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A combined approach using chemical and image analysis to estimate seed maturity for Bordeaux area grapevine

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Abstract

Aim: The phenolic maturity (depending on tannins and anthocyanins) of grapes at harvest is crucial and determines the final quality of wine. The work presented here aimed to characterize the evolution of phenolic maturity of seeds for three varieties by combining macroscopic analysis and chemical analyses of tannins at several phenological stages of interest.

Methods and results: Macroscopic analysis (R software) showed that colour varied dramatically (from green to dark brown) in the two months between bunch closure and fruit maturity. Chemical analysis showed that seed tannins increased from bunch closure to early veraison and decreased after this step until fruit maturity.

Conclusion: These results showed that seed colour variation is correlated to tannin concentration in the seeds.

Significance and impact of the study: There are no easy ways to predict seed phenolic maturity. The aim of this work was to use chemical and image analysis results (usually considered independently) to assess the phenolic maturity of seeds without biochemical analysis such as to establish an optimal harvest date for the most favourable conditions for tannin extraction and, consequently, the organoleptic quality of wine. The originality of this work is to use a combination of seed examination and biochemical composition in terms of tannins (correlation established by PCA). These results will help develop a decision support tool based on a simple seed image acquisition system easily usable by winemakers.

Key words: Grape seeds, tannins, imaging, phenolic maturity

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Introduction

Grape maturity at harvest is crucial and determines the final quality of wine. The maturity of grapevine berries is the combination of optimal technological and phenolic maturity. If technological maturity is well documented and described as sugar and acidity balance (Jones and Davies, 2000; Ollat *et al.*, 2002; Doshi *et al.*, 2006; Castellarin *et al.*, 2016), phenolic maturity depends on various secondary metabolites including tannins (Geny *et al.*, 2003; Ali *et al.*, 2010; Pantelić *et al.*, 2016; De Santis *et al.*, 2017). These phenolic compounds play an important role in red wine organoleptic characteristics, especially the mouthfeel properties like bitterness and astringency. They are also involved in the colour stabilization and evolution of red wine due to their interaction with anthocyanins (Singleton, 1992; Boulton, 2001; Hanlin *et al.*, 2010). Tannins have been partially characterized (Kennedy *et al.*, 2000; Downey *et al.*, 2003; Bogs *et al.*, 2005) and we know that they come from the skin (Cerpa-Calderón and Kennedy, 2008) and seeds of grapes (Kennedy *et al.*, 2000; Downey *et al.*, 2003; Cadot *et al.*, 2006). To determine the phenolic maturity, methods such as chemical analysis as well as pH, anthocyanin or flavan-3ol measurement exist (Glories' method; Ribéreau-Gayon *et al.*, 2006). These methods are effective but require laboratory analyses. During the past 10 years, other subjective methods have been developed to determine phenolic maturity. These experiments are based on the knowledge of oenologists and their ability to taste berries and seeds (Letaief *et al.*, 2013). In 2005, Ristic and Iland (2005) initiated research addressing seed development and tannin accumulation and showed correlations with colour evolution. Fredes *et al.* (2010) used information from this study to develop a comparison method of seed colour against a colour scale. Recently, new approaches such as sensory and instrumental texture measurement or FT-NIR analysis for grape seed maturity characterization have been initiated (Torchio *et al.*, 2012; Letaief *et al.*, 2013; Rodríguez-Pulido *et al.*, 2014; Brillante *et al.*, 2015).

In this paper, both the colour and the tannin composition of grape seeds were analyzed. The originality of our work is to use simple acquisition of numerical data and combine these virtual seed colours with tannin composition (correlation established by principal component analysis (PCA)). This combination allows avoidance of colour scales. These results will help develop a decision support tool based on seed image acquisition.

Materials and Methods

1. Plant material

Experiments were performed during the growing season of 2013 on three grape cultivars (*Vitis vinifera* L. Cabernet-Sauvignon – clone no. 337, Sauvignon blanc – clone no. 242 and Tannat – clone no. 474) from an experimental vineyard in the Bordeaux area (44.791730 N, 0.579145 E), which are known to present different tannin concentrations in wine (Ribéreau-Gayon *et al.*, 2006).

Ten grape clusters were removed at four points, from "bunch closure" to "ripening" (stages 33 to 38 according Eichhorn and Lorenz, 1977), which correspond to key steps in the synthesis of seed tannins (Darné, 1991).

