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ORIGINAL PAPER

Copigmentation, colour and antioxidant activity of single-cultivar red wines

J. Heras-Roger · M. Pomposo-Medina · C. Díaz-Romero · J. Darias-Martín

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Abstract A hundred and thirty-six single-cultivar red wines of different vintages were collected from several wineries in the Canary Islands in order to study the magnitude of the copigmentation phenomenon and the antioxidant activity. The contribution of free anthocyanins, copigmented anthocyanins and polymeric pigments to the colour of wine, as well as the total phenols, the antioxidant activity (2,2-diphenyl-1-picrylhydrazyl, DPPH method) and the chromatic characteristics of the wines were determined. The influence of ageing time and the climatic conditions on these parameters was also studied. The wines made with Merlot, Ruby Cabernet and Syrah cultivars showed the highest parameters of colour, and the largest contribution to the copigmented anthocyanins was from the Ruby Cabernet, Listán negro and Syrah cultivars. The copigmented anthocyanins and the free anthocyanins decrease with the age of the wine, and the antioxidant activity of the samples appears to be related to the total phenol content. An influence of the climatic conditions on colour parameters has been found. The correlation study between parameters suggests that the parameters b^* and L^* could be used as suitable indicators of evolution or oxidation stage of red wines.

Keywords Red wine · Colour · Copigmentation · Antioxidant activity

J. Heras-Roger
Wine Analysis Laboratory of Island Council of Tenerife, C/ Teobaldo Power, 11, 38500 Güimar, Tenerife, Spain

M. Pomposo-Medina · C. Díaz-Romero · J. Darias-Martín (✉)
Department of Chemical Engineering and Pharmaceutical Technology, Food Science and Technology Area, University of La Laguna, 38201 La Laguna, Tenerife, Spain
e-mail: jdarias@ull.es

Introduction

The colour of red wine is a quality parameter which is taken into consideration by producers and consumers [1] and the first organoleptic factor observed by the taster. Many studies have correlated this factor with the general quality of the wine because it can provide useful information about possible deficiencies during the winemaking process [2] and changes in the ageing of the wine [3]. Colour parameters have long been determined by using the Glories indexes, colour intensity ($A^{420} + A^{520} + A^{620}$) and hue ($A^{420}/A^{520}$). In the last decades, CIELab space [4] has also been used for a more accurate wine colour description. Both methods offer information of interest to red winemaking.

The colour of red wine is mainly due to its content of three different groups of compounds: free anthocyanins, polymeric pigments and copigmented anthocyanins [5]. The bright red of young wines is mainly due to free monomeric anthocyanins; however, during wine ageing, the anthocyanins are partly condensated in oligomeric and polymeric pigments which are more stable and show more evolved hues. Nonanthocyanin phenolic compounds (mainly hydroxycinnamic acids, flavanols and flavonols) also affect colour characteristics through copigmentation with anthocyanins [5]. Many factors influence the colour of wines, the first of which is the nature of the phenolic compounds and their reactivity, besides this, there are other factors involved such as pH [6], temperature [7], SO_2, grape variety [8], geographical origin [9], vintage [10], winemaking technique [11] or other compounds present in wine such as iron [12] or potassium [13] among other factors.

There has been growing interest in research into the copigmentation phenomenon in red wine [14–17]. The copigment, normally a colourless organic compound, predominantly associates with the coloured forms of

anthocyanins, displacing their equilibrium towards coloured forms of the flavylium cation. This produces an increase in the colour intensity of red wines. Knowledge of copigmentation is of interest in red wine production because it could produce an improvement of the colour intensity and stability.

The contribution of the copigmentation to the colour is determined according to the Boulton protocol [18]. Moreover, other authors [19] have developed different tests using tristimulus colourimetry to quantify the copigmentation phenomenon.

