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Anthocyanin yield and skin softening during maceration, as affected by vineyard row orientation and grape ripeness of *Vitis vinifera* L. cv. Shiraz

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Running title: Anthocyanin yield and skin softening during maceration of Shiraz grapes

Abstract

Anthocyanin and mechanical properties were evaluated on Shiraz grapes, picked from both sides of North-South and East-West vineyard row orientations at two harvest dates. Wines were made from each combination. The evaluation and evolution of crushed skin mechanical properties during maceration-fermentation, as also affected by grape ripeness, are shown for the first time. No significant differences in anthocyanin content were found in the grapes between the two vineyard row orientations. However, a significant decrease in anthocyanins and berry skin break force (also referred as skin hardness or strength) was found between the two harvest dates. During maceration, a reduction in the crushed berry skin break force of more than 15 % occurred. The intact berries and macerated skins showed similarity in skin break energy values. The anthocyanin profile of the grapes and of the wines prominently displayed malvidin forms, changed mainly by the ripeness level of the grapes.

Key words: row orientation, anthocyanins, texture analysis, skin break force, maceration.

1. Introduction

Wine anthocyanins have a very important role in the production and commercialization of the wine, as they determine the red wine colour; their nature, extraction and final content influence the sensory characteristics and the consumer acceptability of the wine.

The content of anthocyanin compounds in red grapes may vary considerably depending on cultivar, agronomical practices, canopy microclimate, and bunch exposure (Spayd, Tarara, Mee & Ferguson, 2002; Chorti, Guidoni, Ferrandino & Novello, 2010; Hunter, Archer & Volschenk, 2010a). In addition, it is well know that the anthocyanin amount in the wine is dependent, but not strictly correlated, with the grape concentration. Knowing how these factors influence the accumulation of anthocyanins in grapes and their release into the juice/wine during the maceration process is therefore important. However, few studies, some of which on Shiraz grapes, were done on the effect of sunlight exposure on the grape anthocyanin composition. Particular consideration was given to differences within bunches between berries that were exposed to the sun and those facing the interior of the canopy (Pisciotta, Barbagallo, Di Lorenzo & Hunter, 2013) or between bunches directly exposed to sunlight or enclosed in opaque boxes excluding light (Downey, Harvey & Robinson, 2004). In addition, temperature and shading effects on different Merlot vineyard sites (Tarara, Lee, Spayd & Scagel, 2008) and the effect of grape anthocyanin content on the final quality of wines (Ristic et al., 2007) were investigated.

The evaluation of the grape mechanical properties was used in recent years to study modifications in grape texture that may occur under different vine growing conditions (Sato, Yamada, Iwanami & Mitani, 2004; Rolle, Gerbi, Schneider, Spanna & Río Segade, 2011a; Giordano, Zecca, Belviso, Reinotti, Gerbi & Rolle, 2013; Zsófi; Villangó, Pálfi, Tóth & Bálo, 2014) and to monitor the grape ripening process (Maury, Madieta, Le Moigne, Mehinagic, Siret & Jourjon, 2009; Rolle et al., 2011b). Literature on the relationships among vineyard-related characteristics, such as bunch sunlight exposure and berry skin mechanical properties, is scarce. However, a positive relation between these latter parameters and grape anthocyanin content and extractability was observed in wine-like solutions (Rolle, Torchio, Ferrandino & Guidoni, 2012a).

This research aimed to better understand the evolution of the mechanical properties of Shiraz grapes as well as their anthocyanin content and extraction potential, having ripened in different microclimatic environments as induced by changing the vineyard row orientation. Monitoring was done on the intact harvested grape, during the maceration process, and in the respective wines at the end of fermentation. With consideration of the effects of row orientation and grape ripeness level, specific aims can be summarized as: i) chemical and physical evaluation of grapes; ii) evaluation of the changes in skin mechanical properties and anthocyanin concentration of intact berries as well as during maceration; and iii) grape and wine anthocyanin profile.

2. Materials and Methods

2.1. Experimental site and grape sampling. A 3 ha vineyard of *Vitis vinifera* L. cv. Shiraz (clone SH 9C), grafted onto rootstock 101-14 Mgt, was planted in 2003 at the ARC Infruitec-Nietvoorbji experiment farm in the Robertson wine region (33°5' S 19°54' E) of the Western Cape (South Africa) to four

different row orientations and five replicates for each orientation. Only North-South and East-West row orientations were considered in this study. Grapes were harvested in 2012 at two different harvest dates (March 6th and 26th, as the A and the B sample, respectively), The grapes were picked separately on both sides of a vineyard row. For all the analyses, two repetitions of each replicate were used, hence 10 total repetitions for each one of the two row orientations. With each repetition, a sample of 200 berries were randomly sampled in equal amounts from the different parts (top, middle, bottom) of the clusters. This sample was used for the analyses of phenolic and mechanical properties.

