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Characterisation of Bobal and Crujidera grape cultivars, in comparison with Tempranillo and Cabernet Sauvignon: Evolution of leaf macronutrients and berry composition during grape ripening

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Abstract

We present characterisation data for two Spanish autochthonous grapevines, Bobal and Crujidera, in comparison with the well-known cultivars Tempranillo and Cabernet Sauvignon. Microsatellite markers were used for the molecular characterisation of Crujidera grapevines. Leaf macronutrient contents of the four cultivars were evaluated, as well as their changes at different vine developmental stages, and veraison was seen as the most suitable time to evaluate the nutritional status. Quantitative changes in some physiological parameters and the phenolic composition of the four grape varieties were measured during the last month of ripening. Polyphenols, tannins and anthocyanins increased with grape maturation, although the accumulation of these phenolic compounds and their patterns of evolution varied considerably with the cultivar. The biosynthetic potential of these grapes to produce resveratrol largely depended on the grape variety, with a remarkably high content found in Bobal berry skins.

Keywords: *Vitis vinifera* L.; Grapevine cultivars; Ripening; Microsatellites; Macronutrients; Polyphenols; Tannins; Anthocyanins; Extractability; Resveratrol

1. Introduction

Bobal and Crujidera are two indigenous Spanish cultivars, mainly grown in the Valencian Community. Bobal is the grape variety mostly grown in the D.O. Utiel-Requena (Appellation d'Origine) with more than 70% of the total grapevine area of this region. This cultivar has compact medium-to-large sized bunches with large irregular berries and a deep black-coloured skin. Crujidera, on the other hand, is a very scarce cultivar which, despite not being of economic relevance nowadays, has been recently included in *Vitis vinifera* research projects focusing on the maintenance of typical rare varieties. Crujidera is a productive variety with medium-to-large sized clusters of medium compactness, formed by large spherical berries with a bluish-black skin and a juicy-crispy pulp.

The aim of this work was to characterise these autochthonous cultivars, in comparison with the well-known cultivars Tempranillo and Cabernet Sauvignon, which have been more recently introduced into this territory and are acquiring importance in the elaboration of varietal wines from this Valencian area. Firstly, we carried out a molecular characterisation of the available Crujidera plants, as previously identified by their ampelographic characteristics. Microsatellites markers used in our study were those successfully employed by several groups for different genetic or genomic approaches concerning grapevine analysis (Bowers, Dangl, & Meredith, 1999; Bowers, Dangl, Vignani, & Meredith,

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1996; Sefc, Regner, Turetschek, Glössl, & Steinkellner, 1999; Thomas & Scott, 1993).

Since mineral nutrition is an important factor affecting grape composition and wine quality (Delgado, Martín, del Álamo, & González, 2004), we determined the nutrient status of the four cultivars at four different phenology stages. The results of this intercomparison suggest veraison as the most suitable time for nutritional evaluation of grapevine, owing to the fact that leaf mineral composition showed a higher stability at this phenology stage.

The colour and astringency of wines are mainly due to the anthocyanins and tannins extracted from the solid parts of the grape during winemaking. Nonetheless, each grape variety possesses a distinct phenolic composition (de Villiers, Vanhoenacker, Majek, & Sandra, 2004; Ortega-Regules, Romero-Cascales, López-Roca, Ros-García, & Gómez-Plaza, 2006; Ryan & Revilla, 2003) and a particular ease to release these metabolites into must (Glories, 1999; González-Neves, Gil, & Ferrer, 2002; Romero-Cascales, Ortega-Regules, López-Roca, Fernández-Fernández, & Gómez-Plaza, 2005; Saint-Criq de Gaulejac, Vivas, & Glories, 1998a), which are key parameters for the definitive quality of wines. Although these parameters are genetically determined, their expression throughout the grape ripening process is modulated by agricultural and environmental factors (Delgado et al., 2004; Jackson & Lombard, 1993; Pérez-Lamela, García-Falcón, Simal-Gándara, & Orriols-Fernández, 2007; Vian, Tomao, Coulomb, Lacombe, & Dangles, 2006).

We have evaluated the changes observed in various physiological parameters and in the phenolic composition throughout ripening to characterise the Bobal and Crujidera varieties, in comparison with the Cabernet Sauvignon and Tempranillo cultivars. The patterns of variation found in the evolution of total polyphenols, tannins and anthocyanins, as well as in their extractability, are discussed along with results on the resveratrol content in these grapevines analysed.