2. Numerical data acquisition

Twelve grape seeds, randomly selected from each cultivar, were scanned with an Epson scanner (Epson Perfection 3200 Photo) on the ventral side. The statistical software R (R Development Core Team, 2008) was used to analyze the variations in seed colour according to the maturation status and the variations in colour inside the seed. Images were read using the R package EBImage (Pau *et al.*, 2010). The image background was excluded to analyze only the seeds using the R packages EBImage and rtiff (Kort, 2015; Pau *et al.*, 2010). The green frame of the red/green/blue (RGB) images gave the best contrast between seeds of different maturation status. To detect several colours in the image, the histogram of the green frame was seen as a mixture of Gaussian distributions where each Gaussian distribution corresponded to one colour. The R package MCLUST (Fraley and Raftery, 2006) was used to detect from 1 to 3 Gaussian distributions that composed the histogram and estimated the mean, variance and weight in the mixture distribution of each Gaussian. For each seed, the number of pixels contained in each Gaussian was calculated. The images were recoloured with blue, green or red according to the probability of membership of each pixel to each cluster.

3. Chemical analysis

Extraction of phenolics from seed samples was performed according to Pekić *et al.* (1998). After fine grinding of seed samples (Retsch Ultra Centrifugal Mill ZM200, Retsch GmbH & Co, Haan, Germany), two successive macerations were carried out for 3 h each, at room temperature by stirring 0.5 g of seed powder in 40 mL of MeOH/12 N HCl (99.9:0.1; v/v).

After incubation, total phenols were directly measured at 280 nm after diluting to 1:100. Data were expressed in mg total phenols (tannins) per g fresh weight, in mg per seed or in mg per berry and represent the mean (± standard deviation) of three replicates.

4. Statistical analysis

All results reported here are means ± standard error of the mean of at least three independent experiments. An ANOVA was performed to test the cultivar effect on mean total tannin concentration. The ANOVA test was run using R software. PCA correlation was established between tannin concentration and colour clusters using R software.

Results and discussion

This work aimed to combine macroscopic analysis of the evolution of seeds for three contrasting tannin grape varieties (Cabernet-Sauvignon, Tannat, Sauvignon blanc) and chemical analyses identifying tannin concentrations at phenological stages of interest (bunch closure, beginning of veraison, end of veraison and ripening).

The macroscopic analyses showed a similar pattern of change over time between the seeds, whatever the cultivar: seeds went through a growth phase (during the first two stages studied) characterized by an elongation, a broadening and an increase in seed surface and, towards the end of ripening, a withering that became maximum at maturity (data not shown).

Besides the physical pattern of evolution, the colour varied dramatically in the two months between bunch closure and fruit maturity regardless of the cultivar (Figure 1A). It went from a green to a dark brown pattern, which was quantified using an R program. This program helped to identify three clusters of pixels according to their colour (represented in false blue, green or red colours) in each seed analyzed and to determine the proportion of pixels displaying the same colour (Figures 1B and 2).

In the 2013 vintage, cluster evolution was different for the three grape varieties (Figure 2). Red and green were the two major clusters found (Figures 1B and 2). Cabernet-Sauvignon and Sauvignon blanc had the same progression pattern, with a drastic increase in the proportion of pixels belonging to the red cluster (Figure 1B) and a notable decline in that belonging to the green cluster between the beginning and the end of veraison. This result was more important for Sauvignon blanc (decrease from 92% to 36% of the pixels belonging to the green cluster) than for Cabernet-Sauvignon (75% to 40%). Tannat showed a delay in red cluster changeand at the end of

