Application of the differential colorimetry and polyphenolic profile to the evaluation of the chromatic quality of Tempranillo red wines elaborated in warm climate. Influence of the presence of oak wood chips during fermentation

Belén Gordillo, María Jesús Cejudo-Bastante, Francisco J. Rodríguez-Pulido, M. Lourdes González-Miret, Francisco J. Heredia*

Food Colour and Quality Lab., Dept. Nutrition and Food Science, Facultad de Farmacia, Universidad de Sevilla, 41012 Sevilla, Spain

Belén Gordillo: bgordillo@us.es

María Jesús Cejudo-Bastante: mjcejudo@us.es

Francisco J. Rodríguez-Pulido: rpulido@us.es

M. Lourdes González-Miret: miret@us.es

Francisco J. Heredia: heredia@us.es

**** Corresponding author:***

Francisco J. Heredia

Food Colour & Quality Lab., Dept. Nutrition & Food Science. Facultad de Farmacia. Universidad de Sevilla. 41012-Sevilla, Spain

Tel.: +34 954556495 Fax: +34 954556110

e-mail: heredia@us.es

ABSTRACT

The effect of adding American oak wood chips during fermentation on Tempranillo red wines elaborates in a warm climate has been studied. Our attention was focused on the tristimulus colorimetry, differential colorimetry and phenolic compounds related to wine colour. This technique was applied as an oenological alternative to the conventional winemaking for avoiding the common fall of colour of red wines elaborated in warm climates. The addition of oak wood chips promoted the colour enhancement and stabilisation, producing wines with a notably darker colour and with more bluish tonality. This fact was also related to the significantly higher content of some phenolic compounds. On the basis of the results, it could be affirmed that the addition of oak wood chips during fermentation induced visually perceptible colour changes (by the analysis of ΔE^*_{ab}, $\%\Delta^2 L$, $\%\Delta^2 C$ and $\%\Delta^2 H$), mainly in a quantitative way, and also a lower percentage of diminution of colour.

KEYWORDS: Differential colorimetry; polyphenolic compounds; American oak wood chips; Tempranillo red wine; warm climate.

1. INTRODUCTION

Phenolic compounds are the main chemical substances responsible for the quality of the red wines, with regard to the colour, astringency and bitterness. Anthocyanins, flavonols, flavan-3-ols and polymeric compounds play an important role on these sensory characteristics (Robichaud & Noble, 1990; Sarni-Manchado, Cheynier, & Moutounet, 1999). The anthocyanins extracted from the solid parts of the grape (mainly skin) provide the red wine colour, whereas the presence of other compounds (the so-called copigments), normally colourless, allow improving the colour stabilization of aged wines (Pérez-Magariño & González-San José, 2004) by means of copigmentation reactions, which are covalent interactions between anthocyanins and copigments, giving rise polymeric pigments (Ribéreau-Gayón, Dubourdieu, Donéche, & Lonvaud, 2003). Thus, the content of anthocyanins significantly decreases during ageing, contrarily to that observed for polymeric pigments.

In warm regions, the stressful climate conditions make difficult to obtain high quality red wines, with high intensity and stable colour. This fact normally occurred since the phenolic maturity does not coincide with the technological (sugars) maturity of the grapes, and so, at the moment of harvesting different levels of both phenolic and sugar maturity exist (López, Sánchez, Díaz, Ramírez, & Morales, 2007; Mori, Sugaya, & Gemma, 2005), so that is the grapes have high sugar content but phenolics unripe. Thus, seeds normally remain unripened while the optimal maturity of skins and pulp is reached. Wines made from these grapes, low in pigments and cofactors, are not able to form much copigmentation in the first steps of the winemaking process (Boulton, 2001) and as a result, the colour stabilisation does not correctly develop and, after several months of storage both in bottle or barrels, fall of colour normally occurred. Thus, in these warm regions the seed are normally scarce in tannins, so an extra contribution of

67 tannins could be necessary to reach colour stabilisation, and hence the ageing period
68 could be long lasting.
69 Several authors have studied how colour stabilisation was increased by the addition of
70 tannins, both derived from grape and wood (Bautista-Ortín, Martínez-Cutillas, Ros-
71 García, López-Roca, & Gómez-Plaza, 2005; Vivas & Glories, 1996; Zamora, 2003). In
72 particular, phenolics extracted from wood have been described as compounds having
73 great influence on the colour, astringency and bitterness of the wine. They are also
74 involved in changes that take place during ageing (Del Alamo, Bernal, & Gomez-
75 Cordoves, 2000). The high variety of phenolic compounds present in wood, such as
76 benzoic and cinnamic compounds, and ellagitanins, among others, has been studied in a
77 large extent regardless of the regions and raw materials employed (Fernández De
78 Simón, Cadahía, Conde, & García-Vallejo, 1996, 1999; Mämmelä, Savolainen,
79 Lindroos, Kangas, & Vartiainen, 2000; Sanz, de Simón, Cadahía, Esteruelas, Muñoz,
80 Hernández, et al., 2012). In this sense, several authors have demonstrated that cinnamic
81 acids (such as caffeic and coumaric acids) play an important role in the copigmentation
82 reactions with the anthocyanins - , & Boulton, 2001), being
83 described colour enhancements at 520 nm (Jurd & Asen, 1966).
84 Currently, the use of wood chips have been widely used in winemaking as alternative
85 system to the classic ageing, mainly because barrels take up a lot of space in the winery,
86 their lifetime is not too long and the elaboration of aged wines is quite expensive. The
87 use of chips in winemaking is a legal practice in EU countries since 2006, when EU
88 approved the use of wood chips come exclusively from the *Quercus* genus
89 (Commission Regulation (EC), 2006). The use of oak wood chips as alternative of
90 barrels, and their effect on phenolic compounds and colour characteristics have been
91 widely studied. However, their addition before alcoholic fermentation and the study on

the stabilisation of the colour have been scarcely studied, and even less as alternative in warm climate vinifications. Among those research studies, Gómez García-Carpintero, Gómez Gallego, Sánchez-Palomo, and González Viñas (2012), Rodríguez-Bencomo, Ortega-Heras, and Pérez-Magariño (2009), and Soto Vázquez, Río Segade, and Orriols Fernández (2010)) studied the effect of the pre-fermentative addition of oak wood chips on phenolic composition and chromatic characteristics, and volatile fraction, respectively. However, the addition of oak chips did not favour the reactions involved in anthocyanin stabilisation neither nor in colour increase in bottled wines, suggesting that this technique could be useful for the elaboration of young red wines.

Therefore, with the objective of improving the colour stabilisation of red wines from grapes grown in a warm climate, the main goal of this work was to study the effect of adding oak wood chips during fermentation. The study was performed on red wine made from Tempranillo grape variety, one of the most important red grape cultivars grown in Spain. To date, the addition of oak wood chips during the fermentation/maceration stage has been scarcely studied, even less regarding the effect on colour in relation to a considerable number of phenolics studied. Thus, our interest was focused on the study of phenolic composition (anthocyanins, flavonols, flavan-3-ols, hydroxycinnamic acid derivatives and benzoic compounds), chromatic characteristics by applying differential colorimetry.

2. MATERIAL AND METHODS

2.1. Winemaking

Around 300 kg of grapes of *Vitis vinifera* cv. Tempranillo cultivated in "Condado de Huelva" Designation of Origin, in south-western Spain, were harvested at their optimal ripening stage and in good sanitary conditions. After the grapes were destemmed and crushed, the must was distributed in twelve stainless steel tanks of 220 l. Six tanks were

submitted to the addition of 3 g/l of American oak (*Quercus alba*) wood medium-toasted chips of 1 cm^2 average size (Tonelería Martín y Vázquez, Logroño, Spain) to the must, together with skins, and the other six tanks contained untreated, control wine. For all wines, alcoholic fermentation was spontaneously developed. Skin maceration was developed, manually punching down each tank once a day during 8 days. Subsequently, the malolactic fermentation was induced by inoculation of *Oenococcus Oeni* lactic acid bacteria (>10^{10} CFU *O. oeni*/ml, VINIFERM Oe 104, Agrovin, Spain) at the rate of 14 ml/hl at the end of alcoholic fermentation. This second fermentation ended in three weeks, which was confirmed by HPLC determination of malic acid and lactic acid contents, and the wines were then racked. Experiments were carried out in triplicate.