2. Materials and methods

2.1. Study area

Leaf and berry samples of *V. vinifera* L. cultivars Bobal, Crujidera, Tempranillo and Cabernet Sauvignon were collected in 2005 from a commercial vineyard (Fuenteseca vineyard), located at Camporrobles (UTM 8310, at an altitude of 980 m above sea level) within the D.O. Utiel-Requena (Valencia, Spain, D.O. = certificate of guarantee). The vines, spaced 1.35 m × 2.85 m with a density of approximately 2500 vines ha^{-1}, underwent localised irrigation. Environmental conditions and management practices were identical for the different vine plots evaluated in this study. According to the FAO (Food and Agriculture Organisation of the United Nations), the soil is petric Calcisol with 50% sand, 30% lime and 20% clay, 2% organic matter content, electric conductivity of 0.2 dS m^{-1}, and a pH of 8.2.

2.2. Microsatellite analysis

Leaf samples of Crujidera were collected from several vines grown in the Fuenteseca vineyard. Fresh leaves were surface cleaned, frozen in liquid nitrogen, and transferred to be frozen at −80 °C. Two Crujidera accessions from "El Encín" *Vitis* germplasm bank (IMIA, Alcalá de Henares, Spain) were used to assess the trueness-to-type of the tested plants.

DNA was extracted using the EZNA Plant DNA Miniprep Kit (Omega Bio-Tek Inc., Norcross, GA). A total of 12 microsatellite loci, as previously described, were synthesised and employed in this study: VVMD7, VVMD27, VVMD28 (Bowers et al., 1996, 1999); VVS2, VVS29 (Thomas & Scott, 1993); ssrVrZAG21, ssrVrZAG29, ssrVrZAG47, ssrVrZAG62, ssrVrZAG64, ssrVrZAG79, ssrVrZAG112 (Sefc et al., 1999). Fluorescently labelled primers were used for PCR amplification, according to the protocol described by Martín, Borrego, Cabello, and Ortiz (2003).

2.3. Leaf sampling and nutrient analyses

Leaves were collected according to previous methods employed for foliar diagnosis (Delgado et al., 2004). Sampling took place in the months of June, July, August and September 2005, coinciding with various vine phenology stages (bloom, post-bloom, veraison, and pre-harvest, respectively). Three separate samples of 20 leaves were collected for each sampling data and cultivar. Leaves were then surface washed, dried, and pulverised. For nutrient analysis, samples were digested with nitric acid using a heating digestor (DK 20 Velp Scientifica srl, Usmate, Italy). Potassium (K), calcium (Ca) and magnesium (Mg) concentrations were measured by atomic absorption spectrometry (SpectrAA220 FS, Varian Inc., Palo Alto, CA), and phosphorus (P) was determined by spectrophotometry (Spectroquant, Merck, Darmstadt, Germany). Total nitrogen content was determined on untreated pulverised samples using combustion analysis (EA 1110 CNHS; CE Instruments, Milan Italy). Three replicate measurements were performed per sample.

2.4. Berry sampling and analyses

Sampling took place after veraison (August 15) at three stages of the ripening process: August 30, September 15, and October 1 (harvest). For each sampling data and cultivar, two grape clusters were collected from both sides of 10 vines located in different rows within each cultivar plot. Berries were separated from clusters and various representative samples of 100 berries were randomly collected for each cultivar, and were immediately frozen and stored at −20 °C until analyses were performed.

Total soluble solids (expressed as °Brix) were determined by refractometry. Colour intensity ($OD_{420} + OD_{520} + OD_{620}$) and total tannin content measurements

were performed by spectrophotometry, according to the methods proposed by Glories (1984) and Ribéreau-Gayon and Stonestreet (1966), respectively. For these analyses, seeds were manually separated from grape samples, and berries homogenised for 2 min. The resulting slurry was filtered and the juice obtained centrifuged at 3000*g* for 5 min, prior to the analyses.

For grape skin determinations, representative samples of each cultivar were manually peeled, and the skins were used to measure total polyphenols, expressed as the total polyphenol index (TPI), total anthocyanins, and the anthocyanin extractability index (AEI), as proposed by Saint-Criq de Gaulejac, Vivas, and Glories (1998b). Resveratrol extraction from berry skins and HPLC determinations of *trans*-resveratrol were performed according to the methods developed by Romero-Pérez, Lamuela-Raventós, Andrés-Lacueva, and de la Torre-Boronat (2001).

3. Results and discussion

3.1. Characterisation of Crujidera vines by microsatellite markers

Microsatellites have been used in this study as molecular markers to identify and assess the trueness-to-type of an old plantation of grapevines growing in a commercial vineyard (Fuenteseca vineyard, Camporrobles, Valencia, Spain). According to their morphological characteristics, these grapevines have been catalogued as the *V. vinifera* Crujidera cultivar.

