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Influence of the physiological stage and the content of soluble solids on the anthocyanin extractability of *Vitis vinifera* L. cv. Tempranillo grapes

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ABSTRACT

The influence and the extent of the anthocyanin extractability from grape skins at different physiological stages and different soluble solids contents within each stage were investigated. For this purpose three different physiological stages (pre-harvest, harvest and over-ripening) and three soluble solids contents (22, 24 and 26 °Brix) within each stage were taken into account using *Vitis vinifera* L. cv. Tempranillo.

This study revealed the influence of physiological stages and soluble solids contents on the amounts of anthocyanins. The most important factor was the physiological stage although the soluble solid contents effect was also relevant, especially for anthocyanins monoglucosides. The highest contents in anthocyanins were found at pre-harvest, following by harvest and finally at over-ripening. Within each physiological stage, the relative abundance of acylated derivatives was influenced by the soluble solids contents. The percentages of acylated anthocyanins tend to decrease as the soluble solids contents.

Differences in the anthocyanin extractabilities were highly influenced by the physiological stages and in a lesser extent by the soluble solids contents. The lowest extraction percentages were obtained in pre-harvest samples whatever soluble solids contents. Over-ripening samples present the lowest contents of anthocyanins but these compounds are easily released from tissues to the hydroalcoholic solution providing the highest extraction percentages. Generally, non-acylated anthocyanins were better extracted than the acylated anthocyanins.

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1. Introduction

Phenolic composition of grapes has an important role in the definition of organoleptic properties of wines [1–3]. Anthocyanins are responsible for the colour of red wines and their interactions with other phenolic compounds largely determine the colour changes observed during ageing [4].

Grapes are a nonclimateric fruit and berry growth follows three phases, where two phases of rapid growth were separated by a plateau in-between. According to Coombe [5] phase I can be characterized by a rapid increase in the berry volume and seeds almost fully grown. Phase II is characterized by a decrease in the growth rate. Finally, phase III, is the ripening stage and it is characterized by the second period of berry growth, when sugars are rapidly accumulated. Biosynthesis and accumulation of anthocyanins in grape skins start at veraison (the interception between phases II and III) and present a maximum around harvest but a decline in these compounds has also been described just before harvest or during over-ripening [5]. Factors such as variety, growing region, climate,

and growth conditions could influence the levels of anthocyanins [6–11].

It is generally accepted that extractability of anthocyanins from grape skins increases throughout grape ripening as consequence of the degradation of the cellular wall by pectolytic enzymes [12]. In addition, differences on contents of polysaccharides based on galactose and arabinose, together with changes on the cellulose content and degree of methylation of pectins might also be responsible for the different extractabilities of these phenolic compounds [13]. Cell wall composition is also responsible for its physical properties which are also linked to the anthocyanin extractability. Some studies have considered the relationship between anthocyanin extractability and grape skins mechanical parameters such as hardness, thickness, and break force [14–19]. The highest influence on anthocyanin extractability was attributed to vineyards of origin and little influence of the levels of soluble solids was observed [16]. Moreover, anthocyanin extractability has also been studied throughout ripening using *Vitis vinifera* L. cv. Shiraz and it remained constant whatever the physiological stage of berries [20]. On the other hand, the anthocyanin extractability of Galician red cultivars (Sousón, Brancellao, Ferrol, Merenzao, Mouratón and Mencía) increased during the ripening step until reaching a maximum value, and then it began to diminish. In addition, grape variety has shown a

Table 1
Characteristics of berries.

Sample	Berry weight (g)	Skin weight (g)	Skin moisture (weight %)	Seed weight (g)	Seed moisture (weight %)
P-22	1.86	0.37	67.8	0.09	28.8
P-24	2.03	0.44	66.4	0.08	27.3
P-26	1.86	0.36	63.6	0.07	25.9
H-22	2.03	0.54	72.0	0.08	27.9
H-24	2.07	0.47	69.0	0.08	24.0
H-26	1.99	0.36	64.0	0.07	22.9
O-22	1.64	0.35	69.8	0.08	28.5
O-24	1.74	0.47	70.8	0.08	25.4
O-26	1.83	0.37	65.8	0.07	22.9

great influence on extractability of anthocyanins in these Galician cultivars [21]. This varietal influence on anthocyanins extraction has been previously described for Cabernet Sauvignon, Merlot, Shiraz and Monastrell cultivars [22].

Furthermore, at each ripening stage soluble solids of berries present a Gaussian bell-shaped distribution and this heterogeneity of grapes which may influence the final wine composition and quality and therefore it should be considered at harvest [23].

The aim of this study was to determine the influence and the extent on the anthocyanin extractability of different physiological stages and soluble solids heterogeneity within each stage. For this purpose three different physiological stages (pre-harvest, harvest and over-ripening) and three soluble solids contents (22, 24 and 26 °Brix) within each stage were taken into account using *V. vinifera* L. cv. Tempranillo which is the most often used variety to produce quality red wines in Spain.