2.2 Oenological Parameters

The conventional oenological parameters (pH, total and volatile acidity, free and total SO_2, malic and lactic acids and reducing sugars) were performed according to the Official Methods established by European Union (UE, 2003).

2.3. Spectrophotometric colour measurement

The whole visible spectrum (380-770 nm) was recorded at constant intervals ($\Delta\lambda$=2 nm) with a Hewlett-Packard UV-vis HP8452 spectrophotometer (Palo Alto, CA), using 2 mm path length glass cells and distilled water as a reference. The CIELAB parameters (L^*, a^*, b^*, C^*_{ab}, and h_{ab}) were determined by using the original software CromaLab© (Heredia, Álvarez, González-Miret, & Ramírez, 2004), following the Commission Internationale de L'Eclariage's, CIE, recommendations (CIE, 1986): the CIE 1964 10° Standard Observer and the CIE Standard Illuminant D65. Euclidean distance between two points in the three-dimensional space define by L*, a*, and b* were used for calculating colour differences (ΔE^*_{ab}): $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

The percentage contributions of copigmented anthocyanins to the total wine colour at pH 3.6 were determined following the method proposed by Boulton (1996). Wine samples were first adjusted to pH 3.6.

2.4. HPLC-DAD analysis of phenolic compounds

HPLC separation, identification and quantification of phenolic compounds were 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). Prior direct injection, the samples were filtered through a 0.45 μm Nylon filter (E0034, Análisis Vínicos, Spain). All analyses were made in triplicate.

The anthocyanin identification was carried out following the method proposed by Heredia et al. (2010). Anthocyanins were separated using a Zorbax C18 column (250 x 4.6mm, 5 μm particle size) maintained at 38 ºC. 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-6% B; 10-15 min 70%A-30% B; 15-25 min 60%A-40%B; 25-35 min 55%A-45%B; 35-40 min50%A-50%B; 40-42 min 40%A-60% B; 42-43 min 94% A-6% B. 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 nm by comparing the areas and the retention times with the malvidin 3-glucoside standard, and anthocyanin concentration was expressed as mg/l.

The method used for the identification of the phenolic compounds (flavan-3-ols, flavonols, hydroxycinnamic acid derivatives and other low molecular weight phenolic compounds), was a modification of the method described by Castillo-Muñoz, Gómez-Alonso, García-Romero, and Hermosín-Gutiérrez (2007). These individual phenolic compounds were separated using a Zorbax C18 column (250 x 4.6mm, 5 μm particle

size) maintained at 40 ºC. 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: 0-5 min 94% A-6% B; 5-10 min 89%A-11% B; 10-15 min 80%A-20%B; 15-20 min 77%A-23%B; 20-25 min 74%A-26%B; 25-30 min 60%A-40% B; 30-35 min 50%A-50% B; 35-38 min 40%A-60%B; 38-46 min 94%A-6%B. The flow rate was 0.63 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 280, 320 and 360 nm by comparing the areas and the retention times with the gallic acid, caffeic acid, and quercetin standards, respectively. Phenolic compounds concentration was expressed as mg/l. Total anthocyanins, flavonols and flavan-3-ols were calculated as sum of individual phenolic compounds identified by HPLC. Folin-Ciocalteau reagent was used for the analysis of total phenolics (Singleton & Rossi, 1965).

2.5. Statistical Analysis

Statistical analysis was carried out by using Statistica version 8.0 software (Statistica, 2007). Univariate analysis of variance (Tukey test) was applied to discriminate among the means of chemical data and by pairs of control wines and wines with addition of oak wood chips for each studied point. Moreover, multivariate analysis of data (linear discriminant analysis, LDA) was performed in order to classify wine samples according to phenolic compounds and colour parameters. This method was applied to the set of data consisting of 72 rows (wine samples) and 29 columns (individual phenolic compounds and colorimetric variables).

3. RESULTS AND DISCUSSION

The effect of the fermentative addition of oak wood chips on the colour (by tristimulus colorimetry), differential colorimetry and polyphenolic composition of Tempranillo red

191 wines elaborated in warm climate has been studied. The study of the changes in the
192 colour and polyphenols occurring along different stages of the vinification process is
193 critical to establish the moment at which the applied technique have a greater impact in
194 the quality of the wines. Therefore, an exhaustive follow-up of the alcoholic
195 fermentation process and subsequent stabilisation stage allows us to acquire valuable
196 information on the changes in colour of the wines and polyphenolic compounds. In this
197 sense, several points were considered: the initial point or grape crushing (day 0),
198 different points of the skin-maceration stage (days 2, 4, 6 and 8) and of the stabilisation
199 stage (days 15, 20, 30, 45, 50 and 60).

200 ***3.1. Conventional Analytical Data***

201 The general composition of control wines (CW) and wines with the addition of oak
202 wood chips (OW) for each tank was determined. For both wines, the pH values were
203 similar and volatile acidity values were below the limit (1.2 g/L) established by EU. The
204 low values of reducing sugars (between 2-3 g/L) denoted the correct development of the
205 alcoholic fermentation. Moreover, the values of malic and lactic acids evidenced the
206 correct development of the malolactic fermentation (malic acid < 0.1; lactic acid around
207 2.2 g/L). Adequate values of free and total SO_2 were obtained for both wines (around 40
208 and 160 mg/L of free and total SO_2, respectively).

209 ***3.2. Identification of Polyphenolic Compounds***

210 In this research, several types of polyphenolic compounds have been identified, like
211 benzoic acids, hydroxycinnamic acid derivatives, flavan-3-ols, flavonols and
212 anthocyanins. The hydroxycinnamic acid and benzoic acid derivatives and flavan-3-ols
213 compounds identified were the expected, well-known, compounds usually present in
214 wine (Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez, 2007). Among the
215 flavonols, conjugated (3-glycosides) forms of myricetin, quercetin, isorhamnetin,

syringetin and laricitrin were identified (Castillo-Muñoz, Gómez-Alonso, García-Romero, Gómez, Velders, & Hermosín-Gutiérrez, 2009; Castillo-Muñoz, Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez, 2007). Native grape anthocyanins were detected in Tempranillo red wines (Cejudo-Bastante, Pérez-Coello, & Hermosín-Gutiérrez, 2011; Gordillo, López-Infante, Ramírez-Pérez, González-Miret, & Heredia, 2010), including non-acylated, acetylated and *p*-coumaryolated anthocyanins of the five expected anthocyanidins (delphinidin, cyanidin, petunidin, peonidin and malvidin).

3.3. Pigment evolution

Regardless of the addition of oak wood chips, the two Tempranillo red wines showed the same polyphenolic profile. However, quantitatively, the results showed that the fact of adding oak wood chips during fermentation provoked a positive effect on the content of anthocyanins, flavonols and phenolic acids. That fact was observed during the first steps of the stabilisation stage.

Figure 1 showed the evolution of the content of anthocyanins (as sum of individual anthocyanins by HPLC) and total phenols (by Folin Ciocalteau) in different stages of the vinification process: in must, during fermentation and along the stabilisation stage. As expected since both wines were elaborated under the same extraction conditions (temperature and time), it can be observed that control wines (CW) and wines derived from the addition of oak wood chips (OW) showed the same phenolic pattern evolution: the anthocyanic and total polyphenolic contents appreciably increased during fermentation/maceration, and remains almost constant or slightly decreased during the time of stabilisation studied. While the anthocyanins content was similar for both wines (CW and OW), as can be seen in Figure 1, the total phenols content was higher for OW wine, especially during the maceration stage where the chips transfer their compounds.