2.2. Grape must analysis and vinification. At each harvest, two batches of approximately 80 kg of grapes were picked from each parcel, hence a total of 10 batches were sampled for each row orientation. Each grape batch was treated separately. Grapes were transported to the ARC Infruitec-Nietvoorbji Stellenbosch experimental cellar, where they were destemmed and crushed. For each vinification, the obtained volume was measured and then entirely placed in 100 L food grade containers. A must sample was taken immediately for the measurement of total soluble solids (by refractometry, in Brix), pH and total titratable acidity, following OIV (2011) methods. Sulphur dioxide (50 mg/L) and di-ammonium phosphate (50 g/hL) were then added to the whole batch of crushed juice before inoculation with rehydrated yeast (30 g/hL VIN 13, Anchor Yeasts, Industria, ZA). The maceration/fermentation of all the samples was conducted in a thermo-controlled room at 24 °C, punching down the pomace cap 3 times a day. Samples of macerated skins and juice/wine were taken from the middle of the pomace cap and from the middle part of the fermenting suspension, respectively, at the second and sixth day of maceration/fermentation. After 7 days the fermented juice/wine was removed and the pomace pressed (2 bar pressure). The juice/wine derived from pressing was recovered and added to the primary wine, after which the wine was maintained at 24 °C until the end of fermentation. When the sugar concentration reached less than 5 g/L, a sample was taken for the subsequent anthocyanin analysis.

2.3. Instrumental texture analysis evaluation. A TA.XTplus universal testing machine (Stable Micro Systems, Godalming, Surrey, UK), equipped with a HDP/90 platform, was used for the evaluation of the berry skin break force in fresh berries and macerated berry skins, performing a puncture test (Letaief, Rolle, Zeppa & Gerbi, 2008). For the latter, a 1-mm custom-built needle probe with a test speed of 1 mm/s was used. Twenty intact berries for each repetition were tested, while for the macerated berry skins sixteen skins were used for each repetition. The fresh intact berries were evaluated with a puncture on the equatorial side, whereas for the evaluation of the macerated skin break force (Fmsk) a cylindrical plastic adapter (15 mm in diameter and 10 mm in height) with a 3-mm hole was placed on the platform to avoid the shift of the sample during the test, thus not touching the probe during the compression and not interfering with the puncture test. The maximum force opposed by the berry skin until penetration was expressed in N and indicated as Fsk or berry skin break force. Using the force-distance graph, the berry skin break energy parameter (Wsk or Wmsk for intact or macerated berry skins, respectively), defined as the area under the force-distance curve from the test start until the registered maximum force peak (Fsk parameter; Letaief et al., 2008) was calculated.

2.4. Anthocyanin extraction from grape skins. Ten berries, randomly selected for each repetition, were weighed. Skins and seeds were then separated and the skins treated for anthocyanin extraction and analysis, following the methods of Di Stefano and Cravero (1991). The skins were immediately

placed in a 40 mL wine-like buffer solution containing 12 % ethanol, 5 g/L tartaric acid, and 2 g/L sodium metabisulphite (to avoid any possible oxidation) and adjusted to pH 3.20 with 1 mol/L sodium hydroxide solution. Skin and seed material was weighed for each repetition. The skins were subsequently homogenized at 8000 rpm for 1 min with an Ultra-Turrax T25 homogenizer (IKA Labortechnik, Staufen, DE) and then centrifuged for 15 min at 3000 × g at 20 °C. The supernatant was then taken for anthocyanin analysis.

2.5. Anthocyanin analysis. Anthocyanin concentration in skin extract, fermenting juice and wine samples was assessed reading the absorbance at 540 nm, using an Ultrospec II spectrophotometer (LKB Biochrom, Cambridge, UK), after dilution with an ethanol:water:HCl 37% 70:30:1 (v/v) solution. Anthocyanin concentration was expressed as mg malvidin-3-O-glucoside chloride (Extrasynthèse, Genay, FR) equivalents per L when referring to the fermenting juice and wine or per kg when referring to the skins (Di Stefano & Cravero, 1991). Relative standard deviation (RSD) for the total anthocyanin index method was found to be 1.41 % in previous works (n = 20; Torchio, Cagnasso, Gerbi & Rolle, 2010).

2.6. Anthocyanin profile analysis. A 2 mL sample (grape extract or wine) was purified using a SPE Bond-Elut C18 cartridge (Varian-Agilent Technologies, Palo Alto, CA, US), the phenolic compounds eluted from the cartridge with methanol, the solution evaporated to dryness, re-dissolved in 1 mL of methanol:water:formic acid 50:40:10 (v/v), and membrane filtered at 0.22 µm. The purified sample was then injected into an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA, US), using a Merck RP-18 Purospher 250 x 4 mm i.d. column (Merck Millipore, Darmstadt, DE) at 1 mL/min flow rate and visible light signal detector set to 520 nm wavelength value. The mobile phase solvents comprised an A [water:formic acid 90:10 (v/v)] and a B [methanol:water:formic acid 50:40:10 (v/v)] solution, operated under the following gradient conditions: from 72% A to 55% A in 15 min., then to 30% A in 20 min., to 10% A in 10 min., to 1% A in 5 min. and back to 72% A in 5 min., with an equilibration time of 5 min. plus injection time. Peak identification was assessed by comparing data available in literature (Pomar, Novo & Masa, 2005). Results were expressed by area percentage of the total anthocyanin forms found (Di Stefano & Cravero, 1991).

