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Trying to set up the flavanolic phases during grape seed ripening: A spectral and chemical approach

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ABSTRACT

Grape seeds were collected in ten different dates and classified in seven groups according to their individual hyperspectral imaging characteristics.

Proanthocyanidin composition was studied using HPLC-MS for oligomers and acid catalyzed cleavage for polymers characterization. The combination of both analysis provided a complete description of the flavanols. Chemometric analysis was performed to summarize the analytical results. None of the considered variables presented statistical differences among all groups. From one to five groups were found for each variable, while three was the most frequent value, consequently three putative stages might be considered the real number of different analytical stages since it is the number of statistically significant groups for the majority of the compounds. This classification could be considered as the first step to optimize the use of seeds in winemaking to minimize the gap between sugar and phenolic maturities, consequence of the global climate change, mainly observed in warm climate.

1. Introduction

Phenolic compounds in grapes are mainly found in seeds and skins. Proanthocyanidins are present in both tissues, but differences in their composition have been reported as a function of the tissue they are found in. In grape skins both procyanidins and prodelphinidins are present but in seeds only procyanidins have been described [1–3]. Moreover, seeds proanthocyanidins present lower polymerization degree and higher galloylation degree than skins proanthocyanidins. These compounds are involved in wine stability, organoleptic properties such as color or astringency and other properties such as antioxidant activity.

Grape phenolic composition is well known to evolve during ripening, and it is important to take this into account in order to choose the optimum vintage moment. The term "phenolic maturity" was been proposed by Glories and Augustin [4] to describe the concentration of phenolic compounds in grapes, both skins and seeds, and the ease with which they are released. As stated above, phenolic compounds are highly related to organoleptic properties of wines and thus, to its quality.

Moreover, grape maturity presents some heterogeneity among different plants of the same field and even in different grapes of the same plant or bunch, which makes difficult to choose the optimum vintage moment [5,6]. During the last years, the span between phenolic maturity and sugar maturity in grapes is increasing due to global climate change [7], primarily in warm areas. The lack of balance among the phenolic and sugar maturities leads to a decrease of the wine quality and stability. This situation causes a great concern among winemakers, and several strategies has been proposed in order to maintain the wine quality despite the span between technological and phenolic maturities. Among the strategies developed to mitigate the new situation different winemaking techniques, such as seed removal or the addition of seeds from over-ripen grapes, can be performed to reduce the negative effects of the abovementioned maturity gap.

Berry development consists of three phases: two successive sigmoidal growth periods separated by a lag phase [8]. The first period of growth lasts from bloom to approximately 60 days afterward, being 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.

According to Kennedy and coworkers [9], grape seed

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proanthocyanidins biosynthesis and accumulation commenced with seed development. They reach their maximum at the beginning of veraison or 1 week after veraison. They suffer a sharply decrease in the first weeks after veraison [10] and they tend to decrease slightly until over-ripening [10,11]. For obtaining high quality red wines, it is important to achieve a good level of proanthocyanidins since it is well known that red wines with high content of proanthocyanins tend to age better than those with lower proanthocyanin content. In young red wines, these compounds are involved in color stabilization by copigmentation reactions [12] and further in the formation of anthocyanins derived compounds [13]. On the other hand, seed proanthocyanidins are also involved in wine astringency development, and their maturity level is important since less mature grape seeds are more astringent and present higher tannic intensity [11].

It is well known that seed grape coloration changes during maturity from green to yellow and finally to brown-grey hues. These changes in seed coat color have been related to developmental changes in berry [10] and can be used as an indicator of berry ripeness.

During grape ripening, seeds undergo several compositional and physical changes, which modify the amount and distribution of extracted flavan-3-ols. The integument is intensively lignified and dehydrated, leading to seed hardening and to a more difficult release of flavanols [10]. Total amount of flavanols present in grape seeds suffers a decrease as ripens progresses [14], and their extractability is also reduced as a result of a higher interaction with cellular components [15] as well as the development of several compositional changes, such as oxidative cross-linking, that would be also related to a lesser extractability [9]. These changes lead to diminish the total amount of proanthocyanidins extracted during winemaking.

Hyperspectral imaging is an emerging and green chemistry technique for non-destructive and rapid food analysis usually carried out in either the visible-short near infrared (vis-NIR; 400–1000 nm) or near infrared (NIR; 1000–1700 nm) spectral regions [16]. Winemakers are continuously looking for high quality wines and the major factors impacting on wine quality are related to winemaking process and cultivar features. Among others, grape variety, maturity or sugar content are typically analyzed in order to determine grape quality, set grape price and classify grapes for a range of produced wines [17].