In order to know the significant differences between both wines, the mean value of all parameters studied was calculated (Table 1), and Tukey test was applied. As can be seen, higher mean contents of all the chemical compounds determined (both individual and total contents) were found for OW wines. Differences were significant ($p<0.05$) for total phenols (CW=1546.6 mg/L versus OW=2364.2 mg/L), total phenolic acids (CW=139.9 mg/L versus OW=184.0 mg/L) and total flavonols (CW=23.3 mg/L versus OW=34.7 mg/L) contents. Also, the addition of oak wood chips lead to significant ($p<0.05$) higher content of some individual phenols such as gallic acid, (-)-epicatechin and the glucuronide and glucoside derivatives of myricetin. These increases could be probably due to the release of those compounds (benzoic acids, flavan-3-ols and flavonols) from the wood, in agreement with the high content previously described in different woods (Alañón, Castro-Vázquez, Díaz-Maroto, Gordon, & Pérez-Coello, 2011; Alañón, Schumacher, Castro-Vázquez, Díaz-Maroto, Hermosín-Gutiérrez, & Pérez-Coello, 2013). In the case of anthocyanins, the total content was widely higher for the wines macerated with oak chips (541.95 mg/L for CW and 627.73 mg/L for OW as average values), although these differences were not significant in any individual anthocyanins identified. The higher levels of anthocyanins and flavonols, and also the higher degree of copigmentation found in wines macerated with oak chips (Table 1) could be maybe consequence of the ellagitannin protective effect (Cano-López, López-Roca, Pardo-Minguez, & Gómez Plaza, 2010), presented those in a large extent in wood.

3.4. Chromatic evolution

The evolution of the CIELAB colour parameters (L^*, C^*_{ab} and h_{ab}) in the course of the vinification process for control wines (CW) and wines with the addition of oak wood chips (OW) was evaluated (Figure 2). Both wines followed similar evolutions during

time, i.e., the values of lightness and hue notably decreased after skin-maceration stage (by 35% and 75º, respectively). Later, it was observed a slightly increased during the stabilisation stage. These changes in colour characteristics could be related to the stabilisation by progressive displacement of copigmentation complexes into polymeric pigments, in agreement with other authors (Gao, Girard, Mazza, & Reynolds, 1997; Gordillo, López-Infante, Ramírez-Pérez, González-Miret, & Heredia, 2010). A contrary evolution was observed for chroma, which increased by 85% after skin-maceration. Despite the colour difference provoked as a consequence of the addition of chips was not appreciable during fermentation and skin-maceration, significant differences were observed during the stabilisation stage (Figure 2) when Tukey test was applied by pairs.

Wines with the addition of oak wood chips showed a perceptible and significant ($p<0.05$) darker colour (lower values of lightness, L^*; 66.2 against 70.8 units CIELAB) and a higher chromatic intensity (higher values of chroma, C^*_{ab}; 30.9 against 26.2 units CIELAB), and those wines showed slightly more bluish tonalities (lower values of hue, h_{ab}; -1.60° against 0.47°). These results were in agreement with Soto Vázquez, Río Segade, and Orriols Fernández (2010), who demonstrated that chroma in wines with the pre-fermentative addition of oak wood chips significantly increased after fermentation and bottling. The fact that this tonalities were kept for a longer time in OW could be related to the higher amounts of bluish forms of anthocyanins (glucosides and 3-acetyl-glucosides) (Heredia, Francia-Aricha, Rivas-Gonzalo, Vicario, & Santos-Buelga, 1998). That fact could be clearly shown in the (a*b*)-colour diagram (Figure 3). The different location of the wines conventionally elaborated and wines with the addition of oak wood chips, during the stabilisation stage, permits to establish objectively the chromatic characteristics of the wines. It is observed that CW are located in the first quadrant (positive values of a^* and b^*) of the (a^*b^*)-plane, whereas OW are situated in the

fourth quadrant (positive values of a^* and negative values of b^*). According to this, wines derived from the addition of chips had a more purple or red-bluish colour than control wines. The Tukey test applied by pairs on a^* and b^* shown significant differences in days 45, 50 and 60 (data not shown). Therefore, wines with the addition of oak wood chips evolve in a positive way, in comparison with wines elaborated conventionally. The addition of oak wood chips provoked a positive effect not only on the colour density (chroma) but also on the colour stability. This fact means that, once skin-maceration finished, the losses of colour density of OW was smaller (around 27%), and stable during the time (lightness decreased by 8%, and hue by 2.6º). The improvement of wine structure by the addition of oak wood chips could be due to the several compounds released from the wood chips (especially ellagitannins). Their presence might favour the formation of new pigments derived from anthocyanins, that ensure colour stability by an increase of blue hues, which in turn leads to a decrease of the yellow ones evolution (Vivas & Glories, 1996).

With the aim of evaluating the colorimetric implications of the addition of oak wood chips during skin-maceration stage, the mean colour difference (ΔE^*_{ab}) among both kind of wines in each studied points were calculated (Figure 4). Taking into account that ΔE^*_{ab} of up to 3 CIELAB units indicates colour differences appreciable to the human eyes (Martínez, Melgosa, Pérez, Hita, & Negueruela, 2001), it was confirmed that the presence of oak wood chips during fermentation/maceration did not led to colour differences visually appreciable between both wines ($\Delta E^*_{ab}<3$). However, once fermentative process finished and during the time of stabilisation studied (20, 30, 45, 50 and 60 days), the values of ΔE^*_{ab} progressively increased from 5 to 8 CIELAB units, hence clearly exceeding the visual appreciation threshold. Due to the increase of colour variation was visually appreciable after maceration, the role of each colour attribute

315 respect $\Delta^2E^*_{ab}$ was calculated (as percentage of the quadratic increases of lightness,
316 chroma and hue). Thus, it was proved that the addition of oak wood chips during skin-
317 maceration mainly affected to colour in a quantitative way, with similar quadratic
318 variations of lightness and chroma, and practically negligible of hue ($\%\Delta^2L$ = 47.4,
319 $\%\Delta^2C$ = 50.3, $\%\Delta^2H$ = 2.3).

320 ***3.5. Linear Discriminant Analysis (LDA)***

321 Linear discriminant analysis was applied with the aim of establishing which of the
322 variables were better for discriminating among samples, and carrying out an exploratory
323 tool to uncover unknown trends in the data. A forward stepwise LDA was performed to
324 differentiate the two sample groups (control wines and wines with the addition of oak
325 wood chips), on the basis of phenolic compounds and colour characteristics. Concretely,
326 the angular coordinates of the CIELAB space (L^*, C^*_{ab} and h_{ab}) and the individual
327 phenolic compounds (monomeric anthocyanins, benzoic acids, hydroxycinnamic acid
328 derivatives, flavan-3-ols and flavonols) were used as discriminant variables. The rest of
329 the colour variables were not included in the LDA in order to avoid redundant
330 information. This statistical analysis was performed according to the Wilks' λ statistic
331 to choose the descriptors that best distinguished the different wines. A *F* statistic is
332 computed from the partial λ values, leading to a *p* level. The maximum discriminatory
333 power corresponds to minimum *p* level values. Due to the major significant differences
334 were observed during the stabilisation stage; as previously commented only samples of
335 this stage were considered for the statistical analysis.