The grape variety, the ageing time of wines and the production zones where the vines are cultivated are only some of the factors to consider, because of their influence on the final phenolic composition of red wines. The colour, the copigmentation level and the antioxidant activity of wines are also determined by the final phenolic composition.

There are few data about how the following factors—copigmentation, colour and antioxidant activity—are related to each other. In the present study, several parameters have been determined associated with the colour, antioxidant activity and copigmentation in young and shortly aged bottled red wines produced with single-cultivar grapes from the main wine producers of the Canary Islands, in order to characterize them and establish relationships between all the analysed parameters.

Materials and methods

Wine samples

A hundred and thirty-six single-cultivar red wines of the Canary Islands (Spain), produced during the period 2006–2012, were used in the present study. All the samples were provided by the local Denomination of Origin Certification Councils, to ensure the geographical origin and the grape cultivar authenticity of the wines. The cultivars used were as follows: Listan negro, Negramoll, Baboso, Tintilla, Vijariego, Merlot, Ruby cabernet and Syrah.

Analytical methods

Conventional parameters

Conventional physicochemical parameters with enological significance were determined (Table 1). The methodology used for analysis was in accordance with official methods [20]. Glucose–fructose and malic and lactic acid were determined according to the OIV method [21]; tartaric acid was determined by reaction with ammonium metavanadate and measuring the absorbance at 500 nm. All determinations were performed in duplicate.

Copigmentation and colour

Copigmentation

Copigmented anthocyanins, free anthocyanins and polymeric pigments were determined and calculated according to Boulton [18]. This method modifies the method described by Sommers and Evans [22], introducing the copigmented anthocyanins in the determination. Wine samples were firstly adjusted to pH 3.6. Total wine colour is assumed to be A^{acet}, 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^{SO_2}, 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^{20}, the absorbance measured at 520 nm of the wine sample diluted 1:20 with a buffer solution (24 ml of ethanol is added to 176 ml distilled water, dissolve 0.5 g of potassium bitartrate into the solution. The pH of solution is adjusted to 3.6 with HCl or NaOH solutions as needed). The reading is corrected for the dilution by multiplying by 20, and this 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: percentage of colour due to copigmented anthocyanins $= [(A^{acet} - A^{20})/A^{acet}] \times 100$; percentage of colour due to free anthocyanins $= [(A^{20} - A^{SO_2})/A^{acet}] \times 100$; percentage of colour due to polymeric pigments $= (A^{SO_2}/A^{acet}) \times 100$.

Chromatic characteristics

Ultraviolet and visible spectrophotometric determinations were carried out using a Lambda 11 PerkinElmer spectrophotometer. Absorbance at 280 nm (total phenols) was measured. Colour density ($A^{420} + A^{520} + A^{620}$) and colour hue ($A^{420}/A^{520}$) were determined, and spectra from 380–780 nm, to calculate the tristimulus values and the CIELab space, were recorded. The CIELab parameters h^*, L^*, C^*, a^* and b^* were determined following the recommendations of the Commission Internationale de L'Eclariage [23] and OIV [4]: the 10° Standard Observer and the Standard Illuminant D65. The colour of the wines was determined by the Colvin software of PerkinElmer.

Antioxidant activity

Antioxidant activity was determined using the method described by Brand-Williams [24] modified by Rivero-Pérez et al. [25]. The method measures the

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical activity through the extinction of its maximum absorption at 517 nm. Samples are filtered (0.45 μm pore) and diluted at 1:50 rate in water. One hundred microlitres of the diluted sample reacts with 4.9 ml of 60 μM DPPH (Sigma Aldrich) solution in methanol prepared the same day. Oxidative reaction takes place for 2 h, and after this time, A^{517} nm is obtained using a PerkinElmer λ25 UV–vis spectrophotometer. Methanol (Panreac, Spain) is used to adjust zero, and every sample has been analysed in triplicate, only values with less than 10 % variability have been considered. The results are expressed as mM Trolox equivalent antioxidant activity using a calibration curve determined in our laboratory.