We carried out a molecular identification based on the use of various sequence tagged microsatellite site (STMS) primers, previously reported by different researchers for grapevine analysis. Our results demonstrated that the studied plants presented the same genotypic pattern, as shown in Table 1. This genotypic profile, represented by the allelic size of the 12 microsatellite loci employed in our analyses, was also identical to that of the two Crujidera accessions used as a true variety. They share the same alleles at all the loci evaluated, and the cumulative probability of the 12 microsatellites was estimated in the order of 2.6×10^{-13}. These results confirm the identity of the plants analysed and validate the previous ampelographic determinations of Crujidera grapevines. These findings allow the use of the available plant material for future propagation programmes of this grape variety.

STMS have been successfully employed in grapevines for cultivar identification and characterisation (Martín et al., 2003), detection of synonymies and homonymies (Ulanovsky, Gogorcena, Martínez de Toda, & Ortiz, 2002) or to assess the geographic origin of cultivars (Sefc et al., 2000).

3.2. Changes in leaf macronutrient contents at different vine phenology stages

The results provided in Table 2 show the variations in macronutrient contents found in the leaves of Bobal, Cabernet Sauvignon, Tempranillo and Crujidera cultivars analysed at four different vine phenology stages: bloom, post-bloom, veraison, and pre-harvest. In general, our data are in accordance with values previously reported for grape vines. Foliar concentrations of N, P and K, in the four varieties analysed, were at their highest at the beginning of the sampling period (June), and declined progressively during summer until pre-harvest (September); the opposite trend occurred for Ca, while leaf Mg concentration did not show any variation throughout the vine-growing season (Table 2). This pattern of variation is similar to those reported for *V. vinifera*, as well as for other distinct plant species (Roca-Pérez, Boluda, & Pérez-Bermúdez, 2006; Schreiner, 2005), which indicated that the foliar concentration of elements such as N, P and K decreased throughout a seasonal cycle, whereas Ca content increased, as a result of the leaf aging process.

All these results suggest that the decrease in N, P, and K is mainly due to the retranslocation of these mobile macroelements to different sink organs, like fruits, that grow during this period. Moreover, the increase in the contents of structural and non-structural leaf compounds, produced from spring to autumn during the process of leaf development, also contributed to the reduction in the percentages of N, P, and K observed in our analyses. Despite this dilution effect, Ca concentrations in the leaves increased throughout the sampling period, due to the low mobility of this macroelement in phloem and its high availability in the soils where the studied grape vines were grown.

Of the possible ratios between macronutrients, only the seasonal variations of N/P are pointed out and shown (Fig. 1). The seasonal pattern of the N/P ratio showed a drastic increase from June to September, which along with other processes, could be related to a higher retranslocation of P rather than N. Throughout the leaf aging process, the P requirements for RNA are greatly reduced and nucleic-acid phosphate can, therefore, be mobilised from leaves and retranslocated to sink organs, leading to higher N/P ratios (Güsewell, 2004). This ratio has been used as a diagnostic indicator of N saturation and/or of the vegetative growth limitation by these nutrients, as well as to identify thresholds of nutrient limitation. The N/P ratios for

Table 1
Sizes of the alleles, in base pairs, for each of the 12 loci analysed in the Crujidera cultivar

VVMD7	VVMD27	VVMD28	ZAG21	ZAG29	ZAG47	ZAG62	ZAG64	ZAG79	ZAG112	VVs2	VVs29
236	180	240	204	111	158	186	138	244	226	140	170
240	180	250	214	111	158	190	142	254	231	142	170

Table 2
Macronutrient contents (% dry weight) in leaves of different grape cultivars collected throughout the growing season (bloom, post-bloom, veraison, and pre-harvest)

