Research Note

Relationship between Skin Break Force and Anthocyanin Extractability at Different Ripening Stages

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Abstract: The extractability of anthocyanins from winegrapes with different skin hardness at two different ripening stages was evaluated. Skin hardness was assessed by texture analysis, a rapid and low-cost analytical technique. Grape berries of cv. Brachetto were calibrated according to their density estimated by flotation in 10 different salt solutions. Skin hardness was measured via a puncture test, and two groups of berries with different skin hardness were selected for each ripening stage: soft (<0.40 N) and hard (>0.50 N). Anthocyanin skin extraction was evaluated in a hydroalcoholic model solution and the kinetics of skin dissolution was monitored. For each ripening stage, the harder skin had greater anthocyanin extractability. Significant interactions between ripening stage and skin hardness were found in the composition of individual anthocyanins present in the extract.

Key words: extractability, anthocyanin, skin hardness, texture analysis, puncture test

The visual characteristic of red wine is mainly due to anthocyanins that are present in the grape skin at harvest and that dissolve in the red wine during the maceration/fermentation process (Amrani Joutei and Glories 1995, Romero-Cascales et al. 2005a). Anthocyanins accumulate gradually in berry skins during ripening (Fernández-López et al. 1992), and their total amount at harvest depends on variety (i.e., pink, red, or black skin variety) and many other factors, including environmental parameters and agronomic practice (Guidoni et al. 2008). The types and the amounts of various anthocyanins in grape skins (their "profile") determine the color of resulting wines, but their extraction from the grape skin into the wine depends on the tendency of the berry skin to release them (González-Neves et al. 2004, Romero-Cascales et al. 2005b). The extractability of anthocyanins increases throughout grape ripening as a consequence of cell wall degradation by pectolytic enzymes (Ribéreau-Gayon et al. 2000). Differences in polysaccharides based on galactose and arabinose, the cellulose content, and the degree of methylation of the pectins could be responsible for the difference in anthocyanin extractability (Ortega-Regules et al. 2006, 2008).

Several studies have attempted to define the best method to evaluate polyphenolic compounds in grapes and their ease of release. These analytical methods are generally rather complex and often require long analysis times (Cagnasso et al. 2008). Furthermore, some data interpretation may be difficult (Venencie et al. 1998).

Texture analysis is a current analytical technique used for measurement of the physical properties of food. For winegrapes, the literature contains studies on modifications of some grape textural properties during ripeness (Abbal et al. 1992, Robin et al. 1997, Lee and Bourne 1980, Grotte et al. 2001) and the influence of climate and growing location on mechanical behavior (Le Moigne et al. 2008).

The aim of this work was to examine the relationship between skin hardness, determined by puncture test, and the ease of extractability of anthocyanins in berries at different ripening stages. The study was carried out on Brachetto, an important aromatic red grape variety used for production of sweet sparkling wines (6–7.5% vol ethanol) and grown in Piedmont (northwest Italy). The ease and rapidity of extraction are important qualitative parameters for Brachetto grapes. These aromatic red grapes are subjected to very short fermentation-maceration (24–48 hr) because the product obtained must not exceed 3.5% vol ethanol before the second fermentation (*prise de mousse*). Reduced maceration times, low fermentation temperature (17–18°C) to preserve aromatic components, and low alcohol concentrations frequently lead to reduced pigment extraction and wines with unsatisfactory color. Knowledge of the extractability of anthocyanin compounds measured via berry skin hardness could permit harvesting at an optimal stage of ripeness and suitable operative choices in the prefermentation and maceration phases.

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Materials and Methods

Grape samples. In 2007 *Vitis vinifera* var. Brachetto grape samples were harvested twice during ripening (with an interval of 10 days) from several vineyards located in the Asti and Alessandria provinces within the Brachetto d'Acqui DOCG. Berries were calibrated according to density (i.e., total soluble solids). Density was estimated by flotation of berries in 10 different salt solutions (from 100 to 190 gL^{-1} NaCl) such that the difference in total soluble solids of two consecutive batches of berries was ~17 gL^{-1} (i.e., 1% vol in potential alcohol) (Fournand et al. 2006). Two ripening stages were studied: A (250 ± 8 gL^{-1} sugar) and B (184 ± 8 gL^{-1} sugar).

