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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 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 known that the anthocyanin amount in the wine is dependent, but not strictly related, 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, 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 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 3ha vineyard of *Vitis vinifera* L. cv. Shiraz (clone SH 9C), grafted onto rootstock 10-14 Mgt, was planted in 2003 at the ARC Infructuur experiment farm in the Robertson wine region (33°5' S 19°54' E) of the Western Cape (South Africa).

different row orientations and five replicates for each orientation. Only North 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 Nietvoorbij 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 (refractometry, in Brix), pH and total titratable acidity, following OIV (2011) methods. Sulphur dioxide (50 mg/L) and diammonium phosphate (50 g/hL) were then added to the whole batch of crushed juice before inoculation with rehydrated yeast (30 L V13, Anchor Yeasts, Industria, ZA). The maceration/fermentation of all the samples was conducted in a 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 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 forming a puncture test (Letaief, Rolle, Zeppa & Gerbi, 2008). For the latter, a 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 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 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 1% ethanol, 5 g/L tartaric acid, and 2 g/L sodium metabisulfite (to avoid any possible oxidation) and adjusted to pH 3.20 with 0.1 M 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 UltraTurrax 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:formic acid (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 1.4% 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, 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 RP18 Purospher 250 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 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). Tukey's 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 15.6 and 54.0mg for the first and second harvest dates 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 EastWest row orientation, but it did not influence berry and skin weight (Table

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 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; 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 orientation 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 EastWest 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 NorthSouth 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 to assess the mechanical properties of the berries, a needle shaped probe, similar to the one 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 (F_{sk}) and break energy (W_{sk}) 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 W_{sk} 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 week period before harvest, the berry skin break energy showed a clear decrease with increasing ripening only in one of the three parcels, while the other two showed only a trend (Maury et al. 2009). On Nebbiolo grapes, during a 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 their ripeness 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 & 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%).

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

- Hunter, J.J.; Archer, E.; Volschenk, C.G. (2010a) Vineyard management for environment valorisation. *Proceedings VIII International Zoning Congress*, June 2010, Soave, Italy, 3-15.
- Hunter, J.J.; Volschenk, C.G.; Bonnardot, V. (2010b) Linking grapevine row orientation to a changing climate in South Africa. *Proceedings Servitus Interfructa International Congress (Cultivation methods in a changing climate)*, March 2010, Stuttgart, Germany, 7, 60.
- Jogaiah, S.; Striegler, K.R.; Bergmeier, E.; Harris, J. (2012) Node cluster exposure to sun on fruit composition of 'Norton' grapes (*Vitis estivalis* Michx) in Missouri. *International Journal of Fruit Science*, 12, 410-426.
- Letaief, H.; Rolle, L.; Zeppa, G.; Gerbi, V. (2008) Assessment of grape skin hardness by a puncture test. *Journal of the Science of Food and Agriculture*, 88, 1567-1575.
- Mattivi, F.; Guzzon, R.; Vrhovsek, U.; Stefanini, M.; Velasco, R. (2006) Metabolite profiling of grape: flavonols and anthocyanins. *Journal of Agricultural and Food Chemistry*, 54, 7692-7702.
- Maury, C.; Madieta, E.; Le Moigne, M.; Mehinagic, E.; Siret, R.; Jourjon, F. (2009) Development of a mechanical texture test to evaluate the ripening process of Cabernet franc grapes. *Journal of Texture Studies*, 40, 511-535.
- McCarthy, M.G.; Coombe, B.G. (1999) Is weight loss in ripening grape berries cv. Shiraz caused by impeded phloem transport? *Australian Journal of Grape and Wine Research*, 5, 17-21.
- Mori, K.; Goto, Yamamoto, N.; Kitayama, M.; Hashizume, K. (2007) Loss of anthocyanins in red wine grape under high temperature. *Journal of Experimental Botany*, 58, 8, 1935-1945.
- OIV (2011) *Compendium of international methods of wine and must analysis*. Organisation Internationale de la Vigne et du Vin, Paris, FR.
- Ortega-Regules, A.; Romero-Cascales, I.; Rodríguez-García, J.M.; López-Roca, J.M.; Gómez-Plaza, E. (2006) A first approach towards the relationship between grape skin-wall composition and anthocyanin extractability. *Analytica Chimica Acta*, 563, 263-268.
- Pisciotta, A.; Barbagallo M.G.; Di Lorenzo, R.; Hunter, J.J. (2013) Anthocyanin variation in individual 'Shiraz' berries as affected by exposure and position on the rachis. *Vitis*, 52, 111-115.
- Pomar, F.; Novo, M.; Masa, A. (2005) Varietal differences among the anthocyanin profiles of 50 red table grape cultivars studied by high performance liquid chromatography. *Journal of Chromatography A*, 1094, 344-351.
- Ristic, R.; Downey, M.; Mand, P.; Bindon, K.; Francis, L.; Herderich, M.; Robinson, S. (2007) Exclusion of sunlight from Shiraz grapes alters wine colour, tannin and sensory properties. *Australian Journal of Grape and Wine Research*, 13, 536-545.
- Rolle, L.; Torchio, F.; Zeppa, G.; Gerbi, V. (2009) Relationship between skin break force and anthocyanin extractability at different ripening stages. *American Journal of Enology and Viticulture*, 60, 93-97.