Element	Sampling	Bobal	Tempranillo	Cabernet Sauvignon	Crujidera
N	June 15	4.25 ± 0.13	5.17 ± 0.12	3.97 ± 0.03	4.42 ± 0.17
	July 15	2.93 ± 0.10	3.45 ± 0.10	2.96 ± 0.15	3.56 ± 0.02
	August 15	2.78 ± 0.02	3.02 ± 0.05	2.73 ± 0.08	2.71 ± 0.04
	September 15	2.40 ± 0.06	2.52 ± 0.08	2.31 ± 0.03	2.48 ± 0.05
P	June 15	0.42 ± 0.04	0.52 ± 0.04	0.35 ± 0.01	0.49 ± 0.01
	July 15	0.24 ± 0.01	0.31 ± 0.02	0.23 ± 0.01	0.35 ± 0.02
	August 15	0.20 ± 0.03	0.22 ± 0.03	0.19 ± 0.04	0.23 ± 0.02
	September 15	0.16 ± 0.01	0.18 ± 0.01	0.14 ± 0.02	0.18 ± 0.01
K	June 15	1.34 ± 0.05	1.37 ± 0.02	0.89 ± 0.01	1.24 ± 0.02
	July 15	1.20 ± 0.01	1.35 ± 0.01	0.56 ± 0.03	1.06 ± 0.03
	August 15	0.56 ± 0.02	0.61 ± 0.02	0.54 ± 0.02	0.70 ± 0.02
	September 15	0.45 ± 0.05	0.55 ± 0.01	0.35 ± 0.04	0.47 ± 0.03
Ca	June 15	0.62 ± 0.02	0.60 ± 0.05	0.99 ± 0.03	0.94 ± 0.07
	July 15	0.89 ± 0.09	1.01 ± 0.11	1.09 ± 0.05	1.01 ± 0.08
	August 15	1.46 ± 0.12	1.42 ± 0.17	1.22 ± 0.15	1.67 ± 0.09
	September 15	2.26 ± 0.13	2.31 ± 0.21	2.10 ± 0.09	2.46 ± 0.18
Mg	June 15	0.17 ± 0.03	0.24 ± 0.05	0.28 ± 0.05	0.25 ± 0.09
	July 15	0.18 ± 0.03	0.23 ± 0.08	0.29 ± 0.04	0.23 ± 0.02
	August 15	0.27 ± 0.02	0.24 ± 0.03	0.30 ± 0.07	0.21 ± 0.01
	September 15	0.28 ± 0.07	0.31 ± 0.07	0.37 ± 0.10	0.26 ± 0.05

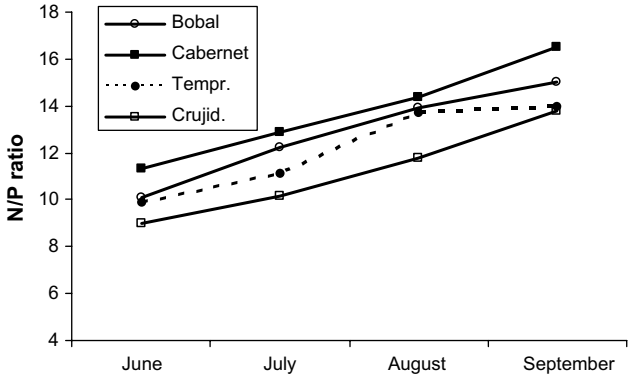

Fig. 1. N/P ratios in leaves of different grape cultivars collected throughout the growing season (bloom, post-bloom, veraison, and pre-harvest).

the four grape cultivars studied are similar to the average ratios of terrestrial plant species (N/P = 10–13) at their natural field sites (Güsewell, 2004); the leaves of the vines only exhibited a deep starvation of P at harvest time (Table 2 and Fig. 1).

Bloom is usually the standard stage for collecting leaf samples for diagnostic purposes in grapevine. However, its short duration often makes the adequate collection and analysis of leaf samples difficult. Besides this, our results showed a marked variability in mineral concentrations among the four cultivars at this stage, whereas a higher stabilisation in the leaf macronutrient levels at veraison was observed (Table 2). These findings suggest veraison as the most suitable time to evaluate the nutritional status of grapevines, due to the higher stability of the leaf mineral composition at this phenology stage.

3.3. Evolution of various physiological parameters during the ripening of Bobal, Tempranillo, Cabernet Sauvignon and Crujidera grapes

Ripening of the four grape varieties studied was assessed at three sampling dates during the last month of growth (15, 30 and 45 days after veraison), by measurement of berry weight, °Brix and colour intensity. A comparison of the data obtained from the four cultivars for each sampling date is presented in Table 3.

At harvest, berry weight was significantly higher in the Bobal grapes, reaching 285 g per 100 berries, whereas the smallest fruits were those of the Cabernet Sauvignon and Tempranillo vines, with 103 g and 134 g, respectively. The increase in weight was gradual throughout the ripening process, but became moderate in the four varieties. However, significant increases were only observed across the sampling dates in the smallest berries (Cabernet Sauvignon and Tempranillo) during the first two weeks of September. Grape size is of fundamental importance, since anthocyanins are synthesised in the skin. Consequently, a larger berry weight results in a lower skin-to-flesh ratio and the compounds are diluted (Ortega-Regules et al., 2006).

