into phenolic families varies as
a function of the assayed factors.

The results indicated that the maceration time and the propor-
tion of chips applied during the alcoholic fermentative maceration
had a significant influence (p-values of 0.018 and 0.02, re-
spectively) on the extraction of some phenolic families. The interaction
effects between the factors were significant for the sum of antho-
cyanins and flavan-3-ols content, which are abundant phenolic
compounds in red grapes and oak chips, respectively
(Waterhouse, 2002). Although there was no interaction effect on
the total content of phenolic acids, it was significant for the sum of
benzoic acids, which are also grape/oak chips-related phenolic
compounds (Cabrita, Barrocas Dias, & Costa Freitas, 2011;
Waterhouse, 2002). These significant interactions indicate possible
synergistic or antagonistic effects of the studied factors both in pig-
ments and copigments of wines.

In particular, the interaction effects showed the stronger influ-
ence (higher level of significance, p < 0.001) of the maceration time
on most of the individual anthocyanins and phenolic acids at skin
removal, while the chip dose stronger influenced the benzoic acids
and flavan-3-ol extraction.

It can be observed that shorter maceration times led to wines
(C5, S5, and D5) with higher contents of anthocyanins (mainly glu-
cosides and acetates; p < 0.001) and phenolic acids (mainly gallic,
t-caftaric-protocatechuic, and t-coumaric acids) than longer macera-
tion time (C10, S10, and D10), which were in contrast richer in
flava-3-ols (mainly (+)-catechin and epicatechin). The decrease of
the anthocyanin content in extended macerations is in agreement
with the results reported by other authors (Cheynier et al., 2006;
González-Neves et al., 2010; González-Sáez et al., 2014; Ivanova
et al., 2011; Sacchi et al., 2005). This effect could be explained by
the different reactions involving anthocyanins and other phenolic
compounds easily diffused from the skin and pulp into the must in
the first days of maceration. These competing processes such as
oxidation, hydrolysis, condensation or polymerisation make
them to be degraded or transformed progressively into new poly-
meric compounds (González-Neves et al., 2010). At the same time,
oak chips fragments and solid parts of grapes might adsorb these
compounds provoking a slowly decrease in their concentration
from the earlier stages of vinification, especially when maceration
is extended over time (Del Barrio-Galán et al., 2015; Gordillo et al.,
2014). On contrast, longer maceration time (10 days) positively
affected the extraction of some flavan-3-ols whose diffusion from
skins and seeds into the must is favoured with higher alcohol con-
tent, sulphur dioxide, temperature, and contact time (Canals et al.,
2008; Jensen, Blachez, Egebo, & Meyer, 2007; Quijada-Morín,

Regarding the chips treatment, it seemed that the effect on the
extraction of phenolic compounds varied according to the macera-
tion time applied. In shorter maceration times (5 days), wines
made in contact with oak chips (S5 and D5) had higher total con-

teins of anthocyanins, benzoic acids and flavan-3-ols than trad-

with oak chips during fermentation (Gordillo et al., 2014; Zimman
et al., 2002). Thus, lower chip doses combined with higher maceration times
led to wines (S10) with similar pigment content that traditional
macerated wines but significantly richer in some copigments such as
GC, procyanidin B3, (-)-epicatechin, gallic and ellagic acids.

On contrast, the increase of chip dose in the fermentation mash
at longer maceration time produced a slightly decrease in some
individual phenolic compounds that negatively influenced the
total content of the phenolic families in D10 respect to S10 wines.

Finally, the maceration time and the chip dose applied did not
influence the extraction of the individual flavonol compounds
and so their total content in wines.

Figs. 1 and 2 shows the evolution of the total anthocyanin con-


tent (mg/L ± SD, n = 3) in 5 and 10 days macerated wines (respec-
tively), and the percentage of copigmentation and polymerisation,
during 6 months of storage. After pressing, a marked decrease of anthocyanins was observed in all wines but
the pigment stability was influenced by the interaction of the mac-
eration time and chip dose. It can be observed that the lowest pig-
ment loss corresponded to S5 wines (30%), that is, when the lower
maceration wine was combined with the lower chip dose (Fig. 1A).
On the contrary, the highest decreases in total anthocyanins corre-
sponded to T10 and D10 wines (45% and 40% of total pigment loss,
respectively), that is, when the higher maceration time and chips
dose was applied (Fig. 2A).

