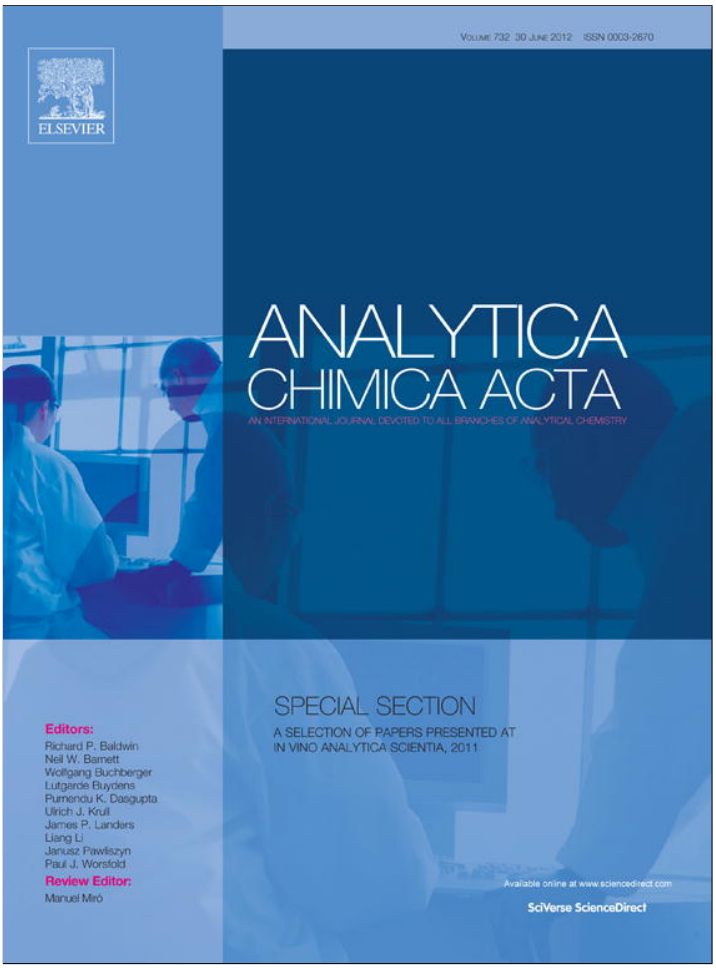

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Influence of climatic conditions on the phenolic composition of *Vitis vinifera* L. cv. Graciano

Raúl Ferrer-Gallego, José Miguel Hernández-Hierro*, Julián C. Rivas-Gonzalo, M. Teresa Escribano-Bailón

Grupo de Investigación en Polifenoles. Unidad de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno, E 37007 Salamanca, Spain

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ABSTRACT

The influence of different climatic conditions on the phenolic composition of grape skins and seeds of *Vitis vinifera* L. cv. Graciano – an autochthonous cultivar from Rioja and Navarra regions (Spain) – was evaluated during ripening in a separate way. Graciano grapes from two different vineyards with different climatic conditions and from two different vintages (2008 and 2009) were analysed. Clear differences between phenolic maturity pattern of grape skins and seeds were observed. In this context, it may be important to evaluate the phenolic maturity of seeds and skins in a separate way in order to decide the optimal harvest time. It was also noticeable that the effect of vintage (mainly due to changes in climatic conditions) may affect the changes in the phenolic composition of both grape skins and seeds. Although in a lesser extent, the effect of the vineyard was also observable, and it was especially relevant in vintages with irregular climatic conditions such as 2008 vintage.

In a second strand, results obtained from the phenolic composition of grape seeds and skins at harvest, oenological parameters at harvest and climate conditions during vegetative stage were evaluated and relationships among the aforementioned variables were revealed.

1. Introduction

As occurs with other agricultural products, chemical composition of grapes depends on several factors. It is well known that cultivar, climate, soil, water availability, cultural practices and degree of maturity have a significant effect on nutrient and metabolite concentrations in several crops, including grapes.

Grapes require certain maturity ranges depending on variety, growing region and the style of wine to be made. The measurement of total soluble solids is a well-established parameter for basic grape quality assessment. However, an appropriate sugar maturity is not enough to ensure the quality of grapes and therefore the quality of the red wines obtained. One of the major factors affecting red wine quality is the phenolic maturity degree of grapes at harvest time [1]. A number of sensory attributes of wine are directly associated with phenolic compounds which come from grape skins and seeds [2–4]. Anthocyanins and flavonols are only present in grape skins whereas proanthocyanidins are present in skins and seeds [1]. Seed proanthocyanidins are made up of (+)-catechin, (−)-epicatechin and (−)-epicatechin-3-*O*-gallate units [5,6], whereas skin proanthocyanidins also contain (−)-epigallocatechin [7].