Texture analysis of berry skin. A puncture test was carried out on the side of all berries (~200) present in each one of the two ripening stages defined by flotation (see above). Measurements were made using a Universal Testing Machine TAxT2i Texture Analyzer (Stable Micro System, Godalming, Surrey, UK) equipped with a HDP/90 platform, needle probe (P/2N), and 5 kg load cell. Tests were performed at 1 mms^{-1} and berry skin break force (F_{sk}), expressed in Newton (N), was determined (Letaief et al. 2008a). All data acquisition was made at 400 Hz, using Texture Expert Exceed software, version 2.54. For each ripening stage, two groups of berries with different skin hardness were selected: M, soft (0.20–0.40 N) and D, hard (0.50–0.70 N).

Anthocyanin extraction in hydroalcoholic solution. Sixty berries (three replicates of 20 berries) belonging to each of the four groups were used for studying anthocyanin extractability. The groups were high sugar, hard (AD), high sugar, soft (AM), low sugar, hard (BD), and low sugar, soft (BM). The berry skins were removed manually from the pulp and dried with absorbent paper. They were quickly immersed in 75 mL model solution consisting of ethanol/water (3:97 v/v) to simulate extraction conditions during industrial production and 100 mgL^{-1} $K_2S_2O_5$ to limit oxidation of phenolic compounds throughout extraction (Fournand et al. 2006) and 5 gL^{-1} tartaric acid and adjusted to pH 3.20 with 1 N NaOH. The kinetics of extraction were monitored at regular intervals: 10, 20, and 30 min and 1, 2, 4, 8, 12, 24, and 48 hr.

Spectrophotometric and HPLC analysis. A spectrophotometric method was used to evaluate the total anthocyanin index (TAI) of hydroalcoholic solutions and berry skins before extraction (Di Stefano and Cravero 1991). Fresh berry skin and, at the end of extraction, the residual solid berry skin were quickly immersed in 75 mL of a buffer solution containing 12% v/v ethanol, 600 mgL^{-1} $K_2S_2O_5$, 50 mgL^{-1} NaN_3, and 5 gL^{-1} tartaric acid and titrated to pH 3.20 by adding 1 N NaOH. After homogenization with an UltraTurrax T25 (IKA Labortechnik, Staufen, Germany), the extract was centrifuged (1126 *g*, 10 min, 20°C). The supernatant was then used for analysis. Analysis of individual anthocyanins was performed after filtration using a Sep-Pak C18 cartridge (Waters Corporation, Milford, MA) and elution with methanol. The chromatograph system was a P100 pump equipped with an AS3000 autosampler (Spectra Physics Analytical, San Jose, CA), a 20-mL Reodyne sample loop, a LiChroCART column (25 cm x 0.4 cm i.d.) (Merck, Darmstadt, Germany) packed with LiChrosphere 100 RP-18 5-µm particles (Alltech, Deerfield, IL), and a Spectra Focus Diode Array Detector (Spectra Physics) operating at 520 nm. The following conditions were used: solvent A, 10% v/v formic acid in water; solvent B, 10% v/v formic acid with 50% v/v methanol in water. These solvents were passed through a 0.20-µm filter. The solvent flow rate was 1 mL/min and column temperature was 20°C. The solvent program used was 72% A to 55% A over 15 min, to 30% A over 20 min, to 10% A over 10 min, to 1% A over 5 min, to 72% A over 3 min. An equilibrium time of 10 min was used (Rolle and Guidoni 2007). Data treatment was carried out using the ChromQuest chromatography data system (ThermoQuest, San Jose, CA). Identification of the free forms of anthocyanins in the berry skin extract was performed by comparison with external standards (delphinidin-3-*O*-glucoside chloride, malvidin-3-*O*-glucoside chloride, peonidin-3-*O*-glucoside chloride, petunidin chloride, cyanidin chloride; Extrasynthèse, Genay, France); the acylated forms of anthocyanins were identified by comparing the retention time of each chromatographic peak with available data in the literature (Di Stefano et al. 1995). The percentages of individual anthocyanins were determined by comparing the area of each individual peak with the total peak area.

Statistical analysis. Statistical analysis was performed using Statistica for Windows, release 7.1 (StatSoft, Tulsa, OK). A two-way factorial analysis of variance was performed to define the interaction between ripening stage and skin hardness.