2. Material and methods

2.1. Grape samples

V. vinifera L. cv. Tempranillo red grape samples were collected from a vineyard located in Lleida (Spain) which is under the influence of Mediterranean climate. The samples were collected at three different physiological stages during 2009: pre-harvest (August 25), harvest (September 16) and over-ripening (October 5). In the studied samples notations **P**, **H** and **O** were used to indicate pre-harvest, harvest and over-ripening respectively. Thirty clusters were collected at each stage and their grapes were sorted according to their density. The density of grapes was estimated by flotation of berries in different NaCl solutions (130–150, 150–170, and 170–190 g L^{-1}) corresponding to different solids contents (22, 24 and 26 °Brix respectively). Table 1 shows some berry characteristics (i.e. berries, skins and seeds weights and moisture percentage of skins and seeds) obtained from 100 grapes.

2.2. Anthocyanins extractions

Grape skins were separated manually from the whole grapes and two subsamples were taken from each sample, one for the exhaustive methanolic extraction and the other one for the hydroalcoholic extraction.

2.2.1. Exhaustive methanolic extraction

Ten grams of grape skins were macerated at 4 °C in methanol containing 0.1% of HCl 12 M until colourless of skins. Methanolic phases were successively pooled, a few milliliters of water were added and the extract was concentrated under vacuum at 30 °C until methanol was removed and finally made up to 100 mL with ultrapure water. The aqueous extract was diluted 1:2 with HCl 0.1 M, filtered through 0.45 μm pore-size filters and directly

injected into the chromatographic system to determine anthocyanins.

2.2.2. Hydroalcoholic extraction

Ten grams of grape skins were macerated in 100 mL of model wine solution (4 g L^{-1} tartaric acid, 12.5% ethanol, adjusted at pH 3.6 with NaOH 0.5 M). The vials were kept at 25 °C and darkness throughout the experiment. At regular intervals (1, 3, 5 and 7 days) 250 μL of extraction medium were taken, diluted 1:2 with HCl 0.1 M, filtered through 0.45 μm pore-size filters and directly injected into the chromatographic system to determine anthocyanins.

2.3. Chromatographic analysis

Chromatographic analyses were performed on an Agilent Technologies 1200 Series RRLC equipped with an auto-injector, 1200 binary pump SL, column heater, diode array detection (DAD) and data treatment station. A Varian® C18 column (2 mm × 50 mm, 2.8 μm particle size) thermostatted at 35 °C was used. Solvents were (A) 0.1% trifluoroacetic acid, and (B) 100% HPLC grade acetonitrile. The elution profile was as follows: from 10% to 10.6% B for 3 min, from 10.6% to 15% B for 0.5 min, from 15% to 20% B for 0.5 min, from 20% to 22.4% B for 3 min and from 22.4% to 40% B for 0.5 min. The flow-rate was 1.1 mL min^{-1} and the injection volume was 20 μL. The UV–vis spectra were recorded from 220 to 600 nm with a bandwidth of 2 nm. The preferred detection wavelength was 520 nm. Anthocyanins were identified according to their spectroscopic and chromatographic features which had been previously acquired in our laboratory and the quantification was carried from the peak areas at the aforementioned preferred detection wavelength. All analyses were performed in triplicate. Chemical structures of the analysed anthocyanins and their retention time are provided in Fig. 1.

2.4. Analysis of chromatic parameters

An Agilent Technologies UV–vis spectrophotometer 8453 was used for scanning between 380 and 770 nm at 1 nm intervals with a 2 mm path-length quartz cell. At the first and seventh days an aliquot of hydroalcoholic extracts was taken and its spectrum was recorded. Reference blank measurements were made with the cuvette filled with model wine solution. CIE 1964 10° standard observer and the CIE D_{65} illuminant were used as references to calculate the tristimulus values recommended by the Comission Internationale de l'Éclairage [24]. The CIELAB space was used and parameters measured included: Lightness (L^*), red–green coordinate (a^*, $-a^*$), yellow–blue coordinate (b^*, $-b^*$). From a^* and b^* coordinates another two colour parameters are defined: the hue angle (h_{ab}) and chroma (C_{ab}^*) which indicate qualitative and quantitative aspects of colour respectively. Calculations were made using the CromaLab® software [25].
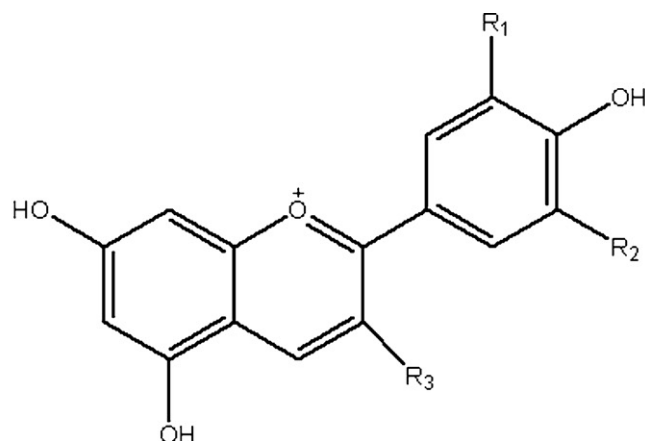
N°	Rt$_{min}$	Compound	R$_1$	R$_2$	R$_3$
1	0.934	Delphinidin-3-*O*-glucoside	-OH	-OH	-*O*-glucose
2	1.511	Cyanidin-3-*O*-glucoside	-OH	-H	-*O*-glucose
3	2.164	Petunidin-3-*O*-glucoside	-OCH$_3$	-OH	-*O*-glucose
4	3.380	Peonidin-3-*O*-glucoside	-OCH$_3$	-H	-*O*-glucose
5	4.371	Malvidin-3-*O*-glucoside	-OCH$_3$	-OCH$_3$	-*O*-glucose
6	5.551	Delphinidin-3-*O*-(6"-*p*-coumaroyl)glucoside(*trans*) + Malvidin-3-*O*-(6"-acetyl)glucoside	-OH -OCH$_3$	-OH -OCH$_3$	-*O*-(6"-*p*-coumaroyl)glucose (*trans*) -*O*-(6"-acetyl)glucose
7	5.914	Cyanidin-3-*O*-(6"-*p*-coumaroyl)glucoside (*trans*)	-OH	-H	-*O*-(6"-*p*-coumaroyl)glucose (*trans*)
8	6.077	Petunidin-3-*O*-(6"-*p*-coumaroyl)glucoside (*trans*)	-OCH$_3$	-OH	-*O*-(6"-*p*-coumaroyl)glucose (*trans*)
9	6.225	Malvidin-3-*O*-(6"-*p*-coumaroyl)glucoside (*cis*)	-OCH$_3$	-OCH$_3$	-*O*-(6"-*p*-coumaroyl)glucose (*cis*)
10	6.626	Peonidin-3-*O*-(6"-*p*-coumaroyl)glucoside (*trans*)	-OCH$_3$	-H	-*O*-(6"-*p*-coumaroyl)glucose (*trans*)
11	6.826	Malvidin-3-*O*-(6"-*p*-coumaroyl)glucoside (*trans*)	-OCH$_3$	-OCH$_3$	-*O*-(6"-*p*-coumaroyl)glucose (*trans*)