2.7. Statistical analysis. Statistical analysis was performed using the statistical software package IBM SPSS Statistics (IBM Corporation, Armonk, NY, US). The Tukey-b test at $p < 0.05$ was used in order to establish statistical differences by one-way analysis of variance (ANOVA).

3. Results and Discussion

3.1. Chemical and physical evaluation of the grapes, harvested from different vineyard row orientations, as affected by ripening

An average difference of 1.8 °Brix was registered between the two harvest dates, with a modification of the titratable acidity content (average decrease of 0.76 g/L, as tartaric acid) and of the juice pH (increase of 0.08 units) (Table 1). These differences were mainly related to the common ripening trend of Shiraz that often reduces the average berry weight, as also found by others (McCarthy & Coombe, 1999; Guidoni & Hunter, 2012). However, no skin weight decrease was observed (Table 1).

The seed weight did not show significant changes during the ripening period; an average of 2.1 seeds per berry were found, with the mean weight contribution of the seeds to each berry being 55.6 mg and 54.0 mg for the first and second harvest date, respectively, corresponding to 3.36 % and 3.49 % of the total berry weight, depending on the harvest date.

The row orientations affected the titratable acidity content, which was higher in the grapes from the East-West row orientation, but it did not influence berry and skin weight (Table 1).

The fresh skin anthocyanin concentration decreased from the first to the second harvest, either when expressed per kg of berries, per berry or per gram of berry skin (Table 2), as a function of the ripeness level; this observed decrease is confirmation of the possibility of a loss of anthocyanins during the last ripening phases (Yamane, Jeong, Yamamoto, Koshita & Kobayashi, 2006; Mori, Goto-Yamamoto, Kitayama & Hashizume, 2007). When normalizing the values per grape berry skin weight it was found that the anthocyanin contents were strongly dependent on the skin attributes rather than the row orientation, and the ripening effect is further perceived. No effect of row orientation on skin anthocyanin concentration was observed.

A study on the effect of different vineyard row orientations on Norton table grapes (Jogaiah, Striegler, Bergmeier & Harris, 2012) in a vineyard located in Hermann (Missouri, US) showed, like in the present study, a significant effect on total titratable acidity of the must. Higher values were found with North-South row orientation compared to East-West and the pH values seemed not in line with the acidity. Also the anthocyanin content in the cited study differed between the two row orientations, with about 20 % higher values found for the North-South row orientation.

In a study on Shiraz grapes Pisciotta et al. (2013) compared the effect of light exposure of the berries in relation to their anthocyanin content. No significant differences between berries facing the internal or external side of the canopy were found when the anthocyanin contents were expressed per skin surface unit. However, significant differences in anthocyanin contents occurred between berries sampled from different bunch portions (apical, median or basal). According to the architecture of the vertical shoot positioned canopy these berries were indeed differently exposed to sunlight.

Results on the mechanical properties of the fresh grapes are shown in Table 2. To assess the mechanical properties of the berries, a needle shaped probe, similar to those used by other authors (Maury et al., 2009), was used in order to minimize the interferences due to the berry compression that naturally occurs when the probe begins to press against the sample. The values registered for the fresh berry skin break force (Fsk) and break energy (Wsk) were almost similar between the two row orientations, whereas a strong decrease from the first to the second ripeness level occurred for both parameters. Therefore, with ripening the resistance of the berry skin against breaking decreased, hence the berry released its contents (pulp and seeds) faster and more easily during crushing and maceration. This can be seen especially with the Wsk value, which decreased by 49 % (from 0.068 ± 0.023 mJ to 0.035 ± 0.026 mJ on average) between the first and second harvest dates. The variability among single measures (relative standard deviation) was quite high for all the samples, as also previously observed on cv. Nebbiolo skins with a similar testing probe (Rolle et al., 2011b).

The evolution of the mechanical properties during ripening was also investigated in other varieties. On Cabernet franc grapes in a 6-week period before harvest, the berry skin break energy showed a clear decrease with increasing ripening only in one of the three measured parcels, while the other two showed only a trend (Maury et al. 2009). On Nebbiolo grapes, during an 8-week monitoring period from the first week after véraison to harvest (Rolle et al. 2012a), the Fsk parameter increased during the whole period and the Wsk parameter showed the same trend until the seventh week.

3.2. Skin mechanical properties and anthocyanin yield during maceration/fermentation

Berry skin mechanical properties, i.e. berry skin break force and thickness, were previously studied in relation to the extractability of phenolic compounds (Rolle, Siret, Río Segade, Maury, Gerbi & Jourjon, 2012b). This far, no studies have been done on the evaluation and evolution of the mechanical properties of crushed skins during maceration, and how the ripening of the grapes (and of the skins themselves) may affect this during the vinification process. The analytical evaluations were carried out on the second and sixth day of maceration, that is at the point when the fermentation yeasts have almost completed the exponential phase and when maximum anthocyanin amount is extracted for macerated Shiraz grapes (Gómez-Míguez & Heredia, 2004).