336 According to *p*-levels and *F*-values, flavan-3-ols ((+)-catechin and (-)-epicatechin) and
337 some flavonols (3-glucosides of isorhamnetin, laricitrin and kaempferol, and 3-
338 glucuronide of quercetin) were the variables included in the discriminant functions. In
339 addition, the colorimetric characteristic of chroma (C^*_{ab}) was also considered as

discriminant function. Therefore, those variables were able to discriminate between both groups of samples (CW and OW) with high levels of significance ($p<0.001$).

According to the classification functions, control wine samples were clearly differentiated from those belonged to wines with the addition of oak wood chips (Figure 5). 95.8% of the samples were correctly assigned. Moreover, the percentage of prediction was 100% for CW samples and 91.7% for OW samples.

4. CONCLUSIONS

It can be concluded that, a priori, the presence of oak wood chips during fermentation and skin-maceration lead to an increase of the content of phenolics that improves the chemical characteristics of the wines for colour stabilisation. Thus, adding oak chips could represent a useful oenological alternative to prevent the excessive and typical loss of pigments in wines obtained from grapes cultivated in warm climates. As a consequence, an enhancement and stabilisation of the colour are produced and they will be long lasting, increasing in value the wines cultivated in those broad regions of warm climate. However, in order to check the stabilisation of the colour and reflect commercial storage conditions, it would be interesting to carry out further studies about storage of several months or years.

ACKNOWLEDGMENT

We are indebted to Consejería de Ciencia, Innovación y Empresa, Junta de Andalucía, Spain (project P10-AGR06331) and Ministerio de Ciencia e Innovación (project AGL2011-30254-C02-02) for financial support, and Cooperativa Vitivinícola Nuestra Señora del Socorro (Rociana, Huelva, Spain) for collaborating with the experiments.

REFERENCES

Commission Regulation (EC). (2006). 1507/2006 of 11 October. Official Journal of the European Union, October 12, 2006, L280/9-L280/11.

Alañón, M. E., Castro-Vázquez, L., Díaz-Maroto, M. C., Gordon, M. H., & Pérez-Coello, M. S. (2011). A study of the antioxidant capacity of oak wood used in wine ageing and the correlation with polyphenol composition. *Food Chemistry, 128*(4), 997-1002.

Alañón, M. E., Schumacher, R., Castro-Vázquez, L., Díaz-Maroto, M. C., Hermosín-Gutiérrez, I., & Pérez-Coello, M. S. (2013). Enological potential of chestnut wood for aging Tempranillo wines Part II: Phenolic compounds and chromatic characteristics. *Food Research International, 51*(2), 536-543.

Bautista-Ortín, A. B., Martínez-Cutillas, A., Ros-García, J. M., López-Roca, J. M., & Gómez-Plaza, E. (2005). Improving colour extraction and stability in red wines: The use of maceration enzymes and enological tannins. *International Journal of Food Science and Technology, 40*(8), 867-878.

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

Boulton, R. B. (1996). A Method for the Assessment of Copigmentation in Red Wines. In *47th Annual Meeting of the American Society for Enology and Viticulture*). Reno, NV.

Cano-López, M., López-Roca, J. M., Pardo-Minguez, F., & Gómez Plaza, E. (2010). Oak barrel maturation vs. micro-oxygenation: Effect on the formation of anthocyanin-derived pigments and wine colour. *Food Chemistry, 119*(1), 191-195.

Castillo-Muñoz, N., Gómez-Alonso, S., García-Romero, E., Gómez, M. V., Velders, A. H., & Hermosín-Gutiérrez, I. (2009). Flavonol 3-O-glycosides series of Vitis

vinifera Cv. Petit Verdot red wine grapes. *Journal of Agricultural and Food Chemistry, 57*(1), 209-219.

Castillo-Muñoz, N., Gómez-Alonso, S., García-Romero, E., & Hermosín-Gutiérrez, I. (2007). Flavonol profiles of Vitis vinifera red grapes and their single-cultivar wines. *Journal of Agricultural and Food Chemistry, 55*(3), 992-1002.

Cejudo-Bastante, M. J., Pérez-Coello, M. S., & Hermosín-Gutiérrez, I. (2011). Effect of wine micro-oxygenation treatment and storage period on colour-related phenolics, volatile composition and sensory characteristics. *LWT - Food Science and Technology, 44*(4), 866-874.

CIE (1986). *Colorimetry*. Vienna, Austria: Technical Report CIE 15.2, 3 ed.

- , E., & Boulton, R. B. (2001). Enhancement of red wine colour by pre-fermentation addition of copigments. *Food Chemistry, 73*(2), 217-220.

Del Alamo, M., Bernal, J. L., & Gomez-Cordoves, C. (2000). Behavior of monosaccharides, phenolic compounds, and color of red wines aged in used oak barrels and in the bottle. *Journal of Agricultural and Food Chemistry, 48*(10), 4613-4618.

Fernández De Simón, B., Cadahía, E., Conde, E., & García-Vallejo, M. C. (1996). Low molecular weight phenolic compounds in Spanish oak woods. *Journal of Agricultural and Food Chemistry, 44*(6), 1507-1511.

Fernández De Simón, B., Cadahía, E., Conde, E., & García-Vallejo, M. C. (1999). Ellagitannins in woods of Spanish, French and American oaks. *Holzforschung, 53*(2), 147-150.

412 Gao, L., Girard, B., Mazza, G., & Reynolds, A. G. (1997). Changes in Anthocyanins
413 and Color Characteristics of Pinot Noir Wines during Different Vinification
414 Processes. *Journal of Agricultural and Food Chemistry*, *45*(6), 2003-2008.
415 Gordillo, B., López-Infante, M. I., Ramírez-Pérez, P., González-Miret, M. L., &
416 Heredia, F. J. (2010). Influence of prefermentative cold maceration on the color
417 and anthocyanic copigmentation of organic tempranillo wines elaborated in a
418 warm climate. *Journal of Agricultural and Food Chemistry, 58*(11), 6797-6803.
419 Gómez García-Carpintero, E., Gómez Gallego, M. A., Sánchez-Palomo, E., & González
420 Viñas, M. A. (2012). Impact of alternative technique to ageing using oak chips
421 in alcoholic or in malolactic fermentation on volatile and sensory composition of
422 red wines. *Food Chemistry*, *134*(2), 851-863.
423 Gómez-Alonso, S., García-Romero, E., & Hermosín-Gutiérrez, I. (2007). HPLC
424 analysis of diverse grape and wine phenolics using direct injection and
425 multidetection by DAD and fluorescence. *Journal of Food Composition and*
426 *Analysis*, *20*(7), 618-626.
427 Heredia, F. J., Escudero-Gilete, M. L., Hernanz, D., Gordillo, B., Meléndez-Martínez,
428 A. J., Vicario, I. M., & González-Miret, M. L. (2010). Influence of the
429 refrigeration technique on the colour and phenolic composition of syrah red
430 wines obtained by pre-fermentative cold maceration. *Food Chemistry, 118*(2),
431 377-383.
432 Heredia, F. J., Francia-Aricha, E. M., Rivas-Gonzalo, J. C., Vicario, I. M., & Santos-
433 Buelga, C. (1998). Chromatic characterization of anthocyanins from red
434 grapes—I. pH effect. *Food Chemistry*, *63*(4), 491-498.