Statistics

All the statistics were performed by means of the SPSS version 17.0 software for Windows. The Kolmogorov–Smirnov test was applied to verify whether the distribution of the variables was normal ($P < 0.05$). When the statistical distribution was not normal, the nonparametric Kruskal–Wallis test was applied to discover any significant differences between the mean values of the groups of samples. Correlation analysis was carried out to study relationships between variables. ANOVA was conducted with cultivars as a random factor in order to establish differences.

Results and discussion

Enological parameters

The data on the conventional composition are shown in Table 1. All parameters are in the normal ranges for red wines. Six samples showed higher values of density than the normal range for dry wines (<1,000 mg/l). These six wine samples were sweet red wines and ranged from 1,010 to 1,050 mg/l. Fifty-two per cent of the wines showed a high ethanol content (ethanol content ≥ 14°), which corresponds with the current trend to obtain red wines with a high phenolic compound content. The pH values were slightly high, and the titrable acidity values were in the range of the normal content found in Spanish wines [26] and Canary wines [27]. Most of the wines analysed showed a sugar content of less than 5 g/l, and only the sweet red wines had more than 10 g/l of glucose and fructose. The malic acid content in red wine decreases after the malolactic fermentation (MLF). When the malic acid content decreases below 2 g/l, it can be deduced that the MLF has been completed. The mean malic acid value obtained in this study, 0.36 mg/l, is in agreement with other investigators such as Díaz et al. [27] and suggests that the MLF has been completed in most of the analysed red wines. However, there are some wine samples that had a malic acid content above 2 g/l, which demonstrates that MLF was incomplete in some winemaking cases. The lactic and tartaric acid contents fall within normal values according to the literature [28].

In general, the conventional parameters were not significantly different according to single-cultivar red wines. The cultivars with lower alcohol contents were, Listan negro, Negramoll, Tintilla and Ruby cabernet, with an alcoholic graduation of around 13.56, and significant differences with the rest of the wines were obtained from the other cultivars.

Table 1 Conventional composition

	Mean value	SD	Range
Density (g/ml)	0.995	0.007	0.990–1.051
Alcoholic degree	14.08	1.34	11.00–19.54
pH	3.73	0.16	3.23–4.05
Total acidity (g/l)	5.40	0.78	4.01–7.59
Glucose + fructose (g/l)	2.83	8.73	0–59.00
Malic acid (g/l)	0.36	0.54	0–3.37
Lactic acid (g/l)	1.49	1.18	0–5.53
Tartaric acid (g/l)	2.00	1.17	0–4.69

Parameters of colour, phenolic compounds and antioxidant activity

Chromatic characteristics

There is a wide range of red wine colours, which can be described as follows: violet red, purple red, garnet, cherry red, ruby red, brick red and red brown. The violet red colour corresponds to the less evolved wines, and the red brown colour to the more evolved wines [29, 30]. A number has been assigned to these colours in the present study, the violet red colour is number 1, the purple red colour is number 2, and so on. These data and other parameters related to the colour of the wines for each single red wine cultivar are shown in Table 2. The wines obtained from Merlot, Ruby cabernet and Shyrah grape cultivars are grouped and significantly differentiated from the rest of wines obtained from other grape cultivars in the values of b^*, L^*, h_{ab} and colour density. The yellow coordinate (b^*) and the angle (h_{ab}) formed by the coordinates a^* and b^* were lower in Merlot, Ruby cabernet and Shyrah wines, which indicates that the colour tonality of these cultivars is closer to the violet red colour. The presence of less evolved colour tones, with a higher contribution of blue colour, is commonly associated with a higher percentage of copigmented anthocyanins [5]. The Merlot, Ruby cabernet and Shyrah wines presented a higher colour density and lower lightness than the rest of