Phenolic composition of grapes varies depending on several factors such as the effect of vintage [8,9], soil [10], temperature [11–13], luminosity [14–16], cultural practices [5,17–26] and developmental stage [15,27–29]. During ripening, changes occur in the phenolic composition of both seeds and skins, which present different trends. It is generally accepted that anthocyanins are accumulated up to a maximum and then decrease. However, the procyanidin content of grape seeds usually has a maximum at veraison and then decreases and remains relatively constant until harvest time [23,30–35], although the opposite trend has also been found [9].

Nowadays, the wine sector is very interested in defining the concept of phenolic maturity and the repercussions of it on the sensory parameters of the obtained wine (colour, astringency, bitterness, etc.). This concept has been applied as an average value of a representative sample from the whole grape [36].

The aim of this study was to evaluate the influence of different climatic conditions on the phenolic composition of grape skins and seeds of *Vitis vinifera* L. cv. Graciano – an autochthonous cultivar from Rioja and Navarra regions (Spain) – during ripening in a separate way. In a second strand, results obtained from the analysis of the phenolic composition (flavanols, flavonols, phenolic acids and anthocyanins) and oenological parameters at harvest (density, total acidity and pH) and climate conditions during vegetative stage (average temperature, accumulated rainfall and accumulated solar

* Corresponding author. Tel.: +34 923 294 537; fax: +34 923 294 515.
E-mail address: jmhhierro@usal.es (J.M. Hernández-Hierro).

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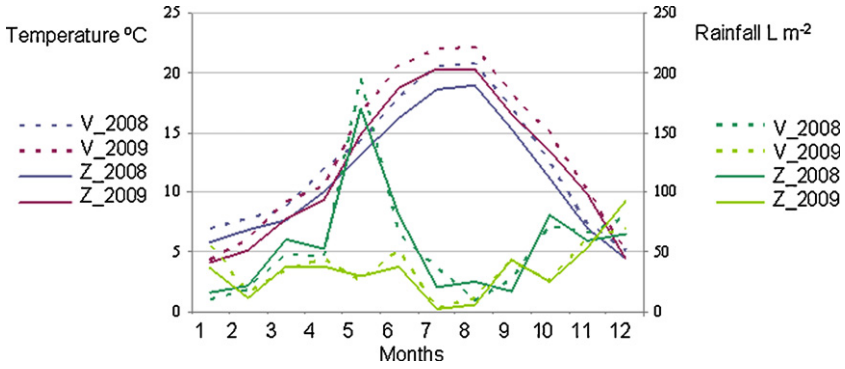

Fig. 1. Monthly average temperature and rainfall for the two studied regions and vintages.

radiation) were evaluated in order to find relationships among the aforementioned variables.

2. Materials and methods

2.1. Samples

V. vinifera L. cv. Graciano grape berries grown in D.O.Ca Rioja (Spain) were used as the source material of phenolic compounds. Samples were collected at two different vineyards. One of them is located in Rioja Alta (vineyard Z), where the Atlantic climate dominates and the other one (vineyard V) is located in Rioja Media, which is characterized by Mediterranean influence. The study was performed for two different years: 2008 and 2009. Red grapes were collected at different developmental stages during berry maturity: from veraison (September 3rd) to over-ripening (November 5th). In the 2008 vintage, seven dates were taken into account for vineyard V and eight for vineyard Z. In the 2009 vintage, the number of dates taken into account was six for vineyard V and seven for the vineyard Z. Sampling was carried out as follows: 300 berries were collected from both sides of vines located in different rows within the vineyard. Edge rows and the first two vines in a row were avoided. Berries were collected from the top, middle and bottom of the cluster and were immediately frozen and stored at −20 °C until analyses were performed.

2.2. Phenolic extraction and determination

Seeds and skins extraction and HPLC-DAD–MS analysis were carried out as described elsewhere in Garcia-Marino et al. [37] and Ferrer-Gallego et al. [38,39]. Briefly, grape seeds and skins were separated manually and submitted to two different extraction procedures with 75% methanol and acidic methanol respectively. In the case of flavanols and phenolic acids from grape skins, an additional clean up procedure using a cationic exchange cartridge (Oasis® MCX) was performed prior to the chromatographic analysis above-stated.