435 Heredia, F. J., Álvarez, C., González-Miret, M. L., & Ramírez, A. (2004). CromaLab,
436 análisis de color. In, vol. Registro General de la Propiedad Intelectual: SE-1052-
437 04). Seville, Spain.
438 Jurd, L., & Asen, S. (1966). The formation of metal and "co-pigment" complexes of
439 cyanidin 3-glucoside. *Phytochemistry, 5*(6), 1263-1271.
440 López, M. I., Sánchez, M. T., Díaz, A., Ramírez, P., & Morales, J. (2007). Influence of
441 a deficit irrigation regime during ripening on berry composition in grapevines
442 (Vitis vinifera L.) grown in semi-arid areas. *International Journal of Food*
443 *Sciences and Nutrition, 58*(7), 491-507.
444 Martínez, J. A., Melgosa, M., Pérez, M. M., Hita, E., & Negueruela, A. I. (2001). Note.
445 Visual and instrumental color evaluation in red wines. *Food Science and*
446 *Technology International, 7*(5), 439-444.
447 Mori, K., Sugaya, S., & Gemma, H. (2005). Decreased anthocyanin biosynthesis in
448 grape berries grown under elevated night temperature condition. *Scientia*
449 *Horticulturae, 105*(3), 319-330.
450 Mämmelä, P., Savolainen, H., Lindroos, L., Kangas, J., & Vartiainen, T. (2000).
451 Analysis of oak tannins by liquid chromatography-electrospray ionisation mass
452 spectrometry. *Journal of Chromatography A, 891*(1), 75-83.
453 Pérez-Magariño, S., & González-San José, M. L. (2004). Evolution of Flavanols,
454 Anthocyanins, and Their Derivatives during the Aging of Red Wines Elaborated
455 from Grapes Harvested at Different Stages of Ripening. *Journal of Agricultural*
456 *and Food Chemistry*, *52*(5), 1181-1189.
457 Ribéreau-Gayón, P., Dubourdieu, D., Donéche, B., & Lonvaud, A. (2003). *Tratado de*
458 *Enología. Microbiología del vino. Vinificaciones*. Hemisferio Sur. Buenos Aires.

459 Robichaud, J. L., & Noble, A.C. (1990). Astringency and bitterness of selected
460 phenolics in wine. *Journal of the Science of Food and Agriculture, 53*, 343-353.
461 Rodríguez-Bencomo, J. J., Ortega-Heras, M., & Pérez-Magariño, S. (2009). Effect of
462 alternative techniques to ageing on lees and use of non-toasted oak chips in
463 alcoholic fermentation on the aromatic composition of red wine. *European Food*
464 *Research and Technology*, *230*(3), 485-496.
465 Sanz, M., de Simón, B. F., Cadahía, E., Esteruelas, E., Muñoz, Á. M., Hernández, M.
466 T., & Estrella, I. (2012). Polyphenolic profile as a useful tool to identify the
467 wood used in wine aging. *Analytica Chimica Acta*, *732*(0), 33-45.
468 Sarni-Manchado, P., Cheynier, V., & Moutounet, M. (1999). Interactions of grape seed
469 tannins with salivary proteins. *Journal of Agricultural and Food Chemistry*,
470 *47*(1), 42-47.
471 Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with
472 phosphomolybdic-phosphotungstic acid reagents. In, vol. 16 (pp. 144-158):
473 American Journal of Enology & Viticulture.
474 Soto Vázquez, E., Río Segade, S., & Orriols Fernández, I. (2010). Effect of the
475 winemaking technique on phenolic composition and chromatic characteristics in
476 young red wines. *European Food Research and Technology*, *231*(5), 789-802.
477 StatSoft Inc. (2007). *STATISTICA (data analysis software system)*. version 8. Tulsa,
478 OK. <www.statsoft.com>.
479 UE (2003). Official Methods to Wine Analyses, Reglamento 440/2003.
480 Vivas, N., & Glories, Y. (1996). Role of oak wood ellagitanins in the oxidation process
481 of red wines during aging. *American Journal of Enology and Viticulture*, *47*,
482 103-107).
483 Zamora, F. (2003). El tanino enológico en la vinificación en tinto. *Enólogos, 25*, 26-30.

FIGURE CAPTIONS

Fig. 1. Total anthocyanin (a) and total phenols (b) contents evolution during vinification in control wines (CW) and wines with the addition of oak wood chips (OW).

Fig. 2. Evolution of CIELAB parameters during vinification: (a), (b) and (c). For each day, asterisks denote significant differences ($p<0.05$), according to Tukey test, between control wines (CW) and wines with the addition of oak wood chips (OW).

Fig. 3. CIELAB colour space (a*b*)-plane for control wines (CW) and wines with the addition of oak wood chips (OW) during stabilisation stage.

Fig. 4. Colour variation (ΔE^*_{ab}) between control wines (CW) and wines with the addition of oak wood chips (OW) during vinification.

Fig. 5. Scatterplot of the canonical variate obtained by LDA: control wines (CW) and wines with the addition of oak wood chips (OW) during the stabilisation stage.

Table 1

Table 1. Mean values of concentration (mg/L) and standard deviations (n=33) for the polyphenolic compounds identified by HPLC-DAD, and the percentage of copigmentation (%CA), in control wines (CW) and wines with the addition of oak wood chips (OW).

	CW			OW		
	Mean		SD	Mean		SD
Total phenolics (Folin Ciocalteau)	1546.6	±	796.94 *	2364.2	±	736.82
Total anthocyanins	541.95	±	170.21	627.73	±	164.53
Total glucoside derivatives	397.10	±	117.68	458.61	±	120.62
Total acetate derivatives	59.91	±	14.31	69.42	±	15.41
Total *p*-coumaric derivatives	84.93	±	26.19	99.69	±	27.20
Total phenolic acids	139.92	±	61.05 *	184.00	±	55.44
Total flavan-3-ols	13.79	±	8.35	16.52	±	7.51
Total flavonols	23.26	±	15.64 *	34.73	±	14.28
%CA (Boulton)	21.12	±	9.41	23.35	±	10.03
Monomeric anthocyanins						
Delphinidin-3-glucoside	53.05	±	15.8	54.74	±	14.21
Cyanidin-3-glucoside	10.98	±	2.2	10.04	±	1.83
Petunidin-3-glucoside	62.57	±	18.32	70.96	±	18.53
Peonidin-3-glucoside	24.85	±	5.56	25.06	±	4.92
Malvidin-3-glucoside	255.33	±	77.85	297.96	±	83.44
Petunidin-3-acetyl-glucoside	15.32	±	3.02	16.91	±	3.07
Peonidin-3-acetyl-glucoside	9.63	±	0.90	10.20	±	1.12
Malvidin-3-acetyl-glucoside	36.78	±	10.17	42.31	±	11.52
Petunidin-3-*p*-coumaroyl-glucoside	17.62	±	4.43	19.94	±	4.61
Peonidin-3-*p*-coumaroyl -glucoside	11.23	±	1.86	11.35	±	1.99
Malvidin-3-*p*-coumaroyl -glucoside	59.19	±	19.50	68.39	±	20.95
Benzoic acids						
Gallic acid	97.89	±	41.07 *	127.48	±	20.15
Hydroxycinnamic acid derivatives						
GRP	8.10	±	2.60	8.24	±	2.36
t-caftaric acid	17.09	±	5.80	19.29	±	5.46
t-coutaric acid	29.09	±	10.39	36.19	±	10.19
p-coumaric acid	0.50	±	0.83	1.03	±	0.80
Flavan-3-ols						
(+)-catechin	21.98	±	11.19	23.16	±	12.46
(-)-epicatechin	6.23	±	3.7 *	10.00	±	4.16
		±			±	
Flavonols						
Myricetin-3-glucuronide	1.71	±	1.29 *	2.57	±	1.37
Myricetin-3-glucoside	10.65	±	6.55 *	15.05	±	6.03
Quercetin-3-glucuronide	4.16	±	2.49	5.60	±	2.53
Quercetin-3-glucoside	5.38	±	2.87	6.97	±	2.73
Laricitrin-3-glucoside	1.86	±	1.67	2.80	±	1.77
Kaempferol-3-glucoside	0.27	±	0.28	0.39	±	0.29
Isorhamnetin-3-glucoside	0.02	±	0.09	0.13	±	0.34
Syringetin-3-glucoside	0.76	±	0.75	1.21	±	0.84

* Asterisk indicates significant difference according to Tukey test ($p<0.05$) between control wines (CW) and wines with the addition of oak wood chips (OW); GRP, Grape Reaction Product (2-*S*-glutathionyl-caftaric acid).