With regard to the contribution of different group of pigments
to the total colour (copigmented and polymeric pigments), the effect varied with the maceration time. In shorter maceration time (5 days), wines made in contact with oak chips (S5 and D5) reached
higher levels of percentage of copigmentation and polymerisation
than traditional macerated wines (C5), which confirm the positive
effect of an oak chips-grape mix maceration in the phenolic struc-
ture of wines (Fig. 1B and C). This fact is in accordance with the
higher extraction of specific colourless oak chips-related com-
pounds that can act as good copigments (Table 1), as previously
reported by other authors with similar maceration time and chip
dose applied (Gordillo et al., 2014; Zimman et al., 2002). In longer
macerated time (10 days), wines made in contact with oak chips
(S10 and D10) reached again higher degree of copigmentation than
traditional macerated wines, C10 (Fig. 2B). However, during the
stabilisation period (from skin removal to the end of the storage),
wines made with higher doses of chips (D10) showed the signifi-
cant (p < 0.05) lowest degree of polymerisation and therefore, the
lower pigment stability (Fig. 2C). This finding could be related with
the higher adsorption of pigment and copigments extracted during
the maceration stage respect to C10 and S10 wines.

3.2. Changes in wine colour

The changes in the colour parameters (L*, Cab, and hab,
mean ± SD, n = 3) during winemaking in 5 and 10 days macerated
wines, grouped by the chip dose, are shown in Figs. 3 and 4, re-
respectively; as well as in Table 1. As can be observed, wine colour and its
stability were noticeably influenced by the maceration treatment
used.
Fig. 1. Evolution of the (A) total anthocyanins, (B) percentage of copigmentation, and (C) percentage of polymerisation, in 5 days macerated wines during winemaking (C5: 0 g/L, S5: 3 g/L, D5: 6 g/L of oak chips).
Fig. 2. Evolution of the (A) total anthocyanins (mg/L ± SD, n = 3), (B) percentage of copigmentation, and (C) percentage of polymerisation, in 10 days macerated wines during winemaking (C10: 0 g/L, S10: 3 g/L, D10: 6 g/L of oak chips).
Fig. 3. Changes in the colour parameters (mean ± SD, n = 3) in 5 days macerated wines during winemaking: (A) $L^*$, lightness; (B) $C^*_{ab}$, chroma; (C) $h_{ab}$, hue angle (C5: 0 g/L, S5: 3 g/L, D5: 6 g/L of oak chips).
Fig. 4. Changes in the colour parameters (means ± SD, n = 3) in 10 days macerated wines during winemaking: (A) $L^*$, lightness; (B) $C_{ab}^*$, chroma; (C) $h_{ab}$, hue angle (C10: 0 g/L, S10: 3 g/L, D10: 6 g/L of oak chips).
Table 2
Colour, lightness, and hue differences (ΔEab, ΔL*, Cab, Δhab) calculated for each wine from the beginning and the end of the maceration and stabilisation period, according to the maceration time (5 and 10 days) and chips dose (Control: 0 g/L; Simple: 3 g/L; Double: 6 g/L of oak chips).