The accumulation of sugars in berries, expressed as °Brix, was progressive and significant across the three sampling dates for the Bobal, Cabernet Sauvignon and Tempranillo varieties (Table 3). This physiological parameter remained more stable in the cultivar Crujidera, although the °Brix of these berries also increased significantly between the first and last samples analysed (Table 3). Compared with Cabernet, it is remarkable to note the high sugar levels found in the autochthonous grape Crujidera.

Table 3
Changes in various physiological parameters of Bobal, Tempranillo, Cabernet Sauvignon and Crujidera grapes collected at three stages after veraison (August 15) throughout the last month of ripening

Sampling	Bobal	Tempranillo	Cabernet Sauvignon	Crujidera
	Weight of 100 berries (g)			
August 30	268 a	118 a	82 a	218 a
September 15	276 a	128 b	96 b	227 a
October 1	285 a	134 b	103 b	232 a
	°Brix			
August 30	14.6 a	15.2 a	16.1 a	18.5 a
September 15	16.8 b	17.8 b	19.8 b	20.5 ab
October 1	19.4 c	19.4 c	22.2 c	21.3 b
	Colour intensity			
August 30	4.9 a	5.3 a	7.8 a	4.3 a
September 15	5.9 b	6.6 b	7.9 a	4.9 ab
October 1	6.3 b	7.0 b	8.1 a	5.2 b

For each cultivar and parameter, values followed by the same letter are not significantly different ($p < 0.05$).

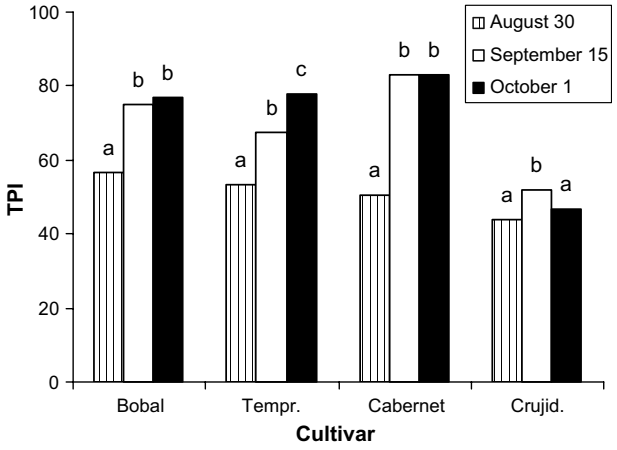

Fig. 2. Total polyphenol index of Bobal, Tempranillo, Cabernet Sauvignon and Crujidera grapes collected at three stages after veraison (August 15) throughout the last month of ripening. For each cultivar, values with the same letter are not significantly different ($p < 0.05$).

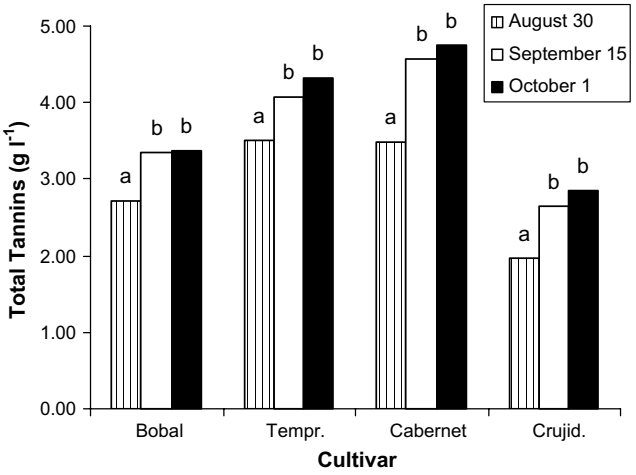

Fig. 3. Total tannin concentrations in Bobal, Tempranillo, Cabernet Sauvignon and Crujidera grapes collected at three stages after veraison (August 15) throughout the last month of ripening. For each cultivar, values with the same letter are not significantly different ($p < 0.05$).

The progressive and parallel evolution of berry weight and sugar content observed for the four grape varieties throughout the last month of ripening is similar to that reported for Cabernet Sauvignon and Tempranillo grapes at different stages of ripening (Ryan & Revilla, 2003). Nevertheless, these results do not coincide with those found in Shiraz berries by Fournand et al. (2006), who reported that once berry weight had reached its maximum level it then decreased, because of water loss, as sugar content increased.