Up to 77 phenolic compounds were determined: 47 proanthocyanidins, 13 anthocyanins, 9 flavonols and 8 phenolic acids.

2.3. Chemometric analysis

Principal component analysis (PCA), an unsupervised pattern recognition technique, was used in order to observe trends in the data indicating relationships between samples and/or between variables. Climatic data, phenolic composition and/or oenological parameters were used as variables in the aforementioned analysis. PCA was applied to the correlation matrix of the original variables [40,41].

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

3. Results and discussion

3.1. Climate conditions

Fig. 1 shows the monthly average temperature and rainfall recorded in 2008 and 2009 vintages in the two studied regions. On the one hand, the average of temperature was slightly higher in region V than in region Z, in both 2008 and 2009 vintages. The 2009 vintage was hotter than the 2008, especially on vegetative period (from April to November). The 2008 vintage was an irregular vintage, abundant precipitation from April to June and cool temperatures in summer. However, 2009 was a typical vintage in these two regions [42]. Other climate parameters were also recorded (data not shown) like wind speed, relative humidity and solar radiation accumulated. These data have been kindly provided by Bodegas RODA (Haro, Spain).

Fig. 2 shows the score plot of PCA of all climate data recorded for the two studied regions and vintages. Results indicate that regions and vintages were noticeably different. It also proves that the influence of vintage (PC 1) is more important than region (PC 2) since PC 1 describes 55% of the variability explained and the second (PC 2) 28%. Samples of 2008 and 2009 are clearly divided for this PC 1. The samples of 2008 vintage are further in PC 1 than samples of 2009; it

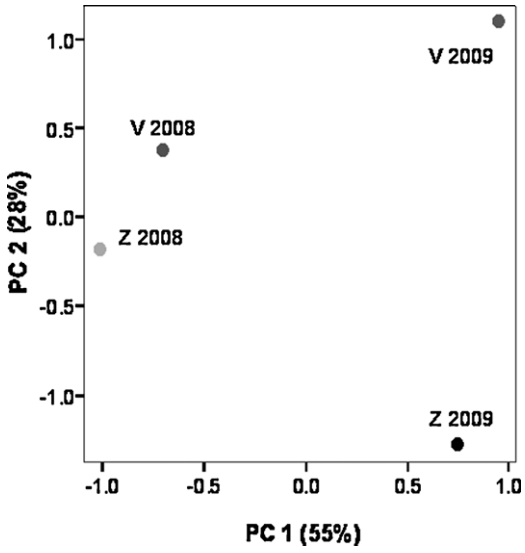

Fig. 2. Score plot of the principal component analysis of all climate data recorded for the two studied regions and vintages.

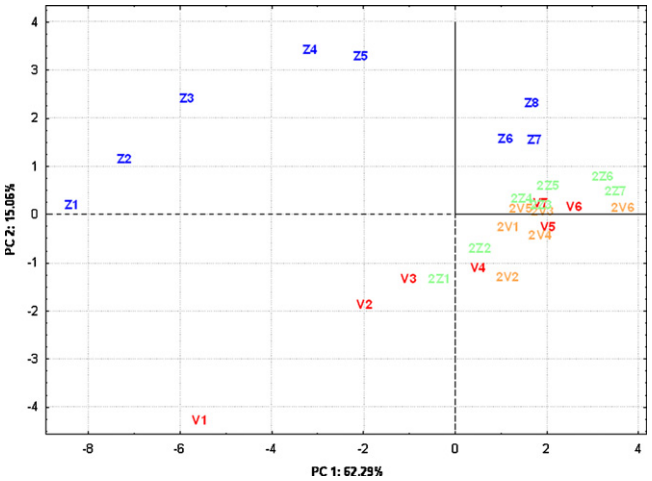

Fig. 3. Projection of grape seed samples on the plane defined by the first and second principal components.