Fig. 1. Chemical structures and retention times of the eleven anthocyanins analysed in grape skin.

2.5. Chemometric analysis

The General Linear Model (GLM) analysis provides a comprehensive set of techniques for analysing any univariate or multivariate data. GLM uses the least square methods of the general linear model to estimate and test hypotheses about effects. Moreover, *post hoc* test (or multiple comparison tests) can be used to determine the significant differences between group means in an analysis of variance setting. In this case, the Tukey HSD test was selected to perform these comparisons.

On the other hand, unsupervised methods are applied to observe trends in the data indicating relationships between samples and between variables. The unsupervised pattern recognition method used for data analysis was principal components analysis (PCA), which was applied to the correlation or the covariance matrix of the original variables [26,27].

The SPSS 13.0 for Windows software package (SPSS, Inc., Chicago, IL) was used for data processing.

3. Results and discussion

3.1. Characteristics of berries

Characteristics of berries were assessed by measurements of berry skin and seed weights, and the percentages of moisture in skin and seed from 100 grapes per sample. Table 1 shows the results as data per single berry. Berry weights increased from pre-harvest to harvest and decreased at over-ripening for all the studied solid soluble contents. This pattern is in good agreement with that described by other authors and it had been mainly attributed to the loss of water [8,20], which could be also detected in our samples. Similar pattern was obtained for skin weights but it cannot be completely

attributed to the same phenomenon since moisture contents of these skins presented a slight increase from pre-harvest to harvest and then remained quite constant.

A decrease of skin moisture was observed from 22 °Brix to 26 °Brix within each physiological stage with the exception of the 24 °Brix over-ripening sample (O-24). Furthermore, seed weights remained quite stable and only slight differences were observed in moisture contents. Since seed development was almost completed at phases I and II of berry growth [5,28] and berries have been collected after these stages, non-important changes in seed physiological characteristics were expected.