<table>
<thead>
<tr>
<th></th>
<th>C5</th>
<th>S5</th>
<th>D5</th>
<th>C10</th>
<th>S10</th>
<th>D10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maceration period</td>
<td>ΔEab</td>
<td>62.7 ± 0.1a</td>
<td>65.0 ± 1.5a</td>
<td>63.1 ± 0.9a</td>
<td>57.3 ± 0.4b</td>
<td>55.8 ± 0.4bc</td>
</tr>
<tr>
<td></td>
<td>ΔL*</td>
<td>±39.7 ± 0.3a</td>
<td>±38.9 ± 1.8a</td>
<td>±39.4 ± 0.2a</td>
<td>±37.9 ± 0.3ab</td>
<td>±37.2 ± 0.2ab</td>
</tr>
<tr>
<td></td>
<td>Cab</td>
<td>±48.4 ± 0.3a</td>
<td>±52.0 ± 0.1b</td>
<td>±40.2 ± 1.4a</td>
<td>±42.7 ± 0.3c</td>
<td>±41.6 ± 0.3c</td>
</tr>
<tr>
<td></td>
<td>Δhhab</td>
<td>±15.2 ± 0.7ab</td>
<td>±15.4 ± 0.3a</td>
<td>±15.0 ± 1.1ab</td>
<td>±13.8 ± 0.7ab</td>
<td>±13.5 ± 0.6b</td>
</tr>
<tr>
<td>Stabilisation period</td>
<td>ΔEab</td>
<td>33.8 ± 1.2a</td>
<td>23.1 ± 1.2b</td>
<td>29.4 ± 1.1ad</td>
<td>20.2 ± 1.2b</td>
<td>24.0 ± 1.9bc</td>
</tr>
<tr>
<td></td>
<td>ΔL*</td>
<td>±19.2 ± 0.8ad</td>
<td>±9.1 ± 2.2b</td>
<td>±15.2 ± 0.3ac</td>
<td>±11.6 ± 1.1bc</td>
<td>±14.4 ± 1.2cd</td>
</tr>
<tr>
<td></td>
<td>Cab</td>
<td>±27.1 ± 1.1a</td>
<td>±20.8 ± 0.6db</td>
<td>±24.6 ± 1.4ab</td>
<td>±16.3 ± 0.8C</td>
<td>±19.0 ± 1.5cd</td>
</tr>
<tr>
<td></td>
<td>Δhhab</td>
<td>±9.6 ± 0.5a</td>
<td>±6.2 ± 0.3b</td>
<td>±8.7 ± 0.4a</td>
<td>±4.3 ± 0.2c</td>
<td>±5.0 ± 0.5bc</td>
</tr>
</tbody>
</table>

Different letters in the same row mean significant differences (ns: no significant; *: p < 0.05; **: p < 0.01; ***: p < 0.001).

At skin removal, the colour extraction was different for each maceration treatment but was coherent with the pigment extraction. As can be seen in Table 1, the interaction effects between the factors were significant for all the colorimetric parameters, except to the lightness (L*). Results showed the stronger influence (p < 0.001) of the maceration time in both on quantitative (L*, a*, b*) and qualitative (b’, h*) parameters, while the chip dose only influenced the quantitative ones (a’, b’). As expected, the higher pigment extraction during fermentative maceration in shorter maceration wines, the significant higher values of chroma (C*) and lower of hue (h*), respect to longer maceration wines. Also, shorter macerated wines showed slightly lower values of lightness (L*), but the differences were not significant for all wines. These results imply darker and more saturated bluish colour at skin removal respect to the extended maceration time (Ivanova et al., 2011).

On the other hand, the combination of increasing chip dose with shorter maceration time led to wines (S5 and D5) with higher values of C* and lower of hue respect to traditional macerated wines (C5). However, these colorimetric differences were only significant for the quantitative colour attribute chroma (C*) between C5 and S5 wines.

On contrast, the opposite effect was observed when longer maceration times were combined with chip dose, since lower values of chroma and higher of lightness were observed in S10 and D10 wines respect to traditional macerated wines (C10). These results were in accordance with the lower pigment extraction of wines macerated in contact with oak chips, especially those made with higher chip dose (D10).

The evolution of colour parameters over time was in agreement with the behaviour of the anthocyanin content of wines, as well as with the contribution of the different group of pigments to the total colour (copigmented and polymeric pigments). For shorter maceration times (5 days), S5 wines with significant higher anthocyanin content, proportion of copigments and copigmentation degree showed a more vivid bluish colour (higher chroma values and lower of hue) than traditional macerated wines or with 6 g/L chips (Fig. 3B and C). At longer maceration time, wines submitted to an oak chips-grape maceration process (S10 and D10 wines) showed lighter and less intense colour than traditional macerated wines, C10 (Fig. 4A and B). These differences were more marked when higher proportion of oak chips were applied (D10 wines). A similar behaviour was observed by Cordillo et al. (2014), when comparing the impact of adding white pomace to red grapes on the phenolic composition and colour stability of Syrah wines from a warm climate. In fact, higher pigment loss and lower degree of polymerisation (Fig. 2A and C) resulted in a net loss in colour in final wines.