Figure 1

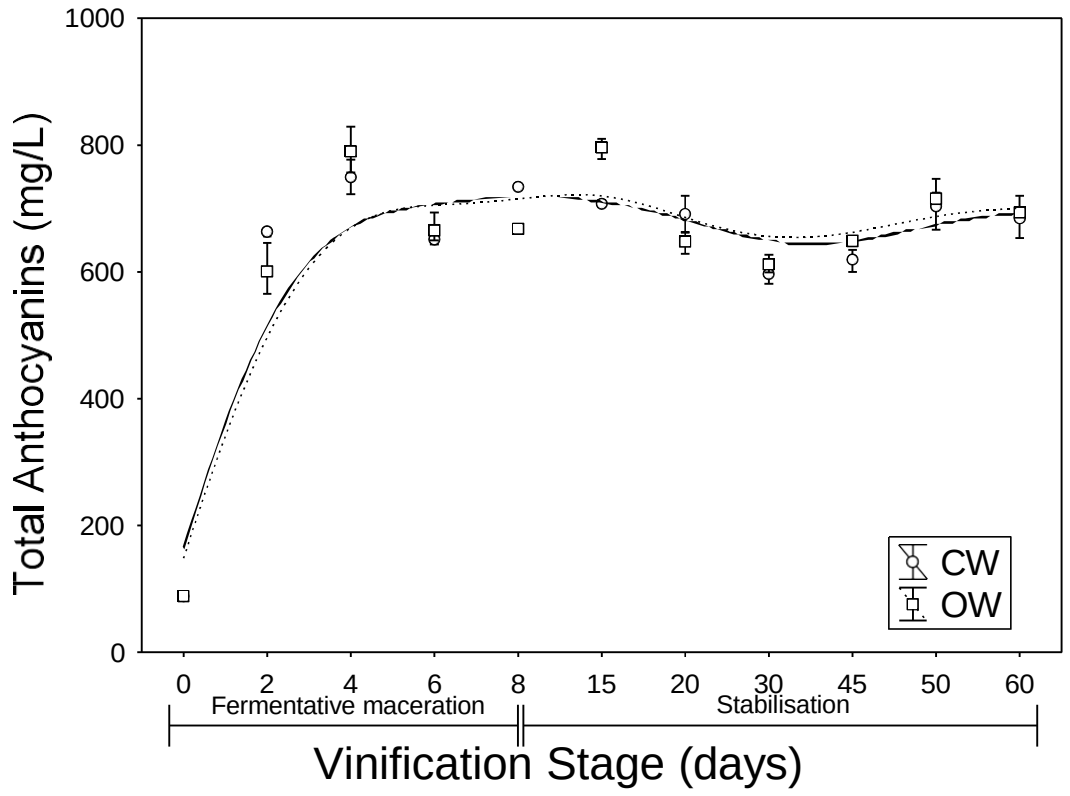

Fig. 1a

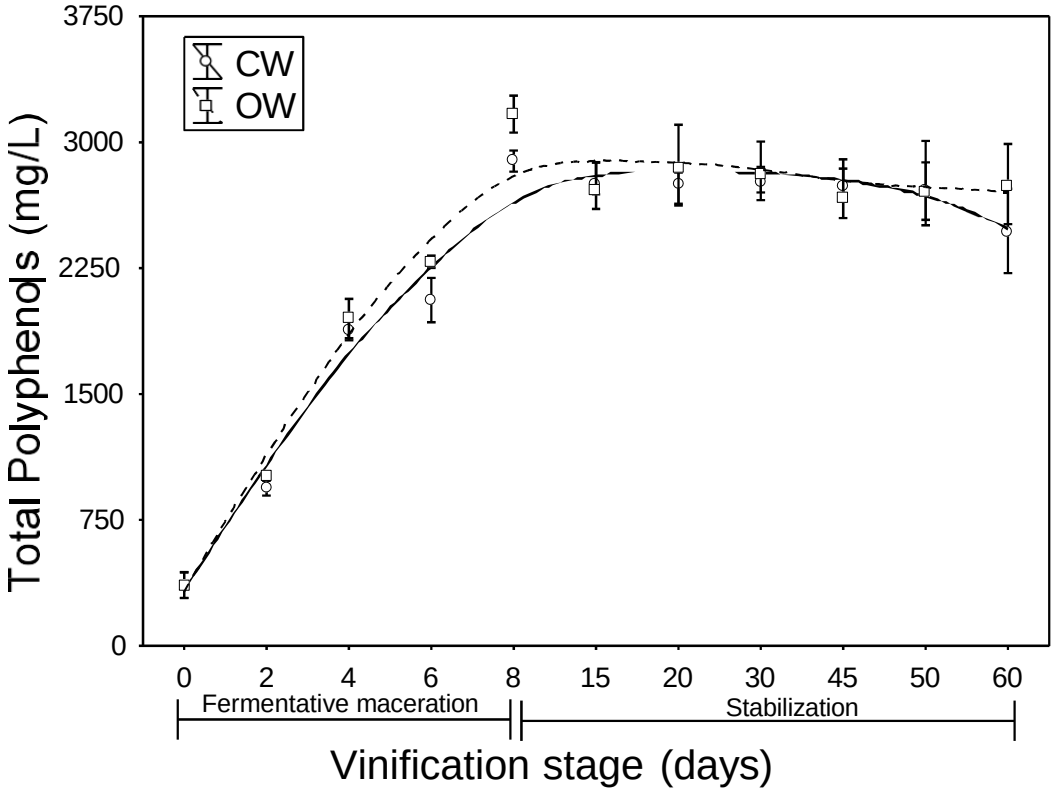
3750
3000
2250
1500
750
0
Total Polyphenols (mg/L)
CW
OW
0
2
4
6
8
15
20
30
45
50
60
Fermentative maceration
Stabilization
Vinification stage (days)

