Influence of wine-grape skin hardness on the kinetics of anthocyanin extraction

UNIVERSITÀ DEGLI STUDI DI TORINO

INFLUENCE OF WINE-GRAPE SKIN HARDNESS ON THE KINETICS OF ANTHOCYANIN EXTRACTION

Luca Rolle[1*] · Fabrizio Torchio[1] · Alessandra Ferrandino[2] · Silvia Guidoni[2]

[1] Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Settore Microbiologia Agraria e Tecnologie Alimentari, Università degli Studi di Torino, Via L. da Vinci 44, 10095 Grugliasco, Torino, Italy.

[2] Dipartimento di Colture Arboree, Università degli Studi di Torino, Via L. da Vinci 44, 10095 Grugliasco, Torino, Italy.

[*]Corresponding author [email: luca.rolle@unito.it; tel: 39 0116708558; fax: 39 0116708549]

ABSTRACT

The main aim of this work was to study in a model hydroalcoholic solution, containing 12% of ethanol and at pH 3.20, the kinetics of anthocyanin extraction from *Vitis vinifera* L. cv Nebbiolo berries of different skin hardness. This mechanical property was evaluated as the breaking skin force measured by Texture Analysis, a rapid and low-cost analytical technique. By TAxT2i Texture Analyzer, a puncture test was carried out on two groups of berries separated according to their density by flotation in order to obtain more homogenous samples and minimize the effect of different stages of ripening of the berries. Among the berries containing 242±8 g/L of reducing sugars in the pulp juice, two groups of berries with different skin hardness were selected: soft (0.26±0.04 N) and hard (0.47±0.05 N). In our experimental conditions, at the end of maceration, the extracts from the higher skin hardness group showed the higher contents of total anthocyanin: +25 mg/L (+9.4%). The anthocyanin profile of extracts, obtained at different extraction times, showed no significant differences among the distribution of different anthocyanins. Only in the early phases of dissolution, did the extracts reveal a dissimilar anthocyanin profiles and in the extracts of hard skins higher percentages of cyanidin and peonidin derivatives were present. Additionally, the evolution of skin mechanical properties from veraison to overripe and the influences of biotype on these parameters at harvest are reported in this work.

Keywords: *Vitis vinifera* L., grapes ripeness, skin mechanical properties, texture analysis, clones, anthocyanin extractability, Nebbiolo, Barolo wine.

INTRODUCTION

Anthocyanins are the pigments responsible for the red colour of grape berries and the respective wines produced from them;[1,2] they play a key role in the formation of polymeric pigments responsible for the stable colour of aged red wines.[3] Anthocyanins are gradually accumulated in the berry skin throughout grape ripening from veraison onwards;[4] their concentrations depend mainly on the cultivar[5,6], but, for the same varieties, can vary widely among different vintages, vineyard practices, climatic conditions, soil features and crop load.[7] In particular, as a function of these aspects, the anthocyanin concentrations of Nebbiolo grapes, one of the most important and well-known Italian vine varieties and the object of this study, can vary between 500 and 900 mg kg^{-1} of grapes.[8-10]

The extraction of anthocyanins during fermentation is conditioned by several factors such as grape variety,[11] use of pectinolytic enzymes,[12] ethanol concentration[13], time and temperature of maceration and other strategies of enological technique.[14,15] However, these pigments are not always easily extracted from skins during winemaking and a low extraction can result in poorly colored wines, even when their amount in the grapes is considered sufficient.

Many studies have been conducted to define the best method to evaluate polyphenolic compounds in grapes and the ease with which they are released from skins. Currently, the cellular maturity index or extractability index (EA), defined by Glories and Augustine,[16] is the method of choice to estimate the extractability of anthocyanins with adequate reliability and to predict the phenol composition and the chromatic characteristics of wines.[17-19] However, the operative protocol to measure EA requires trained technicians and laborious procedures.