The aim of this work is to evaluate the potential of the imaging hyperspectral techniques to differentiate ripening stages in seeds, based on the flavanol composition. To do this, the possible relationship between the homogeneous groups of seeds formed based on the hyperspectral imaging characteristics and the phenolic composition was studied. The differentiation of ripening stages in seeds according to its flavanolic composition could be used to improve the selection of the seeds used for upgrade wine characteristics, providing a non-destructive, fast method for seeds classification. This classification would be the first step of an extended study which would lead with the use of the classified seeds in vinifications with lack of phenolic maturity in order to evaluate which of the groups of seeds would provide better characteristics to the treated wines.

2. Materials and methods

2.1. Grape seed samples

Vitis vinifera L. cv. Tempranillo samples were collected from a vineyard located in the Condado de Huelva Designation of Origin (D.O.) (Andalusia, Spain) which is under the typical climatic conditions of a warm area [18]. The aforesaid cultivar is the most often grown red grape cultivar in Spain for producing quality red wines. Red grapes were collected at different physiological stages during berry maturity in the 2013 vintage: prior to veraison (July 2nd) to over-ripening (August 12th). Ten dates were taken into account for the aforesaid cultivar. Grape seeds were then separated manually from the whole grapes and were immediately frozen and stored at −20 °C until analyses were performed. Prior to the hyperspectral analyses, whole seeds from the ten dates were manually merged into a single sample. Grape maturity presents some heterogeneity among different plants of the same field and even in different grapes of the same plant or bunch. Taking into the account the foresaid heterogeneity, it is desirable to create hyperspectral homogeneous groups, which would be set up regarding the whole composition and not only the phenolic composition. After that, the possible relationship between the homogeneous groups of seeds formed based on the hyperspectral imaging characteristics and the phenolic composition could be studied and putative stages might be considered.

2.2. Near infrared hyperspectral imaging analysis

Equipment and procedure used to image recording are described in detail elsewhere in Rodríguez-Pulido et al. [19]. Briefly, hyperspectral imaging device (Infaimon S.L., Barcelona, Spain) comprised a Xenics® XEVA-USB InGaAs camera (320 × 256 pixels; Xenics Infrared Solutions, Inc., Leuven, Belgium), a spectrograph (Specim ImSpector N17E Enhanced; Spectral Imaging Ltd., Oulu, Finland) covering the spectral range between 900 and 1700 nm (spectral resolution of 3.25 nm). The images were recorded using the abovementioned mirror scanner pushbroom device, a 50 Hz frame rate, an exposure time of 9 ms and the instrument acquisition software SpectralDAQ v. 3.62 (Spectral Imaging Ltd., Oulu, Finland). The whole grape seed samples were thawed and tempered at room temperature and hyperspectral image of sets of 48 individual seeds were recorded. The maximum number of grape seed per image is restricted due to the limitation in the measurement area. Grape seed samples were randomly selected just for hyperspectral measurement. A total of 26 images (one of them contains 32 individual seeds) were recorded. After calibration and segmentation processes, individual spectrum of each grape seed was obtained using Matlab (R2010b; The Math Works, Inc., USA). Noisy wavebands at both extremes of the spectra range were removed and only spectral data in the resulting effective wavelength 950 − 1650 nm regions were used in data analysis due to reduced efficiency outside this range in the used device. These procedures lead to the obtaining of 1232 grape seed spectra, which were combined into the spectral matrix X (X = 1232 samples × Log (1/R) units at 215 wavelengths).

2.3. Chemometrical methods

An unsupervised pattern recognition technique, principal component analysis (PCA), was used in order to provide information about the latent structure of spectral matrix and to find spectral differences among all spectral samples. This method provides information related to spectral outliers (H outliers) and the distribution of samples in the newly-created space. The software used for PCA analysis and spectral pretreatment was Win ISI® (v1.50) (Infrasoft International, LLC, Port Matilda, PA, USA). The k-means clustering algorithm was also used. Given a fixed number of (desired or hypothesized) k clusters, assign observations to those clusters so that the means across clusters (for all variables) are as different from each other as possible. The software used for the aforementioned analysis was STATISTICA 8.0 (StatSoft Inc, Tulsa, OK, USA). Canonical Biplot is a method of multivariate analysis similar to MANOVA (Multivariate Analysis of Variance)

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