- Rolle, L.; Gerbi, V.; Schneider, A.; Spanna, F.; Río Segade, S. (2011a) Relationship between instrumental skin hardness and climate for grapevines (*Vitis vinifera* L.). *Journal of Agricultural and Food Chemistry*, 59, 10624-10634.
- Rolle, L.; Río Segade, S.; Torchio, F.; Giacosa, S.; Cagnasso, E.; Marengo, F.; Gerbi, V. (2011b) Influence of grape density and harvest date on changes in phenolic composition, phenol extractability indices, and instrumental texture properties during ripening. *Journal of Agricultural and Food Chemistry*, 59, 8796-8805.
- Rolle, L.; Torchio, F.; Ferrandino, A.; Guidoni, S. (2012a) Influence of wine grape skin hardness on the kinetics of anthocyanin extraction. *International Journal of Food Properties*, 15, 249-261.
- Rolle, L.; Siret, R.; Río Segade, S.; Maury, C.; Gerbi, V.; Jourjon, F. (2012b) Instrumental texture analysis parameters as markers of table grape and winegrape quality: a review. *American Journal of Enology and Viticulture*, 63, 1-28.
- Rustioni, L.; Rossoni, M.; Failla, O.; Scienza, A. (2013) Anthocyanin esterification in Sangiovese grapes. *Italian Journal of Food Science*, 25, 133-141.
- Sacchi, K.L.; Bisson, L.; Adams, D.O. (2005) A review of the effect of winemaking techniques on phenolic extraction in red wines. *American Journal of Enology and Viticulture*, 56, 197-206.
- Sato, A.; Yamada, M.; Iwanami, H.; Mitani, N. (2004) Quantitative and instrumental measurements of grape flesh texture as affected by gibberellic acid application. *Journal of the Japanese Society for Horticultural Science*, 73, 7-11.
- Spayd, S.E.; Tarara, J.M.; Mee, D.L.; Ferguson, D. (2002) Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *American Journal of Enology and Viticulture*, 53, 171-182.
- Tarara, J.M.; Lee, J.; Spayd, S.E.; Scagel, C.F. (2008) Berry temperature and solar radiation acylation, proportion, and concentration of anthocyanin in Merlot grapes. *American Journal of Enology and Viticulture*, 59, 235-247.
- Torchio, F., Cagnasso, E., Gerbi, V., Rolle, L. (2010) Mechanical properties, phenolic composition and extractability indices of Barbera grapes of different soluble solids contents from several growing areas. *Analytica Chimica Acta*, 660, 183-189.
- Yamane T., Jeong S.T., Yamamoto N.G., Koshita Y., Kobayashi S. 2006 Effects of temperature on anthocyanin biosynthesis in grape berry skins. *American Journal of Enology and Viticulture*, 57, 1, 54-59.
- Zsófi, Zs.; Villangó, Sz.; Pálfi, Z.; Tóth, E.; Bálo, B. (2014) Texture characteristics of the grape berry skin and seed (*Vitis vinifera* L. cv. Kékfrankos) under postveraison temperature deficit. *Scientia Horticulturae*, 172, 176-182.