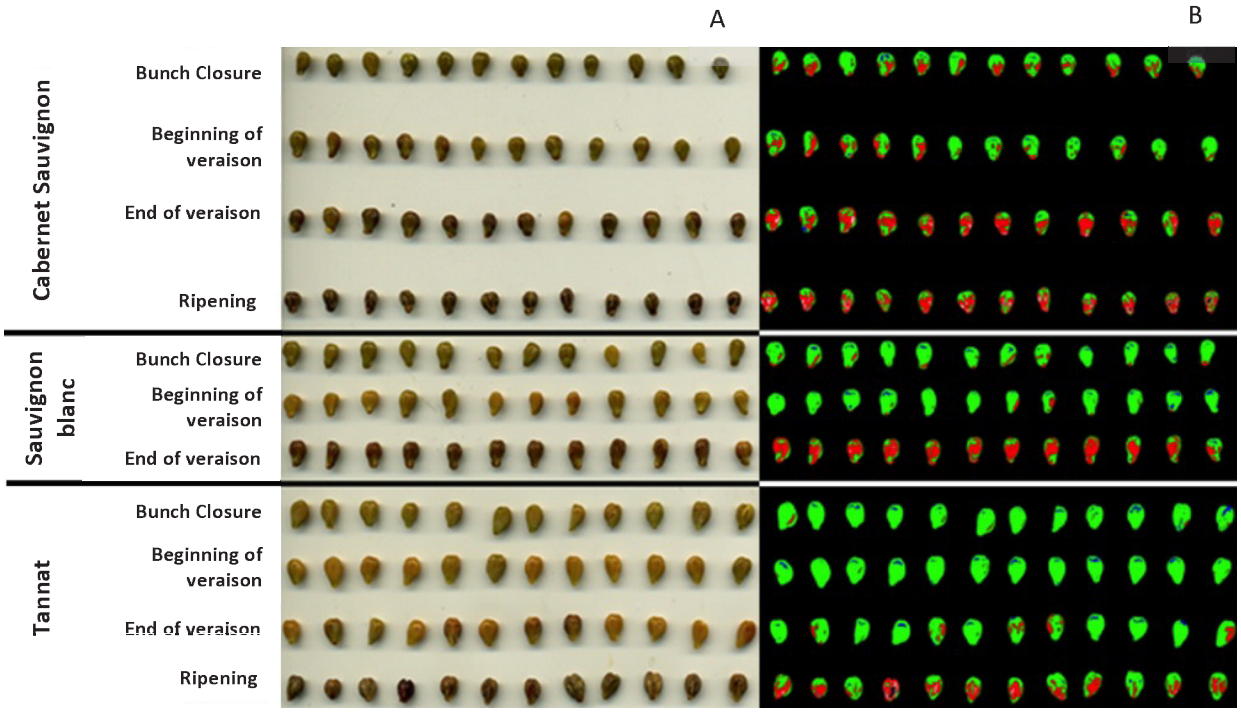

Figure 1. Pictures of seeds from three grape cultivars at four phenological stages
(A) Real seeds and (B) clusters of colours after image treatment.

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veraison, only 15% of the pixels belonged to the red cluster and < 50% (41%) were measured at ripening (Figure 2).

Analysis of grape seed tannin evolution has normally been performed according to Ristic and Iland (2005) by comparison of colours against a colour scale and has been confirmed for different grape varieties (Rodríguez-Pulido *et al.*, 2012a,b; Zuñiga *et al.*, 2014); however, nothing has been done on Bordeaux grape cultivars. Here, extracted colours have been compared to each other. By using digital imagery, the use of a colour scale was unnecessary. Numerical data have already been used in precision viticulture studies (Rodríguez-Pulido *et al.*, 2012a,b; Whalley and Shanmuganathan, 2013; Zuñiga *et al.*, 2014), all of which have investigated ripeness estimation and phenolic maturity of grape seeds using DigiEye® imaging or NIR hyperspectral imaging. However, if these methods are scientifically approved, their adoption by wine growers is still difficult. All these results led us to use a common scanner and software. Literature and our results allow us to consider evidence that artificial colour variation over time could be representative of seed maturity status. But colours alone are not sufficient to estimate phenolic maturity of seeds. Therefore, we determined total tannins to get a biochemical profile of our seeds. Total tannins in seeds increased from bunch closure to veraison and decreased from veraison to fruit maturity in all grape cultivars. Nevertheless, there were some differences between grapevine varieties. Total tannins were 1.5-fold and 1.2-fold higher for Cabernet-Sauvignon than for Sauvignon blanc and Tannat, respectively, in seeds and also in berries at the end of veraison (Figure 3).