Table 3 shows the results of measurements done during maceration. Simultaneously with an increase in anthocyanin extraction from the skins into the juice/wine with further maceration, the crushed skins softened from day 2 to day 6 by 16.1 % and 15.2 % (for grapes harvested at the first and the second ripeness level, respectively) in Fmsk (macerated skin break force) and by 24.8 % and 19.8 %, respectively, in Wmsk (macerated skin break energy). The percentage differences were therefore higher when grapes were harvested earlier at a lower soluble solid content.

Since the samples of macerated skins were very heterogeneous the individual measurements varied considerably. An adequate sample number was therefore necessary to provide meaningful results. The relative standard deviation (RSD) of values was higher in samples of the sixth day of maceration and in those of the second harvest date. The RSD calculated on Fmsk parameter, by each row orientation for the tests done on the first sampling point, was found to be quite stable after 70 measurements (values under 27.5 %).

The need for a large number of repetitions for each sample when analyzing the macerated skin mechanical properties was especially evident when comparing the influence of the different vineyard row orientations on these parameters (Table 4), with the resulting average values quite similar between the two row orientations. The decrease in Fmsk and Wmsk during maceration seemed to be higher for the North-South row orientation when grapes were sampled at the lower ripeness level, whereas for the East-West orientation when grapes were harvested at the higher ripeness level (Table 4). This could display a non-homogeneous response to maceration by grapes picked at different soluble solid content but, at the present time, there is no information about a possible role of the juice soluble solid content or acidity on the skin softening.

The anthocyanin content, expressed in mg/L of juice/wine, was very variable (high RSD) in the East-West row orientation replicates and the total content at the sixth day of maceration and at the end

fermentation was similar for the wines produced with grapes from the two row orientations but higher in the wines produced with the more ripened grapes (Table 4).

During maceration, anthocyanins are extracted in the very first stages by the action of the grape crushing and skin contact time in a mainly non-alcoholic must suspension, eventually with some contribution by the commonly added small amounts of sulphur dioxide. Later, when fermentation has started and the alcohol content, carbon dioxide and higher temperature effects take place, the permeability of the skin cells and membranes increases (Sacchi, Bisson & Adams, 2005), with a further release of anthocyanins and other phenols. These phenomena also bring about a reduction in the berry skin break force and energy, particularly after some days of maceration-fermentation.

In addition to the maceration and fermentation effects, the berry skin break force itself seems to be related to the anthocyanin extractability. When anthocyanin extraction from grapes sampled at a single harvest time and with the same pulp sugar content was compared in model solutions (at 3 % and 12 % (v/v) fixed amount of ethanol, as the common ethanol content reached in the maceration by the vinification protocols that are used with these grape cultivars), higher Fsk values resulted in a higher rate of extraction with either Brachetto (Rolle, Torchio, Zeppa & Gerbi, 2009) or Nebbiolo (Rolle et al., 2012a) cultivars. Nonetheless, few significant differences were found between the percentages of different anthocyanin forms extracted.

The anthocyanin content extracted from the skins and available in the juice/wine at the second harvest date was higher in all the measurements, compared to the macerations performed at the first harvest date (Tables 3 and 4). The total content measured in the fresh grapes of the second harvest date was lower with respect to the first harvest date grapes (Table 2). Therefore, several factors could have influenced the release and extractability of anthocyanin from the skins, including the skin/pulp ratio (Guidoni & Hunter, 2012), the ripening level (Hernández-Hierro, Quijada-Morín, Rivas-Gonzalo, & Escribano-Bailón, 2012) and the skin cell wall modifications (Ortega-Regules, Romero-Cascales, Ros-García, López-Roca, & Gómez-Plaza, 2006; Hernández-Hierro et al., 2013). The variations of the Fmsk and Wmsk values from the first to the second harvest date or from the fresh grape to the crushed skins may also be possible consequences of the skin structure modifications occurring in the skins during ripening.

The value of Fmsk of the macerated skins evaluated at the second day of maceration was found higher than those found when the same test (as Fsk) was done on the fresh berries. This could be related to the shape of the intact berry that forces the skin to be homogeneously stretched due to the effect of the berry contents (mainly pulp). This property is difficult to maintain when analyzing the skins of crushed berries. The testing conditions and equipment were adapted in order to maintain the natural stretching and stability of the skin during the puncture test, but this could give slightly different results in comparison to the intact berry. The Wsk values, instead, were consistent with Wmsk values, and, as expected, a decline of the values was observed from the fresh berry to the macerating skins (Tables 3 and 4), with a minor reduction in the riper berries.