Results and Discussion

The concentrations and relative proportions of various anthocyanins in Brachetto grapes at two ripening stages were determined (Table 1). Riper grapes (250 gL^{-1} sugar; A) had twice the total anthocyanins of less ripe grapes (184 gL^{-1} sugar; B), and individual anthocyanin content was different in the two stages. During ripening, malvidin-3-glucoside and peonidin-3-glucoside generally increase in comparison with other anthocyanidin monoglucosides (Roggero et al. 1986, Jordão et al. 1998). The ratio of peonidin/malvidin derivatives was 0.52 in A and 0.32 in B. Acetylglucoside anthocyaninswere the least abundant class.

Skin break force (F_{sk}) values were determined for the two ripening stages (Table 2). Analysis of variance did not show significant differences between A and B. The high variability of skin break force, under the same conditions of soluble solids present in the berries, is attributable to the different cultural and environmental situations of vineyards from which grapes were taken (Rolle et al. 2006). This variability was part of the experimental design and was intended to provide a high distribution of F_{sk} and to allow the making of two groups of well-characterized berries. Groups M (soft) and D

Table 1 Total anthocyanin content and relative proportions of individual anthocyanins in Brachetto grapes at two ripening stages (A = 250 gL^{-1} sugar; B = 184 gL^{-1} sugar) in 2007. Means of three replicates (average ± standard deviation).

	Total anthocyanins (mg kg^{-1}grapes)			Anthocyanin profile (%)		
	A	B	Signf[a]	A	B	Signf[a]
Total anthocyanin index	598 ± 75	292 ± 76	***			
Simple glucosides	574 ± 2	276 ± 0	***	96.0 ± 0.36	94.6 ± 0.16	**
Acetyl-glucosides	2 ± 0	1 ± 0	**	0.3 ± 0.04	0.3 ± 0.01	ns
Cinnamoyl-glucosides[b]	22 ± 2	15 ± 1	**	3.7 ± 0.33	5.0 ± 0.17	**
Delphinidin derivatives (∑)	45 ± 6	16 ± 2	**	7.5 ± 0.95	5.6 ± 0.59	**
Cyanidin derivatives (∑)	31 ± 2	10 ± 2	**	5.2 ± 0.30	3.3 ± 0.65	**
Petunidin derivatives (∑)	44 ± 4	18 ± 1	**	7.3 ± 0.67	6.0 ± 0.21	**
Peonidin derivatives (∑)	164 ± 14	60 ± 7	**	27.4 ± 2.31	20.6 ± 2.24	**
Malvidin derivatives (∑)	315 ± 15	188 ± 11	**	52.6 ± 2.48	64.5 ± 3.69	**

[a]**, ***, and ns indicate significance at $p \leq 0.01$, $p \leq 0.001$, and not significant, respectively.
[b]Cinnamoyl-glucosides included both *p*-coumaroyl and caffeoyl anthocyanin forms.

(hard) were composed of berries with lower and higher F_{sk}, respectively, than the medium value of 0.43 N. When vineyards are more homogeneous, F_{sk} values at harvest have lower standard deviations and are characteristic of the variety (Letaief et al. 2008b). Grape softening during maturation is the result of significant changes in parietal constituent composition, notably in pulp cells (Ribéreau-Gayon et al. 2000). Structural properties of the cell walls may determine the mechanical resistance of berry skin (Barnavon et al. 2000). This change during ripening can be measured with a compression test and expressed with a rheological parameter such as firmness (Robin et al. 1997, Grotte et al. 2001), but with this type of test, pulp and skin data are aggregate. Lee and Bourne (1980) evaluated the evolution of skin hardness with a penetration-puncture test using a flat probe (0.9 mm diam). This small cylinder is plunged into the tissue and measures the evolution of stress-strain and a mix of compression (under the plunger) and shearing (Roudot 2006). In the puncture test conducted on Brachetto grapes, a needle probe was used to estimate the skin break force while minimizing other possible interferences. From veraison to ripeness, there is an increase in F_{sk}, with a steady or slight decrease around harvest and a new increase in overripe berries. This general pattern varies with cultivar (Letaief 2007).