The spectrophotometric measurements of colour intensity in grape musts showed a progressive and significant increase prior to harvest, except for those musts from Cabernet Sauvignon berries, where colour remained almost constant in the three samples evaluated (Table 3). Despite this pattern of evolution, the musts obtained from the Cabernet grapes always possessed the highest values of colour intensity while, in contrast, Crujidera berries produced the least coloured musts (Table 3).

3.4. Changes in total polyphenols, tannins and anthocyanins during the ripening of Bobal, Tempranillo, Cabernet Sauvignon and Crujidera grapes

Besides the aforementioned physiological data, various parameters related to the phenolic composition of grapes have been determined, to characterise the Bobal and Crujidera varieties, in comparison with Cabernet Sauvignon and Crujidera grapes. The results obtained for the four cultivars, as well as the evolution of these parameters during the ripening process, are shown in Figs. 2–4.

The total polyphenol index (TPI) reflects the combination of the distinct phenolic compounds in grape berries or wines. In the case of grapes, the TPI is widely employed as an indicator of berry quality for wine production. The TPI values found for the three sampling dates (Fig. 2) corroborate the high values previously reported for the well-known Tempranillo and Cabernet Sauvignon varieties, as well as for the autochthonous grape Bobal. These three cultivars presented TPI values ranging between 77 and 83 at harvest. In contrast, the TPI value for the other autochthonous grape, Crujidera, was around 50, which could represent an initial limitation in the use of this variety for winemaking. In relation to the evolution of total polyphenols, different authors agree on a rapid and high accumulation of these metabolites in the berries during the several weeks after veraison, which is followed by a stabilisation phase until ripening, or even by a decrease in polyphenols a few days prior to the grape harvest (González-San José & Diez, 1992; Keller & Hrazdina, 1998; Pirie & Mullins, 1980). It has been reported that the higher or lower activities, from veraison to ripeness, of the numerous enzymes involved either in the biosynthesis or the degradation

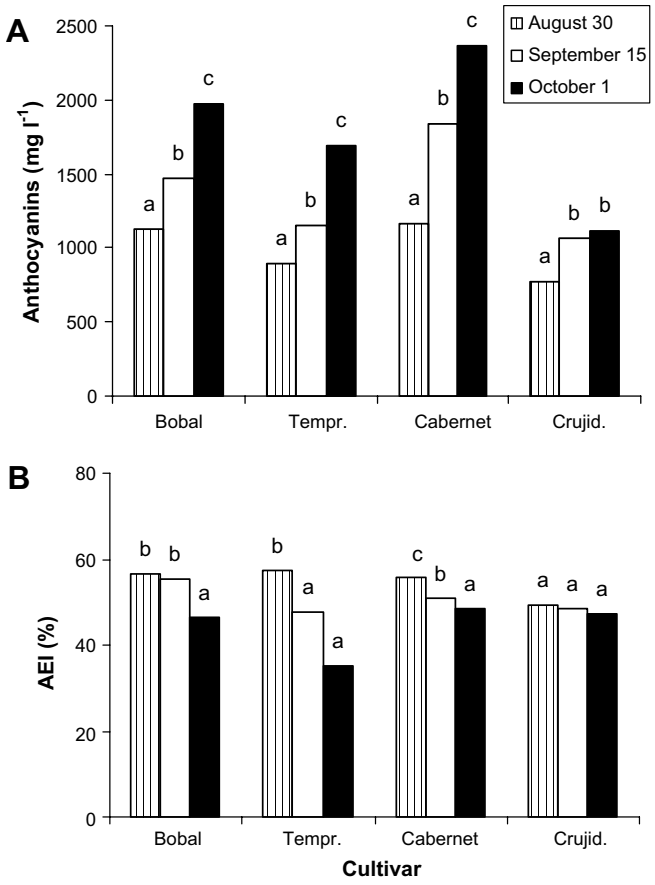

Fig. 4. Anthocyanin concentrations (A) and anthocyanin extractability index (B) in Bobal, Tempranillo, Cabernet Sauvignon and Crujidera grapes collected at three stages after veraison (August 15) throughout the last month of ripening. For each cultivar, values with the same letter are not significantly different ($p < 0.05$).

(peroxidases and glycosidases) of phenolic compounds are responsible for these patterns of evolution (Delgado et al., 2004). In this respect, our results suggest that polyphenol evolution also depends on the *V. vinifera* cultivar; thus, the TPI data showed a final stabilisation of polyphenols in Bobal, Cabernet Sauvignon and Tempranillo, whereas the pattern was different in Crujidera, where these natural products decreased significantly in ripe fruits (Fig. 2).