3.3.Grape and wine anthocyanin profile considering the grape ripening effect and vineyard row orientation

The grape anthocyanin profile, shown in Table 5, was coherent with the typical Shiraz profile (Mattivi, Guzzon, Vrhovsek, Stefanini & Velasco, 2006; Guidoni & Hunter, 2012). The vineyard row orientation did not play an important role; only small differences were found for cyanidin-3-glucoside (which accounts for less than 0.5 % of the total content) and total acetyl-glucoside derivatives. The harvest date, on the contrary had major effects: an increase of the percentage of the cinnamoyl-derivatives was found (Table 5), mainly caused by the malvidin-3-coumaroyl-glucoside content, which increased from 25.7 % to 29.1 % between the two harvest dates, accounting for the majority of the coumaroyl-glucoside forms present in the grapes.

Different conditions of temperature and light exposure of the bunches may alter grape anthocyanin concentration and profile, modifying, in particular, the ratio between di-oxygenated and tri-oxygenated forms and the ratio between free or derivative forms (Bergqvist, Dokoozlian & Ebisuda, 2001; Downey et al., 2004; Tarara et al., 2008; Chorti et al., 2010; Rustioni, Rossoni, Failla & Scienza, 2013), even if not all the studies agreed with these observations (Ristic et al., 2007).

An exclusion of sunlight from Shiraz clusters was found to significantly modify the percentage content of all anthocyanin forms, except delphinidin-derivative values, and also modify the ratios of di-oxygenated/tri-oxygenated forms in favour of the latter (Ristic et al., 2007). In addition, in differently oriented rows modified conditions of bunch exposure to sunlight could be expected with a difference in the photosynthetically active level of disposable radiation (PAR), UV-A and UV-B levels (Grifoni, Carreras, Zipoli, Sabatini, Dalla Marta & Orlandini, 2008; Hunter et al., 2010a; Hunter, Volschenk & Bonnardot, 2010b). The modification of the anthocyanin profile that emerged from our study could be in agreement with the changes of microclimatic condition of the last period of ripening, more than with the different row orientation. Nonetheless, it seems also possible that during the last phases of ripening, or in the over ripeness condition, the individual anthocyanins may have undergone some transformation independently from the light and temperature condition, also taking into consideration the mechanical properties of the berry skins, that, in fact, greatly changed during the ripening process. However, no evidence of this possibility is present in literature.

In particular, as a consequence of the different row orientation, only the cyanidin content was slightly changed, but a percentage reduction of total acetyl-glucoside forms was found at the second harvesting date. The differences found by Ristic et al. (2007) between the total anthocyanin contents expressed in mg per berry were not significant between the shaded and control treatments, as observed also in this study between the samples collected from the different vineyard row orientations. Another study focusing on the effect of light exposure on the acylated content showed significant differences in all the trials between normal and shaded samples with regard to the acylated anthocyanin percentages, and partially to the ratios of acetic acid/p-coumaric acid acylated forms (Rustioni et al., 2013). Furthermore, their evaluation of the final wines did not always show a difference in the acylation percentage between the normal and shaded trials.

A study of Tarara et al. (2008) on Merlot grapes showed that different light exposure by the vineyard (canopy) side (Sun-East and Sun-West trials) affects the amount of anthocyanins found in the berry skin, with important changes in both the total amount (expressed as content per berry skin surface units) and the percentage anthocyanin forms. However, the study analyzed only the two separate sides of a single row orientation, and not a comparison between two row orientations made from grapes taken on both sides, the latter which is a more common practice in commercial vineyards. A

different accumulation of anthocyanins (in g of fresh skin), in relation to the PAR measured, was also found between North and South sides in a vineyard in the San Joaquin Valley (California, US) (Bergqvist, Dokoozlian & Ebisuda, 2001).

Some differences in the anthocyanin profiles of the wines, produced from grapes harvested at two ripeness levels, were found at the end of fermentation (Table 6). Although the content of delphinidin, cyanidin and petunidin-3-glucoside changed significantly, their contribution to the total anthocyanin concentration accounted for less than 3 % altogether, contributing very little to the total colour perception. The malvidin-3-glucoside content marked a reduction of 1.3 % between the two points, but the more interesting effect was observed for the acylated compounds. The decrease of acetyl derivative forms, quantified as a rough 0.8 %, was mainly due to the reduction of the malvidin-3-acetyl-glucoside, which decreased from 24.5 % to 23.8 % (-0.7 %). A possible factor involved in this relative reduction can be the higher extraction of other forms, mainly *p*-coumaroyl and caffeoyl-glucoside forms, due to the grape ripening effect and to the presence of higher percentage contents already found in the riper grapes. Indeed, the sum of these latter forms accounted for a total of 17.1 % in the wines produced from the first harvest and of 19.6 % for the second harvest. The malvidin-3-caffeoyl-glucoside and *p*-coumaroyl-glucoside forms were principally responsible for the relative increase, with a +2.2 % variation between the two ripeness levels (12.0 % and 14.2 % was the contribution in the first and the second harvest wines, respectively). The peonidin-3-coumaroyl-glucoside form also marked a 0.2 % increase between the two points, while the values of other forms (delphinidin-3-coumaroyl-glucoside and cyanidin-3-coumaroyl-glucoside) decreased between the points. The latter two forms together accounted for less than 1% of the total anthocyanin profile of both harvesting points.