Total free anthocyanins were extracted from the four groups of berries (AD, AM, BD, BM) into a model hydroalcoholic solution (Table 3). The extraction of anthocyanins is influenced by ethanol concentration in water-alcohol solution (Canals et al. 2005). The final TAIs of Brachetto grape extracts, although similar, were strongly affected by ripening stage, as the initial anthocyanin contents were different. Skin hardness largely determined anthocyanin release. Hard skins under our experimental conditions released 72.4% (group A) and 70.3% (group B) of the available anthocyanins compared to 62.7% and 62.1%, respectively, for soft skins. Therefore, skins belonging to group AD presented greater capacities for anthocyanin release. Some anthocyanin types presented different behaviors during extraction. After 48 hr maceration, in less ripe B berries, peonidin and cyanidin derivatives were most abundant in hydroalcoholic solutions obtained from hard skin (Table 4). In particular, the higher TAI in group BD is attributable to the greater presence of peonidin derivatives. Less dramatic differences in the anthocyanin profile were revealed between hydroalcoholic solutions of groups AM and AD. In both ripening stages, significant differences in acetyl-glucoside and cinnamoyl-glucoside assignable to a difference of F_{sk} were not observed, likely because, in Brachetto grapes, these coloring pigments are only present in small quantities (5%). Ripening stage, skin hardness, and the interaction between these two factors influences the total amount and type of individual anthocyanin in the final product (Table 5). Therefore, the chemical composition of Brachetto must-wine after 48 hr maceration depends not only on berry ripeness but also on all factors (vintage, environment, vineyard management, clone) that can influence skin hardness (Rolle et al. 2006, Letaief et al. 2008b, Río Segade et al. 2008).

Table 2 Average, minimum, maximum values, and relative standard deviation of skin break force (F_{sk}), expressed in Newton (N), of Brachetto grapes at two ripening stages, as determined by puncture test.

	Skin break force (N)			
	Avg	Min	Max	SD
A (250 gL^{-1} sugar)	0.432	0.246	0.702	0.096
B (184 gL^{-1} sugar)	0.430	0.224	0.651	0.088

Conclusions

For each ripening stage and with a maceration in a model hydroalcoholic solution, Brachetto grapes with a higher skin break force (F_{sk}) produced extracts with higher total anthocyanin. Significant interactions between ripening stage and skin hardness were found in the individual anthocyanin composition of extracts. Hard skins were characterized by increased fragility of the cell walls,

Table 3 Extractability in a model hydroalcoholic solution of total anthocyanin index (TAI) from four groups of berries: AD, AM, BD, BM (A = 250 gL^{-1} sugar; B = 184 gL^{-1} sugar; M = soft skin 0.22–0.40 N; D = hard skin 0.45–0.70 N) (average ± standard deviation) and results of factorial analysis of variance carried out for different extraction times.

	10 min	20 min	30 min	1 hr	2 hr	4 hr	8 hr	24 hr	48 hr
TAI (mg malvidin-3-glucoside L^{-1})									
AM	46 ± 8	85 ± 3	92 ± 17	128 ± 14	191 ± 15	248 ± 4	312 ± 5	410 ± 7	434 ± 24
AD	62 ± 17	89 ± 21	114 ± 21	162 ± 11	241 ± 29	317 ± 30	381 ± 22	475 ± 40	501 ± 32
BM	24 ± 3	45 ± 11	58 ± 13	90 ± 13	116 ± 15	152 ± 11	176 ± 10	195 ± 7	215 ± 11
BD	36 ± 8	65 ± 16	83 ± 19	108 ± 10	141 ± 10	173 ± 8	209 ± 4	234 ± 15	244 ± 8
Ripening stage	**[a]	**	*	***	***	***	***	***	***
Hardness	ns	ns	ns	**	**	**	***	**	**
Ripening stage*hardness	ns	ns	ns	ns	ns	ns	ns	ns	ns
Extraction (%)									
AM	6.6 ± 1.2	12.3 ± 0.4	13.3 ± 2.5	18.6 ± 2.1	27.7 ± 2.2	35.9 ± 0.6	45.0 ± 0.7	59.3 ± 1.0	62.7 ± 3.5
AD	8.9 ± 2.4	12.9 ± 3.0	16.5 ± 3.0	23.5 ± 1.6	34.7 ± 4.2	45.9 ± 4.4	55.1 ± 3.1	68.6 ± 5.7	72.4 ± 4.6
BM	6.0 ± 0.8	13.0 ± 3.1	16.8 ± 3.8	25.8 ± 3.6	33.4 ± 4.4	43.9 ± 3.1	50.7 ± 2.9	56.5 ± 2.1	62.0 ± 3.3
BD	10.5 ± 2.3	18.8 ± 4.6	23.9 ± 5.5	31.1 ± 2.9	40.6 ± 2.9	49.8 ± 2.4	60.1 ± 1.0	67.5 ± 4.4	70.3 ± 2.4
Ripening stage	ns	ns	ns	**	*	*	**	ns	ns
Hardness	*	ns	ns	*	*	**	***	**	**
Ripening stage*hardness	ns	ns	ns	ns	ns	ns	ns	ns	ns

[a]*, **, ***, and ns indicate significance at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, and not significant, respectively.