3.2. Anthocyanins extractions

3.2.1. Exhaustive methanolic extraction

Fig. 2 shows the contents of the different anthocyanins obtained by the exhaustive methanolic extraction coupled to the chromatographic analysis. Data were expressed as chromatographic peak area/skin dry matter (%) to take into account the different levels of moisture in grape skins. The General Linear Model analysis and the Tukey HSD test were applied to these data. Results revealed the influence of physiological stages and soluble solid contents on the amounts of anthocyanins ($p < 0.05$). For each anthocyanin, different letters indicate statistical differences ($p < 0.05$) on its amount, not only among physiological stages but also among soluble solid contents (Fig. 2). This analysis also revealed the existence of interaction between the aforementioned main factors so the influence of one of the factors will depend on the levels of the other one. In all cases the most important factor, which presented the highest sums of squares term in the GLM analysis, was the physiological stage. The influence of physiological stage on the anthocyanins contents has been previously
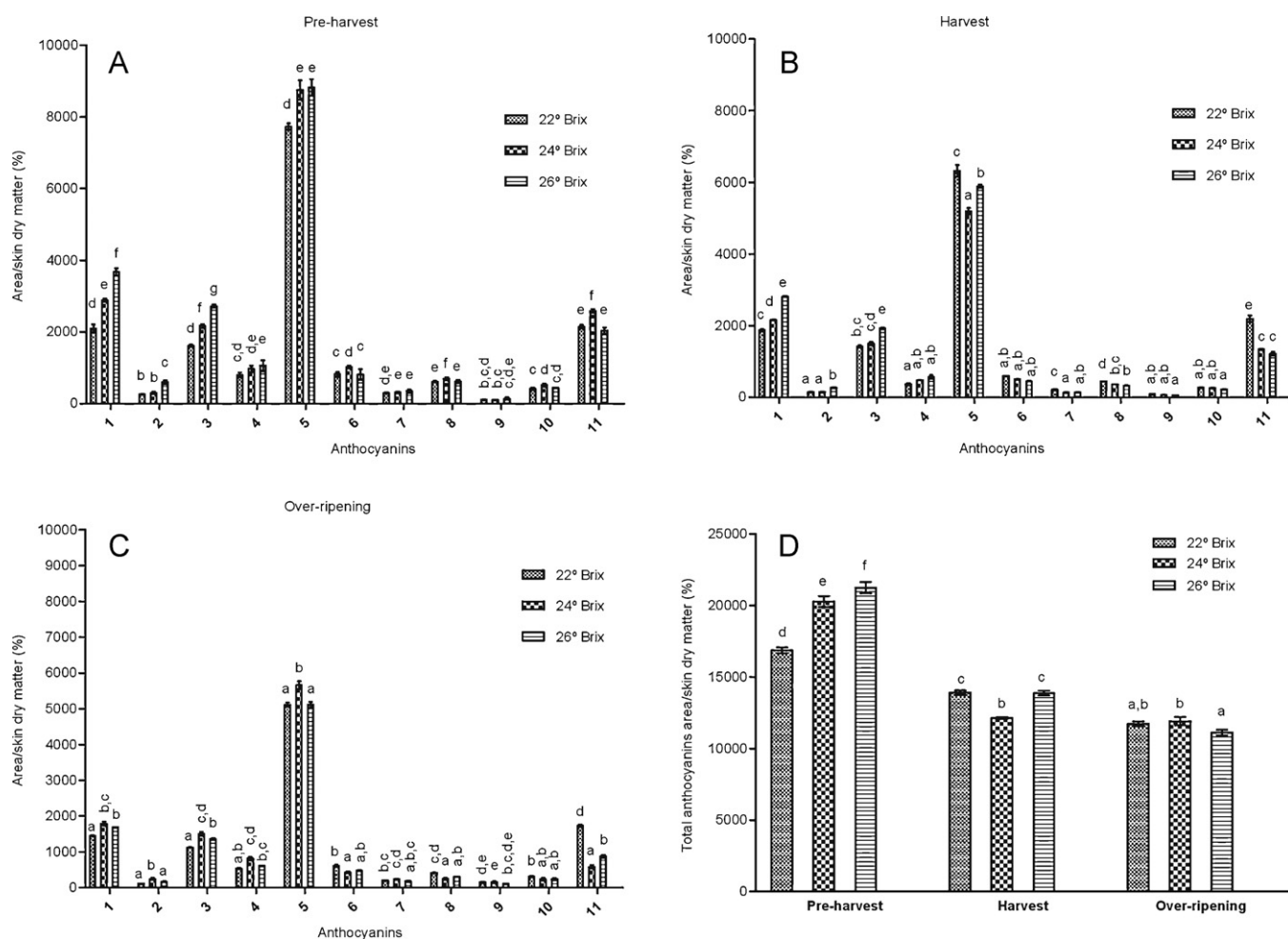
Fig. 2. Contents of the different anthocyanins obtained by the exhaustive methanolic extraction. (A) Pre-harvest, harvest (B), over-ripening (C) and total anthocyanins (D). Statistical analysis: General Linear Model analysis and Tukey HSD test (both $\alpha = 0.05$). For each anthocyanin different letters indicate statistical differences on its amount not only among physiological stages but also among soluble solids contents (°Brix).

described by other authors [8,29]. The soluble solid contents effect was also relevant, especially for anthocyanins monoglucosides, except for malvidin-3-*O*-glucoside which was more influenced by the interaction between the main factors (physiological stage and soluble solids contents). This interaction was also the second important factor for acylated anthocyanins although the influence of soluble solids contents was also presented, with the exception of cyanidin-3-*O*-(6″-*p*-coumaroyl)glucoside (*trans*) and malvidin-3-*O*-(6″-*p*-coumaroyl)glucoside (*cis*). Regarding the total anthocyanins contents (Fig. 2), the most important factors were the physiological stage, the interaction between the physiological stage and the content of soluble solids and finally the content of soluble solids (p <0.05). It is noteworthy that the highest amounts were found at pre-harvest, harvest and finally at over-ripening. This pattern has been previously observed by Ryan and Revilla [8] in different red grape cultivars such as Cabernet Sauvignon and also Tempranillo although these authors did not consider the extent and impact of the content of soluble solids on anthocyanin extractability.