Fig. 1b

Figure 2

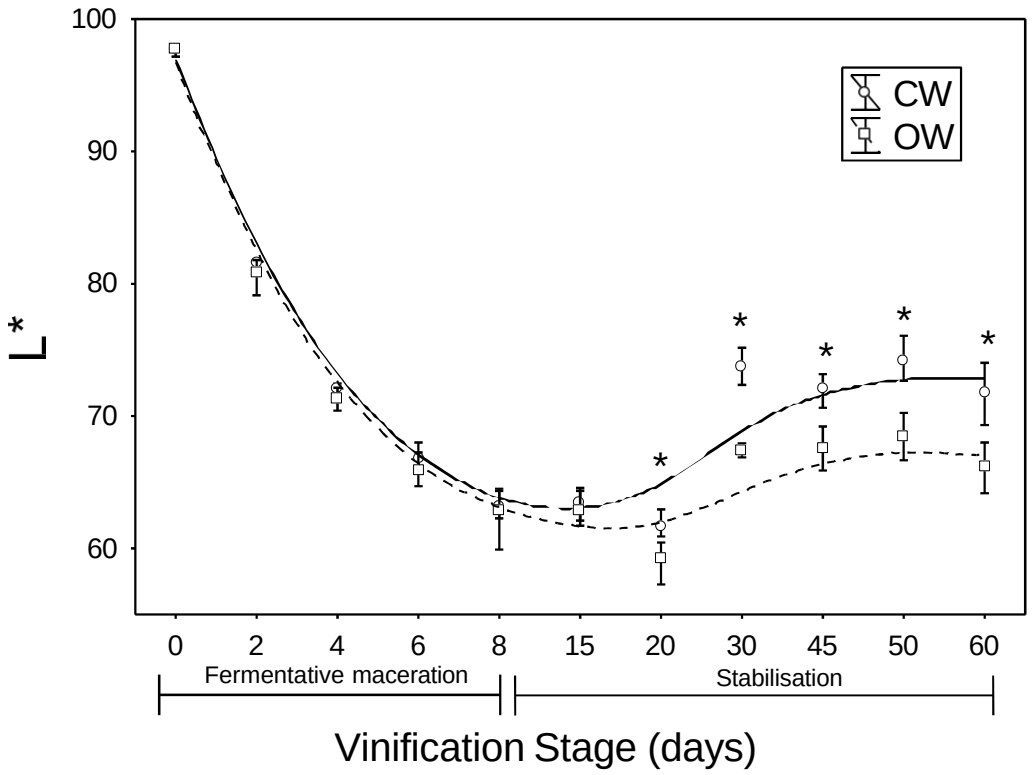

Fig. 2a

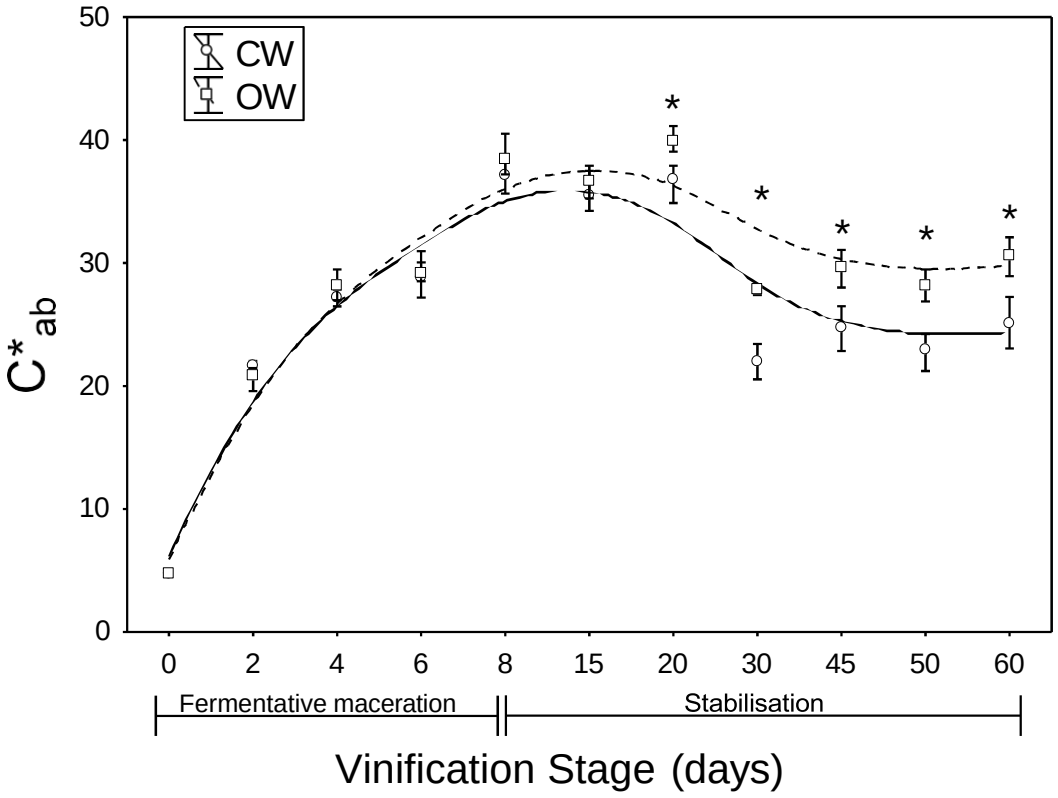
CW
OW
C*ab
50
40
30
20
10
0
0
2
4
6
8
15
20
30
45
50
60
Fermentative maceration
Stabilisation
Vinification Stage (days)

Fig. 2b

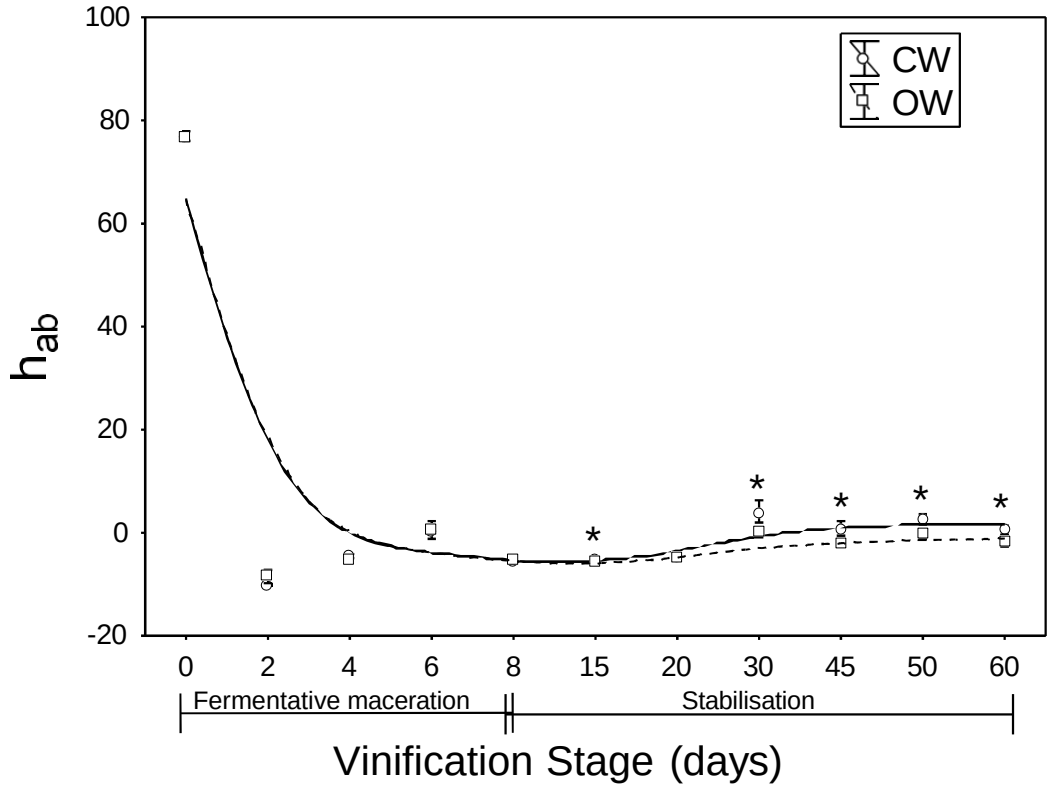
100
80
60
40
20
0
-20
h_{ab}
CW
OW
*
*
*
*
0
2
4
6
8
15
20
30
45
50
60
Fermentative maceration
Stabilisation
Vinification Stage (days)