The total tannin contents of Bobal, Tempranillo, Cabernet Sauvignon and Crujidera berries, and their evolution throughout ripening are depicted in Fig. 3. At harvest, tannin contents were significantly higher in Cabernet Sauvignon and Tempranillo grapes with 4.76 and 4.31 g l^{-1} of total tannins, respectively, whereas the lowest amount (2.85 g l^{-1}) was found in Crujidera fruits. The pattern of variation of tannins was similar in all four grape cultivars analysed since, in all cases, the berries presented tannin concentration values that increased gradually until the time of harvest (Fig. 3). However, the accumulation of tannins following veraison was highly significant until two weeks before harvest, after which these compounds remained virtually stable. This profile is in agreement with results previously reported for various grape cultivars (Delgado et al., 2004; Esteban, Villanueva, & Lissarrague, 2001). Nonetheless, it is difficult to reach definitive conclusions on the evolution of tannins, since other authors observed no major changes (Fournand et al., 2006), or even a decrease (de Freitas, Glories, & Monique, 2000) in total tannins from veraison to harvest.

Total anthocyanin concentrations of Bobal, Tempranillo, Cabernet Sauvignon and Crujidera berries, and the changes observed throughout the ripening process are depicted in Fig. 4A. Although the berries of the four varieties accumulated high concentrations of anthocyanins at the time of harvest (>1100 mg l^{-1}), the ability of the grape to biosynthesise these flavonoids was greatly dependent on the cultivar analysed. Anthocyanin concentrations were very high for Cabernet and Bobal, which reflects the oenological potential of this autochthonous grape. These concentrations decreased in Tempranillo, and reduced to half for those in the skins of Crujidera grapes (Fig. 4A). In relation to the evolution of total anthocyanins during ripening, we observed a continuous biosynthesis of these flavonoids in the skins of the four grapes. Nevertheless, two different patterns can be distinguished; firstly, that shared by the Bobal, Tempranillo and Cabernet Sauvignon grapes, which was characterised by a progressive and significant accumulation of these metabolites until harvest; while in Crujidera berries, an initial significant increase of anthocyanins was followed by a stabilisation phase during the two weeks prior to harvest (Fig. 4A).

Anthocyanins accumulate in berry skins during the ripening of grapes and it is known that each variety of grape has a particular potential to biosynthesise these phenolic compounds. Nevertheless, a comparison of the numerous studies carried out to determine anthocyanin contents in grape berries and to establish the pattern of accumulation during ripening demonstrate that these parameters are affected to a greater or lesser extent by factors such as maturity, climate, soil conditions and management practices (Delgado et al., 2004; Jackson & Lombard, 1993; Pérez-Lamela et al., 2007; Vian et al., 2006). All constant and varying factors affecting the oenological quality of grape clusters, besides the particular methodology employed (i.e., frequency of sampling and analytical procedures) to evaluate the anthocyanins of their berries, seem to be responsible for the differing results reported on this subject in the literature. Conversely, more conclusive results have been obtained when anthocyanins have been evaluated qualitatively. Thus, it has been demonstrated that the anthocyanin fingerprints of distinct grapes cultivars are closely related to their genetic characteristics and, therefore, these qualitative profiles are highly suitable to distinguish grape cultivars or for varietal authentification of wines (de Villiers et al., 2004; Ortega-Regules et al., 2006; Ryan & Revilla, 2003).

The colour of red wines is mainly due to the anthocyanins extracted from the berry skins into musts during winemaking. During this process however, each grape variety

differs in its ability to release these metabolites accumulated in the vacuoles throughout ripening. We have evaluated anthocyanin extractability from Bobal, Tempranillo, Cabernet Sauvignon and Crujidera berries, as proposed by Saint-Criq de Gaulejac et al. (1998b). According to this assay, the anthocyanin extractability index, expressed as a percentage (AEI %), represents the ratio between non-extractable anthocyanins and total anthocyanins. In other words, the lower the AEI, the greater the ease of anthocyanins to diffuse from grape skins. The values obtained in our experiments are seen in Fig. 4B.

At harvest, the lowest AEI value (35.2%) was found in Tempranillo berries, which reflects a low stability of the skin cell walls and membranes at grape maturity. Bobal, Crujidera and Cabernet Sauvignon berries displayed significantly higher indices, ranging from 46.5% to 48.6% (Fig. 4B). Irrespective of the anthocyanin concentrations in these three varieties, very high for Cabernet and Bobal and lower for Crujidera (Fig. 4A), their high extractability indices indicate the difficulty of anthocyanin extraction from these grapes.