Within each physiological stage only pre-harvest samples presented a clear increase of total anthocyanins from 22 to 26 °Brix. The total anthocyanin contents at harvest and over-ripening did not present a clear pattern regarding the content of soluble solids within each physiological stage. Table 2 shows the relative abundance of non-acylated glucoside derivatives in percentages. Non-acylated derivatives were predominant (between 70.9% and

84.1%) and these values are in agreement with those reported in Shiraz [20] and Tempranillo [29]. Moreover, the General Linear Model analysis and the Tukey HSD test were applied to these data and revealed the influence of physiological stages and soluble solids contents on these percentages. This analysis also revealed the existence of slightly interaction between the aforementioned main factors so the influence of one of the factors will slightly depend on the levels of the other one. Generally, the most important factor was the soluble solids contents. Different letters indicate statistical differences on percentages of non-acylated anthocyanins not only among physiological stages but also among soluble solids contents. The percentages of non-acylated anthocyanins increased as the soluble solids contents whatever the physiological state, with the exception of the highest percentage which was found in the 24 °Brix over-ripening sample (O-24). The formation of

Table 2
Percentage of non-acylated anthocyanins.

	Pre-harvest	Harvest	Over-ripening
22 °Brix	74.0[b,c]	72.7[b]	70.9[a]
24 °Brix	74.3[c]	78.2[d]	84.1[g]
26 °Brix	79.4[d,e]	82.6[f]	80.4[e]

Statistical analysis: General Linear Model analysis and Tukey HSD test (both $\alpha = 0.05$). Different letters indicate statistical differences on percentages of non-acylated anthocyanins not only among physiological stages but also among soluble solids contents.

Fig. 3. Extractability. Projection of the samples on the plane defined by the first and second principal components (A) and the corresponding loading plot (B). The numbers in the loading plot correspond to the compound listed in Fig. 1.

acylated anthocyanins is mediated by anthocyanin acyltransferases (AATs), which are anthocyanin-specific and catalyze the transfer of acyl group from activated acyl donors to the sugar moiety of anthocyanins [30–32]. The observed behaviour suggests that the decrease in the acyltransferase activity coincides with the highest levels of soluble solids contents in berries. This phenomenon could be explained as a negative co-correlation, which common factor might be sunlight exposure. Differences in soluble solids contents have been attributed to sunlight exposure which may enhance their photosynthetic accumulation [33,34]. Furthermore, the opposite effect has been observed on the relative percentage of acylated anthocyanins [35–37].

It is also noteworthy that 24 °Brix over-ripening sample (O-24) presents higher moisture content and percentage of non-acylated anthocyanins than expected according to the observed trend. This could suggest the existence of a relationship between them.

3.2.2. Hydroalcoholic extraction

At regular intervals (1, 3, 5 and 7 days) aliquots of each sample were taken and submitted to the chromatographic analysis. Anthocyanin extractability was calculated using the following equation in order to express the anthocyanin extractabilities as percentages of the exhaustive extraction:

$$\text{Extractability (\%)}_{\text{antho}_i} = \frac{(\text{Peak area}_{\text{antho}_i})_{\text{hydroalcoholic}}}{(\text{Peak area}_{\text{antho}_i})_{\text{exhaustive}}} \times 100 \quad (1)$$

The maximum percentages of extraction were achieved at the third day of maceration which is in accordance with Canals et al. [29]. At that time, the anthocyanin amount extracted in the hydroalcoholic solution was, on average, around 60%. This percentage is lower than previously reported in Shiraz which was in the order of the 70% [20]. Furthermore, principal component analysis was used as an unsupervised pattern recognition method and was applied to the covariance matrix constructed from the extraction percentages obtained form each sample throughout the time. Fig. 3 shows the projection of the samples on the plane defined by the first and second principal components and also the corresponding loading plot. The first principal component (PC 1) describes 49.87% of the variability in the data and the second (PC 2) describes 24.03%. In the score plot (Fig. 3A) each sample is represented by a code

indicating the day of extraction, physiological stage and soluble solids content respectively. The evolution of each sample is indicated by a line which links the different steps throughout the extraction time. As stated above, the maximum percentages of extraction were achieved at the third day of maceration and correspond to the more negative values of PC1 scores within the evolution of each sample. This score plot allows the visualisation of differences in the hydroalcoholic extractabilities of anthocyanins which are due to the different physiological stages and to the different soluble solids contents. Over-ripening samples present the most different pattern and all of them are in the zone of negative values of PC1. The highest extraction percentages were obtained in over-ripening samples, i.e. **O22**, **O24** and **O26**, and also in **H24**.