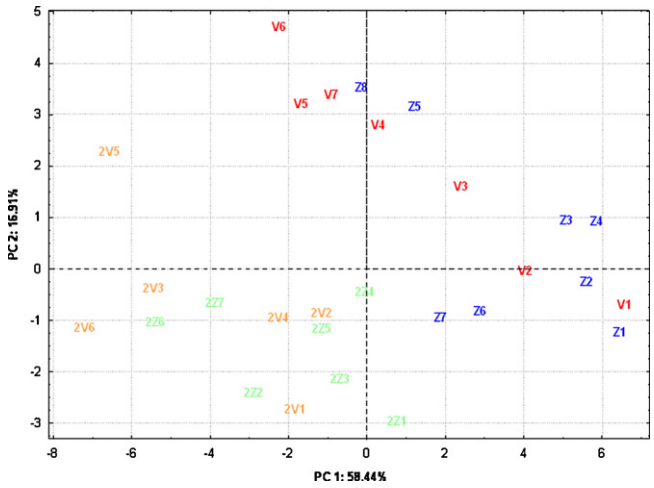

Fig. 4. Projection of grape skin samples on the plane defined by the first and second principal components.

could suggest more heterogeneity in 2008 vintage. Likewise, in PC 2 the variations in samples from vineyard Z are higher than those from vineyard V.

3.2. Phenolic composition of grapes during ripening

3.2.1. Grape seeds

Fig. S1 shows the changes of (+)-catechin and (−)-epicatechin in grape seeds during ripening. The content of (−)-epicatechin was generally higher than the content of (+)-catechin. This pattern is in good agreement with that described by other authors [43]. Taking into account the climate conditions of vintages, the influence in monomers is clear, since the content of monomers in 2008 – an irregular vintage – was higher.

In general, the non-galloylated oligomers compounds in grape seeds showed slight changes during ripening (Fig. S2). Generally, the content of trimers was higher than dimers and tetramers. The content of oligomers showed fewer changes than monomers.

Fig. S3 shows the changes that the content of galloylated compounds undergo in grape seeds during ripening. These compounds suffered a noticeable decrease during this time, especially in 2008. Despite that, similar contents of these compounds have been found at harvest time. Results obtained from the phenolic composition of grape seeds indicate that 2008 (irregular vintage) had higher variations in the phenolic composition of grape seeds than 2009. The content of these compounds in the aforementioned vintage were higher at veraison time which was likely due to its climate conditions.

In order to summarise the evolution of grape seeds samples regarding their phenolic composition, a principal component analysis was performed using 17 variables. These variables corresponded to the phenolic compounds grouped according their basic structures and some individual compounds (Table S1). Fig. 3 shows the projection of grape seed samples on the plane defined by the first and second principal components. Each sample was represented by an alphanumeric code indicating the vintage (i.e. none = 2008; 2 = 2009), vineyard (V and Z) and sampling date respectively. PC 1 describes the evolution of grape seed samples during ripening. Samples located on the left side of this principal component presented the highest content in almost all phenolic compounds; with the exception of the dimer B2 which presented a slightly opposite trend. These samples on the left side correspond to the earlier samples and their temporal evolution indicates the decrease of almost all phenolic compounds analysed in grape seeds during ripening. It is also noticeable that the 2009 vintage presents a more homogeneous pattern. Samples from vineyard Z present more variations in the phenolic content during ripening within each vintage and these variations were noticeable higher in the irregular one (2008 vintage).

3.2.2. Grape skins

Fig. S4 shows the content of anthocyanins and flavonols in grape skins during ripening. Regarding anthocyanins, clear differences between samples from V and Z vineyards can be observed. Within the same vintage, grapes from vineyard V had higher content in anthocyanins than grapes from vineyard Z. Moreover, it seems that the hot summer provoked an advanced accumulation of anthocyanins. Grapes from vineyard V in 2009 vintage show higher content of anthocyanins from the beginning and it was maintained during ripening. This could indicate that an early accumulation of anthocyanins promotes higher amounts of them at the end of the ripeness. Perhaps, this fact is associated to a long vegetative cycle of Graciano. As for total flavonol content of the grape skin, they had a tendency to increase slightly during ripening. Vintages seem to have more influence in these compounds than regions. It is also noticeable that in vineyard Z (2008 vintage) the flavonols and anthocyanins contents were the lowest. This is important to remember that the worst weather conditions took place in this case.

In the two studied vineyards the content of procyanidins had higher values in 2008 likely associated to their irregular climate conditions. However the content of prodelphinidins showed an opposite trend (Fig. S5). Thus, the contents of these compounds were higher when the climate conditions were propitious for a good maturity. This data are in accordance with other studies which shows that berries with more luminosity had higher content of (−)-epigallocatechin [44,45].