The cyanidin-3-glucoside form, which is easily extracted during the first phases of maceration because of the higher diffusion in the must (González-Neves, Gil & Barreiro, 2008), together with the peonidin, delphinidin, and, in minor quantities, petunidin glucoside forms, marked a relative decrease in the wines with respect to the grape proportions, possibly due to degradation reactions occurring during the winemaking process (García-Beneytez, Revilla & Cabello, 2002). This effect, limited to the free glucoside forms, was also previously shown in a study concerning the fermentation-maceration kinetics of Shiraz grapes, where it was found that the extraction of these forms from the skins was almost completed (Guidoni & Hunter, 2012). In this study, conducted on the same vineyards, the percentage of *p*-coumaroyl-glucoside derivative forms in the wine was lower than in the grape because of their minor extractability during maceration/fermentation. A similar result was obtained in the present study but with some differences between the two ripeness levels. Since the softer (more ripe) berries gave a wine profile proportionally richer in *p*-coumaroyl derivatives, a possible contribution of the skin structure and mechanical properties to the rate of anthocyanin extraction from the skin was verified. The different anthocyanin profiles of wines made at the two ripeness levels may also have been caused by the effect of the aforementioned possible degradation and hydrolization (Cheynier, Moutounet & Sarni-Manchado, 1998; García-Beneytez et al., 2002; Canals, Llaudy, Valls, Canals & Zamora, 2005).

4. Conclusions

The vineyard row orientation showed little influence on the grape quality parameters measured in this study. An increase in the total titratable acidity for the East-West orientation, with respect to the North-South, was found. No effect on the mechanical properties of the skins or on anthocyanin concentration was observed. The main differences were related to the ripeness level of the grape. The more ripened grapes showed an increase in soluble sugars and pH, a decrease in the titratable acidity and anthocyanin concentration, and lower values of the skin mechanical properties parameters related to the skin softness, i.e. break force (Fsk) and energy (Wsk).

A decrease of the crushed berry skin strength, evaluated as macerated berry skin break force (Fmsk) and energy (Wmsk), was objectively shown for the first time during skin maceration/fermentation, confirming the general perception of winemakers and cellar workers. The different ripeness levels of the starting grapes affected the skin break force but also the evolution of the mechanical properties of the crushed skin during maceration: the riper grapes showed lower values for all the measurements. The skin break energy parameter in particular gave consistent values between the intact berry and the correspondent macerated skin samples.

Vineyard row orientation had little effect on the anthocyanin profile of the grapes or that of the final wines, whereas the main effect was attributed to the ripeness level. This study helped to further understand the maceration process and could open new possibilities for future studies: as an example, the influence of the grapes different soluble solids content (without the harvest date effect), the influence of viticultural and enological practices, or the application of grape pre-treatments on the skin softening during maceration could be assessed.

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Table 1. Mean values of grape chemical parameters and berry skin weight at harvest.

Harvest date	Vineyard row orientation	°Brix	pH	Titratable acidity (g/L H_2T)	Berry weight (g)	Berry skin weight (mg)
March 6th (A)	North-South (NS)	25.8 ± 0.4	3.92 ± 0.05	4.81 ± 0.17	1.60 ± 0.17	189 ± 21
	East-West (EW)	25.4 ± 0.9	3.85 ± 0.10	5.17 ± 0.22	1.71 ± 0.16	212 ± 33
	Significance	ns	ns	***	ns	ns
March 26th (B)	North-South (NS)	27.3 ± 0.7	3.99 ± 0.03	4.06 ± 0.19	1.50 ± 0.11	206 ± 25
	East-West (EW)	27.5 ± 1.2	3.95 ± 0.07	4.40 ± 0.21	1.59 ± 0.09	218 ± 29
	Significance	ns	ns	**	ns	ns
A	NS+EW	25.6 ± 0.7	3.89 ± 0.08	4.99 ± 0.26	1.65 ± 0.17	201 ± 30
B	NS+EW	27.4 ± 0.9	3.97 ± 0.05	4.23 ± 0.26	1.55 ± 0.11	212 ± 27
	Significance	***	**	***	*	ns

Values are expressed as average ± standard deviation, Samples were taken from 10 field replicates for each harvest date and row orientation combination. The average berry and skin weight were obtained from measuring 10 berries for every replicate (*n*). *, **, *** and "ns" means significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ and "not significant", respectively.

Table 2. Mean values of fresh berry skin break force and energy and anthocyanin content of fresh grapes.