The lowest was obtained in sample **P26**. This may indicate that although technological maturity was reached, these berries did not present the optimal conditions for the maximum extraction of anthocyanins. The extractability of anthocyanins from grape skins may increase throughout grape ripeness as consequence of degradation of cellular wall [12]. Over-ripening samples present the lowest contents of anthocyanins but these compounds are easily released from tissues to the hydroalcoholic solution. From the obtained loadings (Fig. 3B), the original variables most related with the first principal component were the percentage of extraction of malvidin-3-O-(6″-p-coumaroyl)glucoside (trans) (**11**) and peonidin-3-O-(6″-p-coumaroyl)glucoside (trans) (**10**). The high level of extraction of these coumaroyl anthocyanins in over-ripening samples highly contribute to the observed pattern, especially on sample **O24**. The percentages of extraction of the different of non-acylated anthocyanins present a similar pattern among them with the exception of cyanidin-3-O-glucoside (**2**) which presents a pattern more closed to its coumaroyl derivate (**7**). Cyanidin-3-O-glucoside (**2**) presents a great influence on **H24** sample which presents the highest extraction ratio of this compound. Fig. 4 shows the percentages of extraction of non-acylated and acylated anthocyanins at the third day of hydroalcoholic maceration. Generally, non-acylated anthocyanins were better extracted than the acylated anthocyanins although this behaviour was not statistically significant (p > 0.05) in all cases (i.e. **P22** and **H22**). Sample **O24** presented the opposite trend. This behaviour was mainly influenced by the high extraction ratios of malvidin-3-O-(6″-p-coumaroyl)glucoside
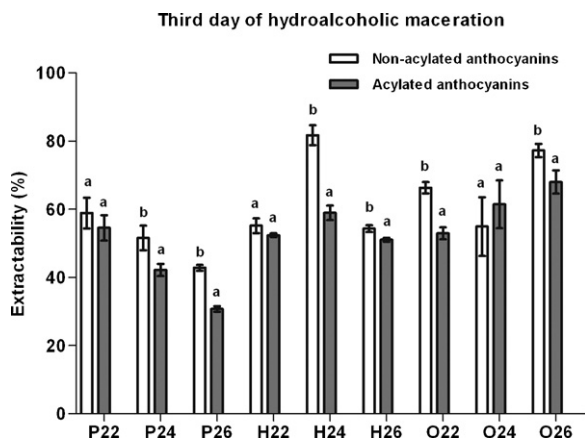
Fig. 4. Percentage of extractability of non-acylated and acylated anthocyanins on the third day of hydroalcoholic maceration. For each sample different letters indicate statistical differences ($p < 0.05$) between the extractabilities of non-acylated and acylated anthocyanins.

(*trans*) (**11**) and peonidin-3-*O*-(6″-*p*-coumaroyl)glucoside (*trans*) (**10**) as stated above. However, the trend of **O24** sample was not statistically significant ($p > 0.05$). It is noteworthy that the highest difference between non-acylated and acylated anthocyanin extractabilities was found in sample **H24** whose non-acylated anthocyanin extractability was highly influenced by the highest extraction ratio of cyanidin-3-*O*-glucoside (**2**) as stated above. The general behaviour presented in our study, which indicates better extractabilities of non-acylated anthocyanins, is consistent with previous studies [20].

3.2.3. Analysis of chromatic parameters

Due to anthocyanins are responsible for the colour of red wines, the chromatic parameters of the hydroalcoholic solutions were obtained in order to assess the impact of the physiological stage and the content of soluble solids on their colour.

The chromatic parameters (i.e. L^*, a^* and b^*) obtained the first and seventh days were represented in a three-dimensional plot (Fig. 5). Moreover, differences between two colour points (ΔE_{ab}^*) were also calculated using the following formula:

$$\Delta E_{ab}^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \tag{2}$$

These colour differences in the CIELAB space are Euclidian distances between their positions in the aforementioned colour space and are represented in by an arrow between two colour points. The human visual threshold is around $\Delta E_{ab}^* = 3$ [38]. Most of the samples presented significant colour differences among them ($\Delta E_{ab}^* > 3$) at the first day of extraction, with the exception of samples **1H22**, **1H26** and **1P22**. At the seventh day all samples were mainly located in three space zones. Each zone presented colour differences (ΔE^*) among samples lower than 3 CIELAB units in the created space. One of the created zones was formed by **7H24** and **7H26** samples, another space zone was formed by **7O22**, **7O24**, **7P22** and **7P24** and finally the last one was formed by **7P26** and **7O26**. The remaining sample (**7H22**) was located alone. The highest ΔE_{ab}^* values between the first and the seventh days of each samples were obtained for **P24** ($\Delta E_{ab}^* = 12.0$), **P22** ($\Delta E_{ab}^* = 9.5$) and **H26** ($\Delta E_{ab}^* = 8.9$) and the lowest for **H22** ($\Delta E_{ab}^* = 1.3$) and **O22** ($\Delta E_{ab}^* = 2.1$) as can be inferred from the length of the arrows which represent the ΔE_{ab}^* vectors. The maximum ΔE_{ab}^* values at each physiological stage were presented for 24 °Brix for pre-harvest and over-ripening samples and 26 °Brix for harvest samples. All pre-harvest samples presented a maximum influence of Δa^* on Eq. (2) whereas over-ripening samples presented their maximum influence on the ΔL^* term. The behaviour in harvest samples did not present a clear pattern.
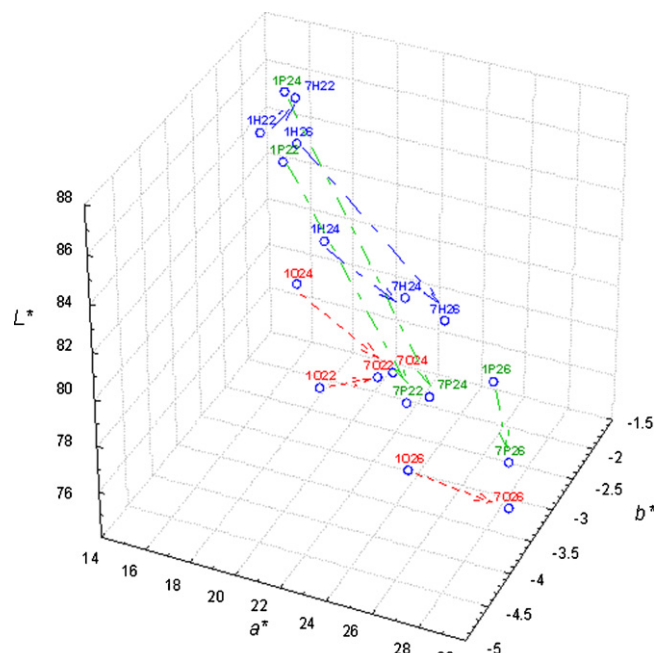