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

Harvest date	Vineyard row orientation	°Brix	pH	Titrateable acidity (g/L TA)	Berry weight(g)	Berry skin weight(mg)
March 6 th (A)	North-South(NS)	25.8 ± 0.4	3.92 ± 0.05	4.81 ± 0.17	1.60 ± 0.17	189 ± 21
	EastWest(EW)	25.4 ± 0.9	3.85 ± 0.10	5.17 ± 0.22	1.71 ± 0.16	212 ± 33
	<i>Significance</i>	ns	ns	***	ns	ns
March 26 th (B)	North-South(NS)	27.3 ± 0.7	3.99 ± 0.03	4.06 ± 0.19	1.50 ± 0.11	206 ± 25
	EastWest(EW)	27.5 ± 1.2	3.95 ± 0.07	4.40 ± 0.21	1.59 ± 0.09	218 ± 29
	<i>Significance</i>	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
	<i>Significance</i>	***	**	***	*	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 replicates. *, **, *** 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
	EastWest (EW)	0.142 ± 0.026	0.070 ± 0.024	943 ± 147	1.60 ± 0.24	7.72 ± 1.66
	<i>Significance</i>	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
	EastWest (EW)	0.084 ± 0.022	0.035 ± 0.022	830 ± 75	1.32 ± 0.13	6.19 ± 1.20
	<i>Significance</i>	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
	<i>Significance</i>	***	***	*	***	***

Values are expressed as average ± standard deviation, for each ripeness level and row orientation combination. n=20 for Fsk and Wsk, n=10 for anthocyanin parameters. *, ** and *** means significance at p<0.05, p<0.01 and p<0.001, respectively.

Table 3. Macerated skin mechanical parameters and relation with anthocyanin content in juice 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	***
	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		
Macerated skin break force as Fmsk (N)	Fresh berries	0.068	± 0.023	0.035	± 0.026	***
	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		
Fresh skin break energy (Wsk, mJ)	Fresh berries	245	± 39a	280	± 32a	**
	2	331	± 63c	370	± 45c	*
	6	281	± 42b	307	± 23b	*
	End of fermentation	281 ± 42b		307 ± 23b		*
Significance ^a		***		***		
Average decrease between points (%)		24.8		19.8		
Macerated skin break energy as Wmsk (mJ)	Fresh berries	0.068	± 0.023	0.035	± 0.026	***
	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)	Fresh berries	245	± 39a	280	± 32a	**
	2	331	± 63c	370	± 45c	*
	6	281	± 42b	307	± 23b	*
	End of fermentation	281 ± 42b		307 ± 23b		*
Significance ^a		***		***		

Values are expressed as average ± standard deviation, for each ripeness level and 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 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 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 (25.8 Brix)			Harvest date B (27.2 Brix)			Significance (between harvest dates, same row orientation) ^c
		North-South (NS) row orientation	EastWest (EW) row orientation	Significance (between row orientations) ^b	North-South (NS) row orientation	EastWest (EW) row orientation	Significance (between row orientations) ^b	
<i>Fresh skin break force (Fsk, N)</i>	<i>Fresh berries</i>	0.140 ± 0.026	0.142 ± 0.026	ns	0.081 ± 0.024	0.084 ± 0.022	ns	
	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		
<i>Fresh skin break energy (Wsk, mJ)</i>	<i>Fresh berries</i>	0.066 ± 0.022	0.070 ± 0.024	ns	0.035 ± 0.029	0.035 ± 0.022	ns	
	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	6	326 ± 36c	338 ± 86b	ns	381 ± 25c	359 ± 57b	ns	***, ns
index in	End of							
juice/wine	fermentation	279 ± 19b	282 ± 60ab	ns	316 ± 11b	298 ± 29a	ns	***, ns
(mg/L)	<i>Significance^a</i>	***	*		***	**		

Values are expressed as average ± standard deviation, for each 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. *, **, *** 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 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
	EastWest	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
	<i>Significance</i>	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
	EastWest	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
	<i>Significance</i>	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
	<i>Significance</i>	***	**	***	ns	*	ns	***

Values are expressed as percentage, average and 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	Σ acetyl-G	Σ 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
	<i>Significance</i>	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
	<i>Significance</i>	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
	<i>Significance</i>	***	*	***	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.