Table 2 Colour parameters for each single red wine cultivar

Cultivar	a^*	b^*	L^*	h_{ab}	Colour density $A^{420} + A^{520} + A^{620}$	Hue A^{420}/A^{520}	Colour number
Baboso ($n = 30$)	38.66	29.21^{b}	20.29^{bc}	28.41^{bc}	9.58^{bc}	0.83^{b}	2.27^{ab}
Listan negro ($n = 29$)	31.50	40.96^{c}	26.14^{c}	29.96^{bcd}	7.61^{ab}	0.78^{b}	2.00^{a}
Merlot ($n = 10$)	36.30	17.05^{a}	9.93^{a}	23.22^{ab}	11.93^{de}	0.82^{b}	1.40^{a}
Negramoll ($n = 13$)	39.26	34.00^{bc}	26.56^{cd}	33.05^{c}	5.96^{a}	1.00^{c}	3.23^{c}
Ruby cabernet ($n = 11$)	31.60	20.06^{ab}	10.14^{a}	20.57^{a}	13.25^{ef}	0.64^{a}	1.2^{a}
Syrah ($n = 12$)	36.77	15.27^{a}	8.90^{a}	21.04^{a}	14.85^{d}	0.65^{a}	1.33^{a}
Tintilla ($n = 14$)	33.65	28.89^{b}	16.28^{b}	26.88^{b}	10.37^{c}	0.79^{b}	1.50^{a}
Vijariego ($n = 17$)	40.13	39.07^{c}	26.48^{c}	34.17^{d}	7.15^{a}	0.84^{b}	3.12^{c}

Results in the same vertical line with the same superscript were not significantly different ($p < 0.05$)

the wines considered here; therefore, they are wines with a higher amount of colouring matter. The wines obtained from the other grape cultivars showed several colour tones, with the exception of wines produced with the Tintilla cultivar which were mostly violet red. An inverse relationship between the wine colour and colour density can be observed. In general, the wines with a lower colour density show more oxidized colour tones. The coordinate a^* and the ratio between the absorbances to 420 and 520 nm were the parameters with less discriminatory power.

Copigmentation

The method developed by Boulton [18] divides the colour of red wine into three categories: the colour due to the copigmented anthocyanins, the free anthocyanins and the polymeric pigments. In young red wines, the fraction of the colour due to copigmentation can be as high as 50 % [5]. In the present study, the Ruby cabernet cultivar had the highest percentage of colour attributable to copigmentation, 31 % on average, followed by the Listán negro, Syrah and Merlot wines with mean values ranging between 16 and 22 %, and the rest of the cultivars showed lower copigmentation levels (Table 3), similar to another study with young red wines [31]. In a previous study with Listan negro wines [16], the contribution percentage to the colour of the copigmented anthocyanins varied between 19.1 and 25.5 % (mean of 22.3 %) after 1 year and between 15.7 and 21.3 % (mean of 18.5 %) after 2 years of ageing. However, it should be mentioned that in another study [17], the Cabernet sauvignon wines showed a copigmentation level ranging from 42 %, at the end of the alcoholic fermentation, to 0 % 9 months later, with Cencibel red wines ranging from 32 to 0 % and the Syrah wines ranging from 44 to 5 %.

As regards colour due to the free anthocyanins, the mean values of the cultivars ranged from 33 to 42 %, and in the case of the polymeric pigments, the mean values were from 27 to 58 %. Wines made with the Vijariego, Negramoll, Tintilla and Baboso cultivar grapes showed the highest colour percentages due to polymeric pigments and had the lowest amounts of copigmented anthocyanins, suggesting that the copigmented anthocyanins synthesized in the first steps of the winemaking process may later produce polymeric pigments. No significant differences in the total phenols between the single-cultivar wines were observed.

Copigmentation levels in wines are clearly and largely determined by the cultivar grape and the ageing time, but there are other factors such as soil, climatic conditions and winemaking techniques that may also influence the copigmentation levels. With respect to the relationship between the copigmentation and tonality, one can observe in Tables 2 and 3 that wines obtained from cultivar grapes with less evolved colour tones generally presented higher amounts of copigmented anthocyanins than wines produced from cultivars with more evolved tones. A relationship between the copigmentation levels and flavonol contents (A^{365}) was not detected.