Fig. 5. Chromatic parameters. Projection of the samples in the three-dimensional space defined by L^*, a^* and b^*.

Fig. 6 shows the evolution of samples on the plane defined by hue angle (h_{ab}) and chroma (C_{ab}^*). Please, note that in this figure segments do not represent ΔE^* values as the parameters represented are h_{ab} and C_{ab}^*. Generally pre-harvest samples presented higher differences between the first and the seventh days of hydroalcoholic maceration in the C_{ab}^* parameter which indicates variations in the quantitative attribute of colour whereas harvest and over-ripening samples presented higher differences in the h_{ab} term which indicates variations in the qualitative attribute of colour. As stated above, the highest differences in the C_{ab}^* parameter were found in pre-harvest samples and they may be related with the lower speed of extraction of phenolic compounds as was previously stated. The different patterns are related not only to the different physiological stages but also to the different soluble solids contents. Nevertheless, no clear relationship between anthocyanins
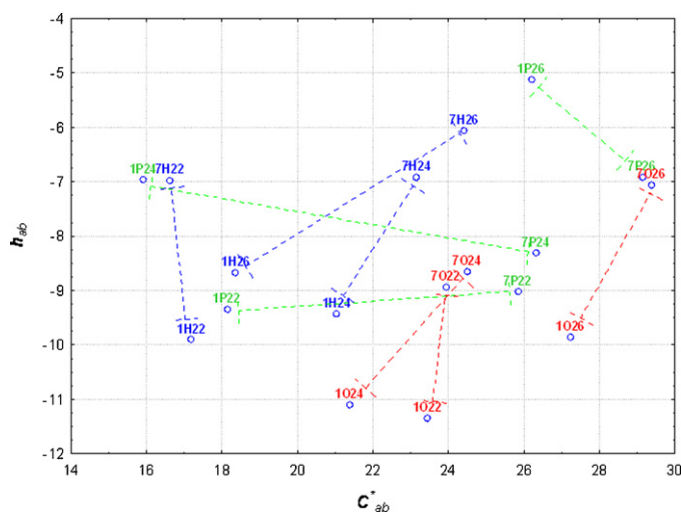

Fig. 6. Chromatic parameters. Projection of the samples on the plane defined by hue angle (h_{ab}) and chroma (C_{ab}^*). Please, note that in this figure segments do not represent ΔE^* values.

extraction and colour patterns has been found. This lack of relationship is in agreement with other studies [20,29] which have attributed this behaviour to copigmentation phenomena or polymeric pigments presence among others.

4. Conclusions

The contents of anthocyanins were highly influenced by physiological stages. On the other hand, the percentages of non-acylated anthocyanins increased as the soluble solids contents whatever the physiological state, with the exception of the highest percentage which was found in a 24 °Brix sample (over-ripening 24 °Brix sample).

The influence and the extent on the anthocyanin extractability at different physiological stages and soluble solids contents heterogeneity within each stage were investigated. Differences among anthocyanins hydroalcoholic extraction were attributed not only to the different physiological stages but also to the different soluble solids contents. This heterogeneity may influence wine composition and quality and therefore it should be taken into account at grape harvest. On the other hand, no clear relationship between anthocyanins extraction and colour patterns has been found probably due to effects such as copigmentation or polymeric pigments among others.

Nonetheless, a comprehensive study should be made in order to evaluate factors, such as different grape varieties, vintages and production areas.