Harvest date	Vineyard row orientation	Fresh berry skin break force as Fsk (N)	Fresh berry skin break energy as Wsk (mJ)	Total anthocyanin index (mg/kg)	Total anthocyanin index (mg/berry)	Total anthocyanin index (mg/g of berry skin)
A (25.6 °Brix)	North-South (NS)	0.140 ± 0.026	0.066 ± 0.022	911 ± 124	1.45 ± 0.17	7.72 ± 1.07
	East-West (EW)	0.142 ± 0.026	0.070 ± 0.024	943 ± 147	1.60 ± 0.24	7.72 ± 1.66
	Significance	ns	ns	ns	ns	ns
B (27.4 °Brix)	North-South (NS)	0.081 ± 0.024	0.035 ± 0.029	841 ± 113	1.26 ± 0.20	6.20 ± 1.17
	East-West (EW)	0.084 ± 0.022	0.035 ± 0.022	830 ± 75	1.32 ± 0.13	6.19 ± 1.20
	Significance	ns	ns	ns	ns	ns
A	NS+EW	0.141 ± 0.026	0.068 ± 0.023	927 ± 133	1.52 ± 0.22	7.72 ± 1.36
B	NS+EW	0.082 ± 0.023	0.035 ± 0.026	836 ± 93	1.29 ± 0.16	6.19 ± 1.15
	Significance	***	***	*	***	***

Values are expressed as average ± standard deviation, for each ripeness level and row orientation combination: n = 200 for Fsk and Wsk, n = 10 for anthocyanin parameters. *, *** and “ns” means significance at $p < 0.05$, $p < 0.001$ and “not significant”, respectively.

Table 3. Macerated skin mechanical parameters and relation with anthocyanin content in juice/wine after 2 and 6 days of maceration and anthocyanin content at the end of the fermentation. The values of mechanical properties measurements on fresh grapes are reported from Table 2.

Parameter	Days of maceration	Harvest date A (25.6 °Brix)	Harvest date B (27.4 °Brix)	*Significance*[b]
Fresh skin break force (Fsk, N)	*Fresh berries*	*0.141 ± 0.026*	*0.082 ± 0.023*	***
Macerated skin break force as Fmsk (N)	2	0.167 ± 0.045	0.137 ± 0.043	***
	6	0.140 ± 0.066	0.116 ± 0.052	***
	Significance[a]	***	***	
	Average decrease between points (%)	*16.1*	*15.2*	
Fresh skin break energy (Wsk, mJ)	*Fresh berries*	*0.068 ± 0.023*	*0.035 ± 0.026*	***
Macerated skin break energy as Wmsk (mJ)	2	0.061 ± 0.037	0.035 ± 0.023	***
	6	0.046 ± 0.042	0.028 ± 0.024	***
	Significance[a]	***	***	
	Average decrease between points (%)	*24.8*	*19.8*	
Total anthocyanin index in juice/wine (mg/L)	2	245 ± 39a	280 ± 32a	**
	6	331 ± 63c	370 ± 45c	*
	End of fermentation	281 ± 42b	307 ± 23b	*
	Significance[a]	***	***	

Values are expressed as average ± standard deviation, for each ripeness level and row orientation combination: n = 160 for Fmsk and Wmsk, n = 10 for the total anthocyanin parameters. Values in columns with different lowercase letters are significantly different ($p<0.05$), Tukey-b test. *, **, *** and "ns" means significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ and "not significant", respectively, between column values ([a]) and row values ([b]).

Table 4. Macerated skin mechanical parameters and relation with anthocyanin content in juice/wine after 2 and 6 days of maceration, and anthocyanin content at the end of the fermentation. Separation by vineyard row orientation of the starting grapes and ripeness level. The values of mechanical properties measurements on fresh grapes are reported from Table 2.

Parameter	Days of maceration	Harvest date A (25.6 °Brix)			Harvest date B (27.4 °Brix)			*Significance (between harvest dates, same row orientation)*[c]
		North-South (NS) row orientation	East-West (EW) row orientation	*Significance (between row orientations)*[b]	North-South (NS) row orientation	East-West (EW) row orientation	*Significance (between row orientations)*[b]	
Fresh skin break force (Fsk, N)	*Fresh berries*	*0.140 ± 0.026*	*0.142 ± 0.026*	*ns*	*0.081 ± 0.024*	*0.084 ± 0.022*	*ns*	
Macerated skin break force as Fmsk (N)	2	0.167 ± 0.045	0.167 ± 0.046	ns	0.133 ± 0.043	0.142 ± 0.043	ns	***, ***
	6	0.137 ± 0.065	0.143 ± 0.068	ns	0.115 ± 0.053	0.118 ± 0.051	ns	***, ***
	Significance[a]	***	**		**	***		
	Average decrease between points (%)	*18.0*	*14.4*		*13.5*	*16.9*		
Fresh skin break energy (Wsk, mJ)	*Fresh berries*	*0.066 ± 0.022*	*0.070 ± 0.024*	*ns*	*0.035 ± 0.029*	*0.035 ± 0.022*	*ns*	
Macerated skin break energy as Wmsk (mJ)	2	0.062 ± 0.038	0.060 ± 0.036	ns	0.032 ± 0.021	0.037 ± 0.024	ns	***, ***
	6	0.046 ± 0.043	0.046 ± 0.041	ns	0.027 ± 0.024	0.029 ± 0.025	ns	***, ***
	Significance[a]	**	**		*	**		
	Average decrease between points (%)	*25.8*	*23.3*		*15.6*	*21.6*		
Total	2	244 ± 22a	247 ± 51a	ns	279 ± 19a	280 ± 43a	ns	**, ns

anthocyanin index in juice/wine (mg/L)	6	326 ± 36c	338 ± 86b	ns	381 ± 25c	359 ± 57b	ns	***, ns
	End of fermentation	279 ± 19b	282 ± 60ab	ns	316 ± 11b	298 ± 29a	ns	***, ns
	Significance[a]	***	*		***	**		