The values of antioxidant activity found in the Canary red wines, expressed as mM Trolox (14.75 ± 6.48 and range 4.56–48.72), were similar to those reported by Rivero-Pérez et al. [25] and Gómez-Gallego et al. [31] in Spanish wines and were higher than the values described by other authors [32–35]. The highest values of antioxidant capacity were observed in wines from Tintilla and Merlot cultivars (Table 3).

Ageing time

The influence of the ageing time can be observed in some data shown in the Table 4. The wines were divided into three groups: more than 3 years of age, 1–3 years and the young wines (less than 1 year). The colour due to the copigmented anthocyanins diminishes with the ageing time in a more marked manner than the free anthocyanin; and on the other hand, the colour due to the polymeric pigments increases, reinforcing the idea that the copigmented

Table 3 Copigmentation and antioxidant activity for each single red wine cultivar

X_C, percentage of colour attributable to copigmentation; X_A, percentage of colour due to the free anthocyanins; X_P, percentage of colour due to the polymeric pigments

Results in the same vertical line with the same superscript were not significantly different ($p < 0.05$)

Cultivar	X_C	X_A	X_P	A_{280}	A_{365}	Antioxidant activity (mM Trolox)
Baboso ($n = 30$)	14.41^{ab}	39.89^{ab}	45.57^{bcd}	60.32^{bcd}	7.77^{a}	14.70
Listan negro ($n = 29$)	22.31^{b}	36.09^{ab}	41.59^{bc}	50.53^{ab}	7.63^{a}	12.63
Merlot ($n = 10$)	15.78^{ab}	40.43^{ab}	43.80^{bc}	64.76^{d}	10.46^{b}	18.02
Negramoll ($n = 13$)	10.96^{a}	37.85^{ab}	51.35^{cd}	47.22^{a}	7.51^{a}	12.33
Ruby cabernet ($n = 11$)	31.39^{c}	41.86^{b}	26.76^{a}	52.60^{abcd}	8.46^{a}	14.98
Syrah ($n = 12$)	22.12^{b}	41.51^{b}	36.45^{ab}	60.33^{bcd}	10.75^{b}	15.49
Tintilla ($n = 14$)	15.22^{ab}	34.48^{ab}	50.30^{cd}	63.29^{cd}	8.11^{a}	18.64
Vijariego ($n = 17$)	9.32^{a}	32.88^{a}	57.80^{d}	52.59^{abc}	6.61^{a}	16.45

Table 4 Influence of the ageing time

Age	X_C	X_A	X_P	L^*	Hue A^{420}/A^{520}	h_{ab}	Colour number
More than 3-year-old wines ($n = 35$)	10.06^{a}	33.34^{a}	56.60^{c}	26.42^{c}	0.94^{c}	32.71^{c}	2.91^{c}
1–3 years wines ($n = 57$)	19.15^{b}	36.56^{a}	44.28^{b}	21.11^{b}	0.80^{b}	28.64^{b}	2.16^{b}
Young wines (1 year or less) ($n = 29$)	20.40^{b}	44.69^{b}	34.91^{a}	13.94^{a}	0.67^{a}	23.95^{a}	1.38^{a}

Results in the same vertical line with the same superscript were not significantly different ($p < 0.05$)

anthocyanins contribute to the formation of polymeric pigments.

Lightness (L^*), which is a quantitative component of the colour, clearly increases with the ageing of the wines, and the colour density (data not shown), another quantitative component, diminishes with the ageing time.

The qualitative colour parameters (qualitative component) include h_{ab}, hue (A^{420}/A^{520}) and the colour, and provide information about the quality or tonality of the colour. The h_{ab} and tonality increase with ageing, which is due to the relative increase in the yellow component. This is reflected in the changes of the colour in wines with ageing, which generally evolve from violet red to tones closer to red brown.