In relation to the evolution of the AEIs during ripening, it is particularly striking to note the opposite patterns observed in Tempranillo and Crujidera grapes. Thus, at the first sampling date (August 30), these varieties possessed the highest and lowest values of extractability, respectively (Fig. 4B). Yet throughout one month of ripening with increasing sugar contents (Table 3), Crujidera grapes did not show any significant change in their AEI, whereas Tempranillo berry ripening favoured increased anthocyanin extractability, as can be deduced from the progressive and significant decreases observed in its AEI values (Fig. 4B). The pattern found for Tempranillo is similar to that reported in previous works (Glories, 1999; Saint-Criq de Gaulejac et al., 1998a), while our results for Crujidera are in agreement with the profiles described for different grapes by González-Neves et al. (2002) and Romero-Cascales et al. (2005), where sugar content changed with no significant changes in the AEI.

The simultaneous evaluation of data for technological maturity and phenolic maturity, usually represented by the measurements of must sugar content and skin anthocyanin extractability, has been suggested as an important tool to establish the most appropriate time of grape harvest, in relation to the type of wine desired. Our results from Tempranillo, Crujidera, Bobal and Cabernet Sauvignon berries support Di Stéfano, Borsa, Bosso, and García (2000), who indicated the need to evaluate the evolution of different ripening parameters for each cultivar and each environment, as no predetermined relationships exist between technological maturity and phenolic maturity.

3.5. Resveratrol contents in Bobal, Tempranillo, Cabernet Sauvignon and Crujidera grapes

Resveratrol, a phenolic phytoalexin of the stilbene family, has also been evaluated in our study. Resveratrol accumulates specifically in the skin of grape berries; it has been demonstrated that its biosynthesis increases in response to different biotic and abiotic stresses affecting the vines (Romero-Pérez et al., 2001). Stilbenes are transferred from skins to musts during maceration, but the final resveratrol concentrations in wines depend on numerous factors. This group of natural products, particularly the form *trans*-resveratrol, has been extensively studied in recent years, due to their beneficial effects for human health, such as their antioxidant activity and protective roles against cardiovascular disorders and carcinogenesis.

Resveratrol extraction and HPLC determinations of *trans*-resveratrol were carried out only on berry skins from grapes collected at maturity. The results obtained (Table 4) showed that resveratrol content varied significantly among the cultivars. The highest value (245 μg g^{-1} dry weight) was found in the Bobal grape; its content was twice that measured in Cabernet Sauvignon and Crujidera, and it was eight times higher than the resveratrol content in Tempranillo grapes. Since the environmental conditions and management practices were identical for the different vine plots, our data clearly demonstrate that resveratrol biosynthesis is closely linked to the genetic characteristics of each cultivar. This finding is in agreement with previous results obtained from the analysis of berry skins or monovarietal wines, where resveratrol concentrations varied considerably and appeared to depend on the grape variety (Abril, Negueruela, Pérez, Juan, & Estopañán, 2005; Romero-Pérez et al., 2001). With regard to resveratrol accumulation detected in our study, it is remarkable to note the very high contents found in the two autochthonous grapes, Bobal and Crujidera, which represent nutritional and therapeutic added values for these cultivars and the wines derived from their berries. Since wines are food-related, it cannot be ruled out that in the near future wine labels will include data about their nutritional–therapeutic properties, besides those describing their particular organoleptic characteristics, bearing in mind that both qualities basically lie in the contents of their phenolic compounds.

This is the first study in which the Bobal and Crujidera grape varieties have been analysed extensively. The results reported herein in comparison with two famous cultivars, Cabernet Sauvignon and Tempranillo, validate and support the excellent oenological results obtained with Bobal grapes, not only for blending, but also for the elaboration of monovarietal aging wines, especially when produced

Table 4
Resveratrol contents in Bobal, Tempranillo, Cabernet Sauvignon and Crujidera berry skins

Grape cultivar	Resveratrol (μg g^{-1} dry weight)
Bobal	245 c
Tempranillo	32 a
Cabernet Sauvignon	115 b
Crujidera	120 b

Values followed by the same letter are not significantly different ($p < 0.05$).

from old vines. Crujidera is also being successfully used to produce distinct red and rosé wines in La Mancha and Valencian regions as part of new projects to recover rare autochthonous varieties. Moreover and in agreement with Sefc et al. (2000), the tendency to plant successful and famous cultivars over all the vine-growing regions of the world should be balanced by the maintenance of the typical varieties of the diverse regions, in order to prevent genetic erosions in this crop.

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