Values are expressed as average ± standard deviation, for each harvest date and row orientation combination: n = 160 for Fmsk and Wmsk, n = 10 for the total anthocyanin parameters. Values in columns with different lowercase letter are significantly different ($p<0.05$), Tukey-b test. *, **, *** and "ns" means significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ and "not significant", respectively, between column values ([a]) and row values ([b,c]).

Table 5. Anthocyanin profile of Shiraz grapes grown in different vineyard row orientations and harvested at two ripeness levels.

Harvest date	Vineyard row orientation	Delphinidin-3-G	Cyanidin-3-G	Petunidin-3-G	Peonidin-3-G	Malvidin-3-G	∑ acetyl-G	∑ cinnamoyl-G
A (25.6 °Brix)	North-South	2.58 ± 0.29	0.44 ± 0.06	3.67 ± 0.24	4.41 ± 0.38	28.2 ± 1.5	24.1 ± 0.7	36.6 ± 1.3
	East-West	2.57 ± 0.32	0.36 ± 0.06	3.64 ± 0.28	4.13 ± 0.53	28.7 ± 1.8	23.6 ± 1.0	37.0 ± 2.1
	Significance	ns	**	ns	ns	ns	ns	ns
B (27.4 °Brix)	North-South	2.09 ± 0.49	0.35 ± 0.06	3.22 ± 0.53	4.34 ± 0.39	27.5 ± 1.1	23.9 ± 0.7	38.6 ± 2.4
	East-West	1.94 ± 0.45	0.33 ± 0.06	3.04 ± 0.45	4.29 ± 0.39	27.5 ± 1.2	22.9 ± 0.4	40.0 ± 2.3
	Significance	ns	ns	ns	ns	ns	***	ns
A	NS+EW	2.58 ± 0.30	0.40 ± 0.07	3.65 ± 0.25	4.27 ± 0.48	28.5 ± 1.6	23.8 ± 0.9	36.8 ± 1.7
B	NS+EW	2.02 ± 0.47	0.34 ± 0.06	3.13 ± 0.48	4.31 ± 0.38	27.5 ± 1.1	23.4 ± 0.8	39.3 ± 2.4
	Significance	***	**	***	ns	*	ns	***

Values are expressed as percentage, average ± standard deviation, n = 10 for each harvest date and row orientation combination. G = glucoside. *, **, *** and “ns” means significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ and “not significant”, respectively.

Table 6. Anthocyanin profile of Shiraz wines at the end of the fermentation, produced from grapes grown in different vineyard row orientations and harvested at two ripeness levels.

Harvest date	Vineyard row orientation	Delphinidin-3-G	Cyanidin-3-G	Petunidin-3-G	Peonidin-3-G	Malvidin-3-G	∑ acetyl-G	∑ cinnamoyl-G
A (25.6 °Brix)	North-South	0.42 ± 0.04	0.13 ± 0.01	2.31 ± 0.11	1.44 ± 0.14	46.3 ± 1.3	31.8 ± 0.5	17.6 ± 1.2
	East-West	0.43 ± 0.08	0.16 ± 0.03	2.34 ± 0.30	1.57 ± 0.21	47.7 ± 1.5	31.1 ± 1.2	16.7 ± 1.2
	Significance	ns	*	ns	ns	*	ns	ns
B (27.4 °Brix)	North-South	0.28 ± 0.03	0.16 ± 0.02	1.96 ± 0.09	1.60 ± 0.14	45.7 ± 0.7	30.8 ± 0.8	19.5 ± 1.3
	East-West	0.29 ± 0.04	0.15 ± 0.01	2.00 ± 0.24	1.56 ± 0.09	45.8 ± 0.8	30.6 ± 0.7	19.6 ± 1.3
	Significance	ns	ns	ns	ns	ns	ns	ns
A	NS+EW	0.43 ± 0.06	0.14 ± 0.03	2.33 ± 0.21	1.50 ± 0.18	47.0 ± 1.6	31.5 ± 1.0	17.1 ± 1.3
B	NS+EW	0.28 ± 0.04	0.16 ± 0.01	1.98 ± 0.18	1.58 ± 0.11	45.7 ± 0.7	30.7 ± 0.7	19.6 ± 1.3
	Significance	***	*	***	ns	**	**	***

Values are expressed as percentage, average ± standard deviation, n = 10 for each harvest date and row orientation combination. G = glucoside. *, **, *** and “ns” means significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ and “not significant”, respectively.