Differential Tristimulus Colorimetry was applied to objectively assess the colour extraction and colour stability of each wine during vinification, and compare them according to the maceration time and the chip doses applied. For this purpose, colour, lightness, chroma and hue differences (ΔEab, ΔL*, Cab, Δhhab) were evaluated during the extraction stage (from the grape crushing to the skin removal) and from the skin removal to the end of stabilisation period (6 months). Results obtained are shown in Table 2.

During the extraction period, wines submitted to shorter maceration times showed higher ΔEab values than longer macerated wines, indicating higher colour variation. The negative values obtained for ΔL* and Δhhab but positive for C* are indicative of an increase of the quantity of colour of wines and to a displacement towards blue hue of wines, which is characteristic of the pigment extraction from grapes to wine. As observed, S5 wine presented the highest values of ΔEab (65.0) and Δhhab (+52 CIELAB u.) but the lowest of Δhhab (−15.4°). This observation means higher colour extraction, colour intensity and bluish tonality; which is in accordance with the colour parameters obtained at skin removal (Table 1), as well as the higher phenolic extraction (pigments and copigments) and degree of copigmentation in S5 wines.

During the stabilization period, according to the sign of ΔL*, ΔCab, and Δhhab, higher ΔEab values are indicative of lower colour stability. In this sense, the combination of shorter maceration times with chip doses increased the colour stability of wines respect of those made with traditional grape maceration (lower ΔEab in S5 and D5 than C5 wines). This positive effect is supported by the higher degree of polymerisation reached at the end of the storage period in wines macerated with oak chips (% Polymerisation = 54% and 57.8% in S5 and D5 versus 50% in C5 wines). However, the opposite effect was observed when longer maceration time was applied.

The combination of increasing chip dose in extended maceration time resulted in higher ΔEab values; and therefore, lower colour stability respect to traditional macerated wines. This behaviour was consistent with the lower degree of polymerisation in S10 and D10 wines at the end of the storage period (% Polymerisation = 51% and 46% in S10 and D10 versus 53% in C10 wines; Fig. 2C). These results are in agreement with those reported by Soto Vázquez et al. (2010) and González-Sáiz et al. (2014) for other varieties; which showed that the application of alternative maceration techniques do not always favour the reactions involved in anthocyanin stabilization in comparison to traditional winemaking due to the significant and complex interferences between process variables.

4. Conclusions

The application of an oak chips-grape mix maceration process during fermentation at the rates studied (3 and 6 g/L) increased the content of some colourless phenolics (flavanols and phenolic acids) that contribute to copigmentation reactions respect to the
traditional grape maceration. Nevertheless, the effect on the pigment extraction depended on the maceration time used, which has remarkably consequences on the quality and stability of the wine colour. The application of oak chips at shorter maceration time (5 days) improved the anthocyanin extraction in young wines and their colour characteristics during stabilisation than traditional macerated wines, especially at lower chips proportion (3 g/L). At longer maceration time (10 days), the addition of chips did not improve the extraction of pigments and colour probably due to a higher adsorption of compounds by the fermentation mash, resulting in lower colour stability. Therefore, the present study could be of great interest for the wine industry since results demonstrated in a comprehensive manner the advantages or disadvantages of applying a oak-chips-grape mix maceration to modulate the phenolic composition and colour characteristics of red wines, especially in warm climate regions. It is also important to remark that the evaluation of oak-related compounds as ellagitannins should be considered in further work since might be a factor about the presence of phenolic compounds in wine.

Acknowledgments

We thank Ministerio de Economía y Competitividad (Project AGL2014-58486-C2) for financial support and the staff of Biology Service (SGI, Universidad de Sevilla) for the technical assistance. Berta Baca-Bocanegra holds a predoctoral research grant from the Universidad de Sevilla (V Plan Propio).

References