Acknowledgements

References

[1] B.S. Sun, T. Pinto, M.C. Leandro, J.M. Ricardo-Da-Silva, M.I. Spranger, Am. J. Enol. Vitic. 50 (1999) 179–184.
[2] G. González-Neves, D. Charamelo, J. Balado, L. Barreiro, R. Bochicchio, G. Gatto, G. Gil, A. Tessore, A. Carbonneau, M. Moutounet, Anal. Chim. Acta 513 (2004) 191–196.
[3] M.V. Moreno-Arribas, C. Polo, Wine Chemistry and Biochemistry, Springer New York, New York, NY, 2008.
[4] R. Boulton, Am. J. Enol. Vitic. 52 (2001) 67–87.
[5] B.G. Coombe, Acta Hortic. 34 (1973) 261–273.
[6] N. Mateus, J.M. Machado, V. de Freitas, J. Sci. Food Agric. 82 (2002) 1689–1695.
[7] R. Mira de Orduña, Food Res. Int. 43 (2010) 1844–1855.
[8] J.-M. Ryan, E. Revilla, J. Agric. Food Chem. 51 (2003) 3372–3378.
[9] M.A Esteban, M.J. Villanueva, J.R. Lissarrague, J. Sci. Food Agric. 81 (2001) 409–420.
[10] M. Downey, N.K. Dokoozlian, M.P. Krstic, Am. J. Enol. Vitic. 57 (2006) 257–268.
[11] S. Guidoni, A. Ferrandino, V. Novello, Am. J. Enol. Vitic. 59 (2008) 22–29.
[12] P. Ribereau-Gayon, Y. Glories, A. Maujean, D. Dubourdieu, Handbookof Enology. The Chemistry of Wine Stabilization and Treatments, John Wiley and Sons Ltd., Baffins Lane, Chichester, 2000.
[13] A. Ortega-Regules, I. Romero-Cascales, J.M. Ros-García, J.M. López-Roca, E. Gómez-Plaza, Anal. Chim. Acta 563 (2006) 26–32.
[14] L. Rolle, F. Torchio, G. Zeppa, V. Gerbi, J. Int. Sci. Vigne Vin 42 (2008) 157–162.
[15] L. Rolle, F. Torchio, G. Zeppa, V. Gerbi, Am. J. Enol. Vitic. 60 (2009) 93–97.
[16] F. Torchio, E. Cagnasso, V. Gerbi, L. Rolle, Anal. Chim. Acta 660 (2010) 183–189.
[17] S. Río Segade, L. Rolle, V. Gerbi, I. Orriols, J. Food Compos. Anal. 21 (2008) 644–649.
[18] S. Río Segade, S. Giacosa, V. Gerbi, L. Rolle, LWT-Food Sci. Technol. 44 (2011) 392–398.
[19] S. Río Segade, E. Soto Vázquez, I. Orriols, S. Giacosa, L. Rolle, Int. J. Food Sci. Technol. 46 (2011) 386–394.
[20] D. Fournand, A. Vicens, L. Sidhoum, J.-M. Souquet, M. Moutounet, V. Cheynier, J. Agric. Food Chem. 54 (2006) 7331–7338.
[21] S. Río Segade, E. Soto Vázquez, E. Díaz Losada, J. Food Compos. Anal. 21 (2008) 599–607.
[22] I. Romero-Cascales, A. Ortega-Regules, J.M. Lopez-Roca, J.I. Fernandez-Fernandez, E. Gomez-Plaza, Am. J. Enol. Vitic. 56 (2005) 212–219.
[23] N. Kontoudakis, M. Esteruelas, F. Fort, J.M. Canals, V. De Freitas, F. Zamora, Food Chem. 124 (2011) 767–774.
[24] Colorimetry, in: Technical Report CIE 15.2, 3rd ed., CIE, Vienna, Austria, 2004.
[25] F.J. Heredia, C. Alvarez, M.L. Gonzalez-Miret, A. Ramirez, CromaLab, análisis de color, CromaLab, análisis de color Sevilla, Spain, 2004.
[26] D.L. Massart, B.G. Vandeginste, L.M. Buydens, P.J. Lewi, J. Smeyers-Verbeke, S.D. Jong, Handbook of Chemometrics and Qualimetrics, Elsevier Science Inc., 1998.
[27] R.G. Brereton, Chemometrics: Data Analysis for the Laboratory and Chemical Plant, J. Wiley, Chichester, West Sussex, England, 2003.
[28] J.A. Kennedy, M.A. Matthews, A.L. Waterhouse, Phytochemistry 55 (2000) 77–85.
[29] R. Canals, M.C. Llaudy, J. Valls, J.M. Canals, F. Zamora, J. Agric. Food Chem. 53 (2005) 4019–4025.
[30] T. Nakayama, H. Suzuki, T. Nishino, J. Mol. Catal. B: Enzym. 23 (2003) 117–132.
[31] L.A. Gould, Anthocyanins: Biosynthesis, Functions, and Applications, Springer, New York, NY, 2009.
[32] F. He, L. Mu, G.-L. Yan, N.-N. Liang, Q.-H. Pan, J. Wang, M.J. Reeves, C.-Q. Duan, Molecules 15 (2010) 9057–9091.
[33] M.O. Downey, J.S. Harvey, S.P. Robinson, Aust. J. Grape Wine Res. 10 (2004) 55–73.
[34] J. Bergqvist, N. Dokoozlian, N. Ebisuda, Am. J. Enol. Vitic. 52 (2001) 1–7.
[35] L. Haselgrove, D. Botting, R. van Heeswijck, P.B. HØJ, P.R. Dry, C. Ford, P.G.I. Land, Aust. J. Grape Wine Res. 6 (2000) 141–149.
[36] J.M. Tarara, J. Lee, S.E. Spayd, C.F. Scagel, Am. J. Enol. Vitic. 59 (2008) 235–247.
[37] E. Chorti, S. Guidoni, A. Ferrandino, V. Novello, Am. J. Enol. Vitic. 61 (2010) 23–30.
[38] J.A. Martinez, M. Melgosa, M.M. Perez, E. Hita, A.I. Negueruela, Food Sci. Technol. Int. 7 (2001) 439–444.