Table 4 Anthocyanin content and profile in the hydroalcoholic solution after 48 hr maceration for four groups of berries: AD, AM, BD, BM (A = 250 gL^{-1} sugar; B = 184 gL^{-1} sugar; M = soft skin 0.22–0.40 N; D = hard skin 0.45–0.70 N).

	AM	AD	Signf[a]	BM	BD	Signf[a]
Anthocyanin content (mgL^{-1})						
Total anthocyanin index	434 ± 24	501 ± 32	ns	215 ± 11	244 ± 8	*
Simple glucosides	421 ± 23	486 ± 31	ns	206 ± 11	235 ± 9	*
Acetyl-glucosides	2 ± 0	3 ± 0	ns	1 ± 0	1 ± 0	ns
Cinnamoyl-glucosides[b]	11 ± 1	12 ± 1	ns	8 ± 2	8 ± 1	ns
Delphinidin derivatives (∑)	30 ± 2	38 ± 3	ns	10 ± 1	10 ± 1	ns
Cyanidin derivatives (∑)	23 ± 0	27 ± 3	ns	5 ± 0	8 ± 0	**
Petunidin derivatives (∑)	31 ± 1	37 ± 3	ns	12 ± 1	12 ± 1	ns
Peonidin derivatives (∑)	140 ± 5	145 ± 12	ns	41 ± 1	76 ± 6	***
Malvidin derivatives (∑)	210 ± 17	254 ± 12	*	147 ± 10	138 ± 2	ns
Profile (%)						
Simple glucosides	97.0 ± 0.07	97.0 ± 0.22	ns	95.7 ± 0.83	96.4 ± 0.43	ns
Acetyl-glucosides	0.5 ± 0.06	0.5 ± 0.07	ns	0.4 ± 0.07	0.3 ± 0.06	ns
Cinnamoyl-glucosides[b]	2.6 ± 0.06	2.5 ± 0.15	ns	3.9 ± 0.77	3.3 ± 0.38	ns
Delphinidin derivatives (∑)	7.2 ± 0.50	7.5 ± 0.18	**	4.7 ± 0.33	4.0 ± 0.32	ns
Cyanidin derivatives (∑)	5.3 ± 0.29	5.3 ± 0.33	ns	2.3 ± 0.03	3.1 ± 0.16	**
Petunidin derivatives (∑)	7.3 ± 0.39	7.4 ± 0.15	ns	5.5 ± 0.09	5.0 ± 0.27	**
Peonidin derivatives (∑)	31.3 ± 1.76	29.0 ± 1.10	*	19.2 ± 1.20	31.1 ± 1.46	***
Malvidin derivatives (∑)	48.9 ± 1.20	50.8 ± 1.58	ns	68.3 ± 0.86	56.8 ± 1.31	***

[a]*, **, ***, and ns indicate significance at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, and not significant, respectively.
[b]Cinnamoyl-glucosides include both *p*-coumaroyl and caffeoyl anthocyanin forms.

Table 5 Factorial analysis of variance carried out on different hydroalcoholic solutions after 48 hr maceration.

Effect	TAI[a]	Simple glucosides	Acetyl-glucosides	Cinnamoyl-glucosides	Delphinidin derivatives (∑)	Cyanidin derivatives (∑)	Petunidin derivatives (∑)	Peonidin derivatives (∑)	Malvidin derivatives (∑)
Profile (mgL^{-1})									
Ripening stage	***[b]	***	***	**	***	***	***	***	***
Hardness	**	**	ns	ns	*	*	*	**	*
Ripening stage*hardness	ns	ns	ns	ns	*	ns	*	**	**
Extraction (%)									
Ripening stage		*	**	**	***	***	***	***	***
Hardness		ns	ns	ns	ns	**	ns	***	***
Ripening stage*hardness		ns	ns	ns	**	**	**	***	***

[a]TAI: total anthocyanin index.
[b]*, **, ***, and ns indicate significance at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, and not significant, respectively.

which allows a greater release of pigments. Nevertheless, there are no specific studies on the relationship between F_{sk} and the different components of skin cell walls. Given the ease and lower cost of the puncture test, skin break force may be an interesting field parameter to provide a more scientific choice of harvesting date.

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