Veraison is the key point for tannin accumulation in grapes. In fact, subsequent to that point, tannins are not accumulated and display a decrease that is cultivar-dependent. For Cabernet-Sauvignon, total tannins decreased 1.5 fold between the end of veraison and harvest (Figure 3A). The slope is less important (1.2 fold) but still significant for Sauvignon blanc and Tannat, in seeds and even more in berries (Figure 3B and C). For Sauvignon blanc, a large proportion of total tannins declined from the start of veraison (12.4 mg/berry) to reach 7.7 mg/berry at the end of veraison (Figure 3C). Overall, the tannin profile in seeds and, as a consequence, in berries, is consistent with literature. In grape seeds, tannin biosynthesis starts with seed development. This synthesis pathway, with the increase in seed tannin synthesis before veraison followed by an important decrease from the end of veraison until harvest, has previously been reported (Romeyer *et al.*, 1985; Katalinić and Maleš, 1997; Kennedy *et al.*, 2000). Our observations are also consistent with profiles obtained for Shiraz (Downey *et al.*, 2003), Sangiovese (Filippetti *et al.*, 2015)and Cabernet franc (Cadot *et al.*, 2006). Taken independently, these results do not predict the level of phenolic maturity necessary to establish an optimal harvest date for the extraction of tanninsand hence for the aromatic quality of wine. So, a combination of macroscopic determination and biochemical measurement by

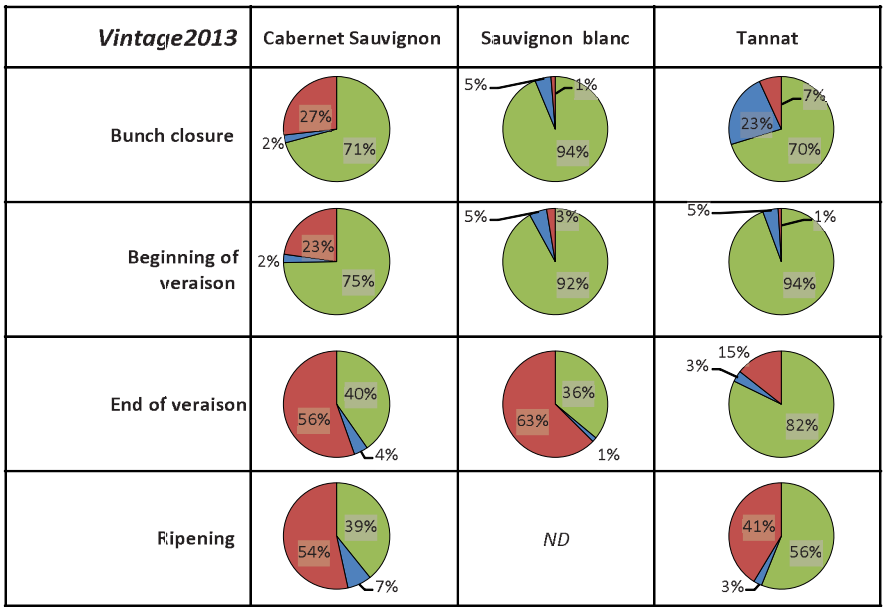

Figure 2. Colour cluster separation of three grape varieties and four phenological stages

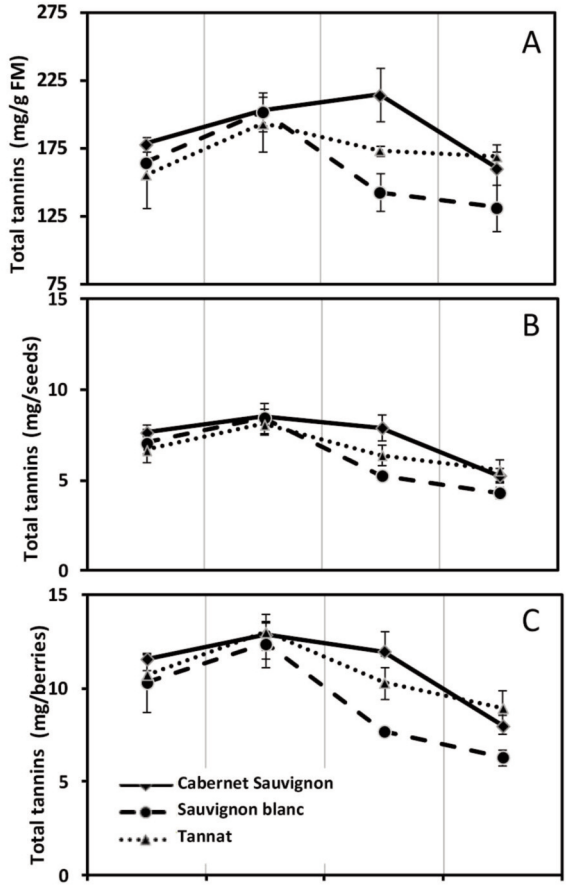

Figure 3. Total tannin determination
Evolution of mean total tannins in (A) mg / g fresh material, (B) mg / seed and (C) mg / berry. For all graphs: Cabernet-Sauvignon is represented by a solid line, Sauvignon blanc by a dashed line and Tannat by a dotted line. BC = bunch closure, BV = beginning of veraison, EV = end of veraison and R = ripening.