Fig. 2c

Figure 3

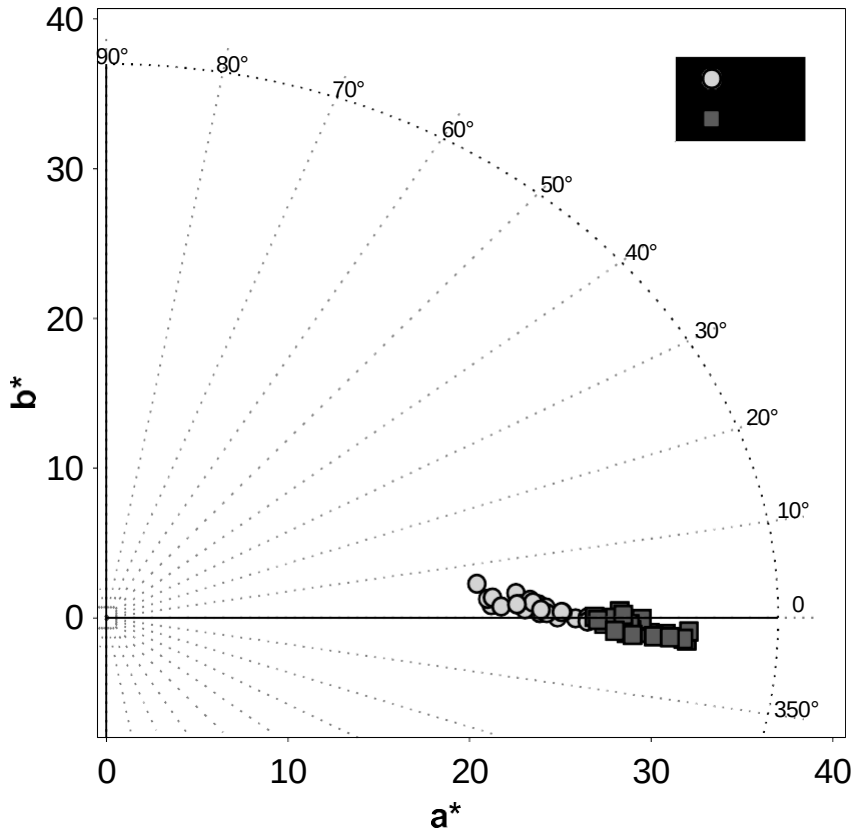

Fig. 3.

Figure 4

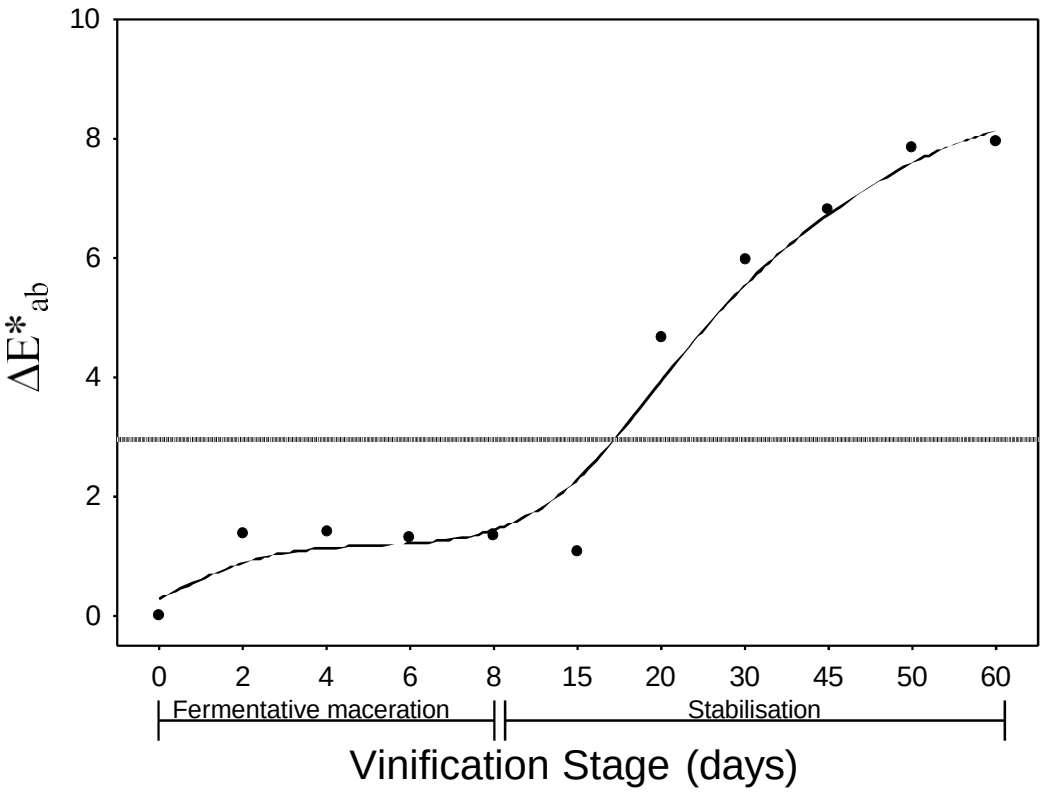

Fig. 4.

Figure 5

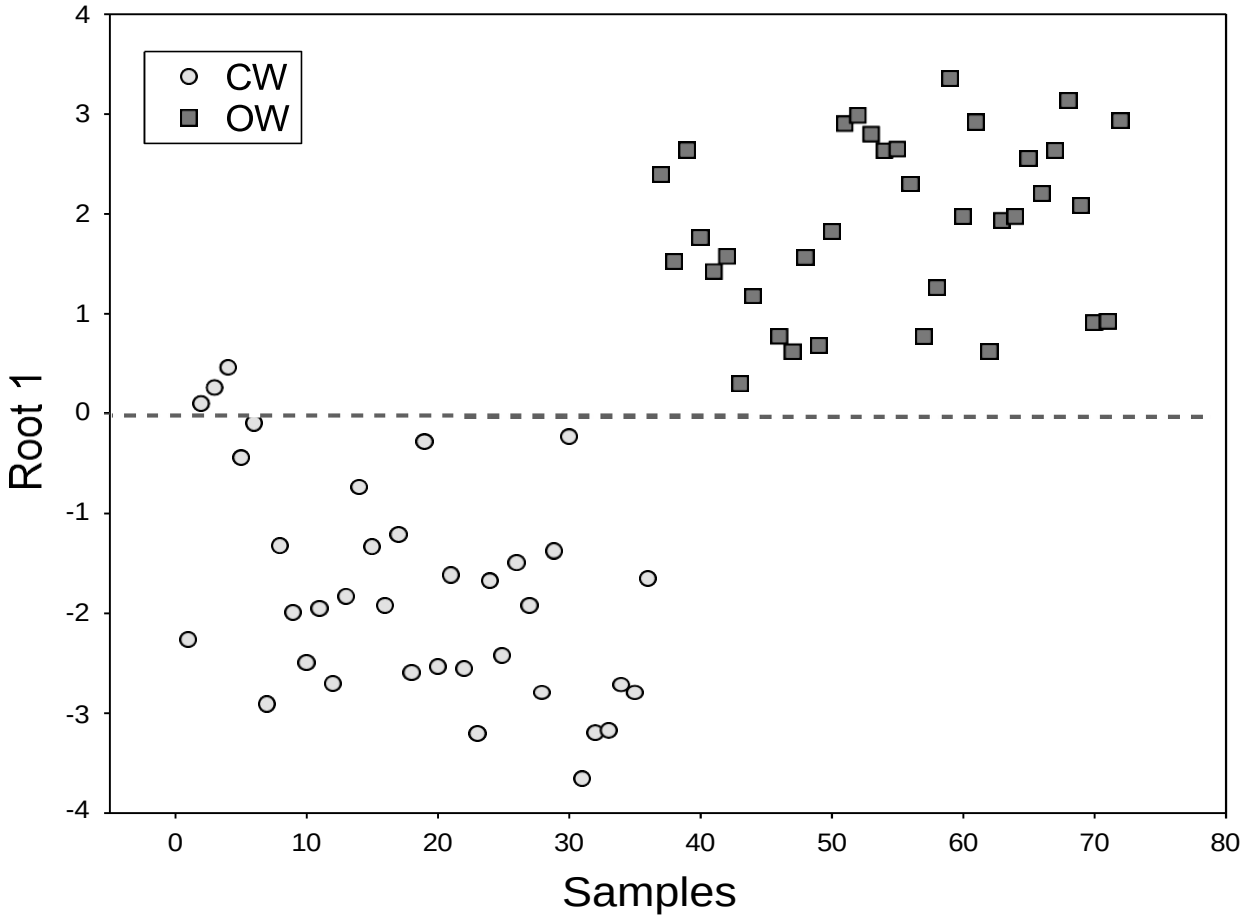

Fig. 5