Principal component analysis was also performed, using 26 variables in this case. These variables corresponded to the phenolic compounds grouped according to their basic structures and some individual compounds (Table S2). Fig. 4 shows the projection of grape skin samples on the plane defined by the first and the second principal components. Each sample was represented by the same alphanumeric code used for grape seeds. PC 1 describes the evolution of grape skin samples during ripening. Samples located on the left side of this principal component presented the higher values in all phenolic compounds of grape skin studied. Samples on the right side correspond to the earlier samples and their temporal evolution toward the left side during ripening indicates the increase in the content of almost all the phenolic compounds studied in the grape skins. It is also noticeable that the 2009 vintage presents a

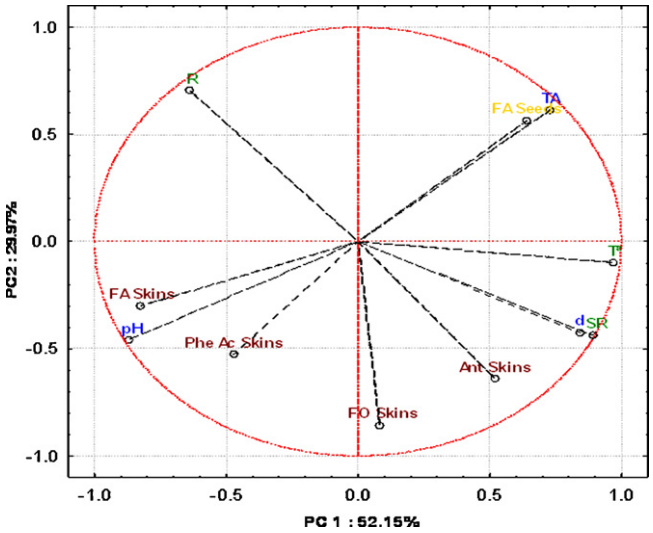

Fig. 5. Loading plot of the principal component analysis of the phenolic composition contents in grape skins and seeds at harvest (flavanols: FA; flavonols: FO; phenolic acids: Phe Ac and anthocyanins: Ant), oenological parameters at harvest (density: *d*; total acidity: TA and pH) and climate conditions during vegetative stage (average temperature: $T^{\underline{a}}$; accumulated rainfall: *R*; and accumulated solar radiation: SR).

more homogeneous pattern. Vineyard V presents more variations in the phenolic compounds during ripening within each vintage. This suggests that the more variations in the phenolic composition of grape skins, the less variation in the phenolic composition of grape seeds. Moreover, a clear separation between vintages in the plane defined by PC 1 and PC 2 is also observed. The separation is more evident than in the case of grape seed. This may indicate that the influence of climatic conditions on the phenolic ripening of grape skins is higher than that of the seeds.

3.3. Phenolic composition, oenological parameters and climatic conditions

In a second strand, results obtained from the phenolic composition analysis at harvest (flavanols: FA; flavonols: FO; phenolic acids: Phe Ac and anthocyanins: Ant), oenological parameters at harvest (density: *d*; total acidity: TA and pH) and climate conditions during vegetative stage (average temperature: $T^{\underline{a}}$; accumulated rainfall: *R* and accumulated solar radiation: SR) were submitted to PCA. Regarding the loading plot (Fig. 5), temperature and solar radiation present a similar trend, which is opposite to the rainfall pattern. Differences between phenolic maturity patterns of grape skins and seeds were also observed. The samples with the highest content of phenolic compounds in the grape seeds at harvest presented the lowest content of phenolic compounds in the grape skins, especially of flavonols and phenolic acids. Moreover, this analysis has also revealed the relationship between phenolic, oenological parameters and climatic variables. Accumulated solar radiation presented a direct relationship with density and in a lesser extent with anthocyanin and flavonol concentrations. On the other hand, accumulated rainfall presented an inverse relationship with the contents of the above-mentioned phenolic compounds (i.e. antocyanins and flavonols) and also with the density of grapes.

4. Conclusions

Regarding the phenolic composition, clear differences between phenolic maturity pattern of grape skins and seeds were observed. In this context, it may be important to evaluate the phenolic maturity of seeds and skins in a separate way in order to decide the optimal harvest time. It is also noticeable that the effect of vintage – mainly due to changes in climatic conditions – may affect the changes in the phenolic composition of both grape skins and seeds. Although in a lesser extent, the effect of the vineyard is also observable and it is especially relevant in vintages with irregular climatic conditions, such as 2008 vintage.

Moreover, results obtained from the phenolic composition analysis, some oenological parameters at harvest time and climatic parameters during the vegetative stage have also revealed the relationship between the aforementioned variables.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.aca.2011.12.072.

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