PCA allows linkage of those two major metrics – colour clusters and tannin composition – of grape seeds. First, PCA allowed demonstration of the correlation between colour clusters and chemical analysis. It showed that the cluster represented in green appeared linked to total tannins (Figure 4A): total tannin measurement was linked to the green cluster (mostly observed at veraison) at nearly 58%. Second, another PCA allowed us to test the correspondence between grape cultivar and phenological stage. It showed that three groups were apparent (Figure 4B). One of them grouped bunch closure and the beginning of veraison (5-6) of Sauvignon blanc and (9) Tannat. The second one grouped bunch closure and the beginning of veraison (1-2) of Cabernet-Sauvignon and the end of veraison (10) of Tannat. Finally, a third group emerged with the end of veraison and ripening (3-4) of Cabernet-Sauvignon and (11) Tannat.

This result is in accordance with the macroscopic analysis, which showed a delay of maturity for Tannat until the end of veraison that disappeared by ripening (Figures 1 and 2).

This first study gives us many interesting results to establish solid perspectives. Although we need to confirm these initial conclusions with seeds from other vintages and cultivars, these preliminary observations show that grape seed colour seems to be linked to phenolic maturity and convince us to persevere in developing a new way to establish the phenolic maturity of grape berry by image analysis using common informatics tools.

Conclusion

These results provide a good framework to help winemakers in determining the ideal maturity stages reflecting both grape seed and berry ripening. Further analysis on other vintages and cultivars are needed to assess the robustness of our results. However, using common informatics tools such as scanner and free software seems to be a powerful decision tool to track cultural evolution of grape bunches in the future.

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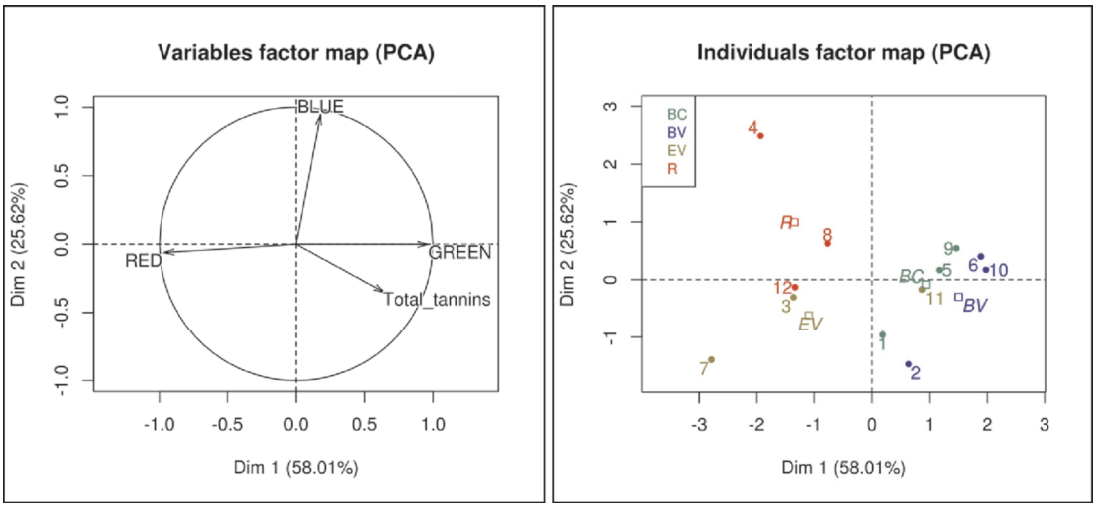

Figure 4. Principal component analysis (PCA) between colours, total tannins and phenological stages
(A) Visualization of correlation between colour cluster and total tannins. (B) Cluster of grape cultivar and phenological stage. 1 to 4 (Cabernet-Sauvignon), 5 to 7 (Sauvignon blanc) and 8 to 11 (Tannat) represent bunch closure (BC), beginning of veraison (BV), end of veraison (EV) and ripening (R), respectively.

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