Influence of climatic conditions

The wine samples used for this study were from the ten appellations in the Canary islands studied here. It is important to emphasize that there are climatic differences between them, in the Lanzarote, Valle de Güimar and Abona appellations, the climate is warmer with higher average temperatures and greater exposure to sunlight. In order to study this influence, the wines were divided into two groups, the warmer group and the rest, and an independent samples test was applied (Levene's test for equality of variances). Significant differences were found in colour and copigmentation parameters. The most significant parameters to differentiate the wines into two groups are shown in Table 5. The warmer group tended to differentiate from the rest of the appellations, with lower values of the b^*, L^*, angle h_{ab}, hue (A^{420}/A^{520}) and polymeric pigments, and higher values of colour intensity, content of flavonol cofactors (A^{365}), free anthocyanins and copigmented anthocyanins. This implies that the wines belonging to these three appellations have a less evolved colour tonality, nearer to violet red, and a more intense colour. Since the proportion of grape varieties varies between the climatic areas, a two-way variance analysis was applied on the selected parameters, using the two climatic regions mentioned above and the variety as factors, in order to try to explain the differences observed. For all the parameters studied, except flavonol content, there is no interaction between the two factors (data not shown). Therefore, it can be deduced that the production region associated with the climatic conditions is an influence on the colour of the wine.

Correlation study

A correlation study between all the parameters studied was performed, and only the most relevant correlations are commented. The yellow coordinate (b^*) is correlated with lightness (L^*) ($r = 0.864^{**}$). The lightness (L^*) showed an inverse correlation with the colour intensity ($r = -0.807^{**}$) and a direct correlation with the angle h_{ab} ($r = 0.825^{**}$) and with the number assigned to the colour ($r = 0.682^{**}$). These results suggest that the parameters b^* and L^* could be suitable indicators of the evolution stage of the red wines. When the value of both parameters increases in a wine, the wine loses colour intensity and evolves to a more evolved colour. The tonality colour or hue (A^{420}/A^{520}) showed a correlation ($r = 0.733^{**}$) with the number

Table 5 Influence of climatic conditions

Results in the same vertical line with the same superscript were not significantly different ($p < 0.05$)

Climatic area	b^*	L^*	Colour density $A^{420} + A^{520} + A^{620}$	h_{ab}	A^{365}
Warm zone ($n = 42$)	20.73^a	14.38^a	11.28^b	24.58^a	8.96^b
Cold zone ($n = 79$)	37.44^b	24.41^b	8.12^a	30.87^b	7.51^a

assigned to the colour and with the percentage of colour due to polymeric pigments ($r = 0.688$**). This could be explained because when the wines age, their polymeric pigment contents increase and evolve to more oxidized colour tonalities.

The antioxidant activity of the analysed wines appears to be highly correlated with the total phenolic index (A^{280}) ($r = 0.638$**). Other authors [36–38] have pointed out that the total phenolic content is closely related to antioxidant activity, which agrees with that observed here, confirming the role of phenolic compounds in the antioxidant activity.

Considering the determinations of the Boulton method [18], the polymeric pigment percentage is inversely correlated with the copigmented anthocyanin percentage ($r = -0.841$**) and with the free anthocyanins ($r = -0.694$**). A clear and direct association between the copigmentation and the evolution stage of the wine can be observed. Wines with a higher copigmentation are less oxidized, i.e. they have a lower amount of polymeric pigments. This fact agrees with that suggested by Boulton [5] about the role of copigmentation in ageing reactions of red wines. As parts of the flavonoid compounds are involved in the copigmentation, they are less likely to be oxidized. Thus, the rates of polymerization would be slower in wines with high levels of copigmentation than an equivalent wine without any copigmentation; therefore, they are wines with a higher stability.

Conflict of interest None.

Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.

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