It is conceivable that physical-mechanical properties of skins assessable by Texture Analysis, a modern analytical technique used for the measurement of the physical characteristics of plant tissue,[20] might be favorably used as extractability markers because of the relationship, determined by multiple linear regression, found among skin hardness and thickness and cellular maturity index.[21] Therefore, the purposes of this work were: i) to determine the kinetics of extraction in berries with different skin hardness; ii) to study the modification of mechanical characteristics of berry skin from veraison to grape overipening and, finally, iii) to evaluate, at harvest, the influence of different biotypes on the mechanical characteristics of berry skin.

MATERIALS AND METHODS

Grapes and sampling

The study was carried out in 2007 in an experimental vineyard located at Neive (Piedmont region, North-West Italy). The mechanical properties of the skin of Nebbiolo CVT 71 clone berries were monitored for eight weeks from veraison (29 August) onwards. Four weeks after veraison (commercial harvest) grapes of the same clone were harvested to assess anthocyanin profile of berry skins, mechanical properties and to monitor anthocyanin extractability during maceration in a model solution. In addition, at commercial harvest, grapes from other Nebbiolo clones (CVT141, CVT180, CVT185 and CVT 308) were picked to evaluate the influence of biotype on the mechanical characteristics of berry skins. For each sample, 400 berries were randomly picked with pedicels.

Mechanical parameters of berry skin

Physical-mechanical parameters of skins were evaluated by the puncture test.[22] A Texture Analyzer (TAxT2i) from Stable Micro Systems (Surrey, UK) equipped with a HDP/90 platform, SMS P/2N needle probe and 5 kg load cell was used. Speed test was 1 mm s^{-1}. All acquisitions were performed at 400 Hz; data were evaluated using the Texture Expert Exceed software package (vers. 2.54 in Windows 2000). The berries were placed on the metal plate of the UTM with the pedicel in a horizontal plane in order to be consistently punctured in the lateral face. The penetration of the needle probe into the berry was 3 mm.[22] From the force-time curves, three parameters were calculated: F_{sk} (N; Berry skin break force), W_{sk} (mJ; Berry skin break energy) and E_{sk} (Nmm^{-1}; Young's modulus of skin).[22,23] The first variable corresponds to the maximum force opposed by the skin at the probe penetration, W_{sk} represents the area under the force-time curve of the puncture test limited between start to F_{sk} value. E_{sk} or Young's modulus of elasticity is a parameter that permits the stiffness of the material to an applied load to be assessed.[23] To measure Sp_{sk} (µm; Berry skin thickness), a piece of skin of almost 0.25 cm^2 was removed at the lateral side of the berry with a razor blade. After calibration of the probe position, the skin thickness was calculated as the distance between the point corresponding to the probe contact with the berry skin (trigger) and the platform base during a compression test.[23] For each test and samples, 30 berries were analyzed.

Assessment of Extractability

Chemicals - HPLC-grade solvents and all other chemicals were purchased from Sigma (Milan, Italy). Solutions were prepared in deionized water produced by a Purelab Classic system (Elga Labwater, Marlow, United Kingdom). Anthocyanin standards (Delphinidin 3-*O*-glucoside chloride, Malvidin 3-*O*-glucoside chloride, Peonidin 3-*O*-glucoside chloride, Cyanidin 3-*O*-glucoside chloride) were supplied by Extrasynthèse (Genay, France).

Texture analysis – The skin hardness of each berry used in this experiment was determined by puncture test (see above) using F_{sk} parameters for their classification. To minimize the effect of different contents of soluble solids on extractability results,[4] the 400 berries of CVT 71 were calibrated according to their density. This was estimated by flotation of berries in ten different salt solutions (from 100 to 190 g L^{-1} NaCl) so that the difference in total soluble solids of two consecutive batches of berries was about 17 gL^{-1} (i.e., 1 vol % in potential alcohol).[4] Berries containing 242 ± 8 gL^{-1} sugars in the pulp juice were used and within them two groups of berries with different skin hardness were selected: S, soft (0.26±0.04 N) and H, hard (0.47±0.05 N). This sugar content is that at which the Nebbiolo grapes are usually harvested for the production of Barolo and Barbaresco Denomination of Origin wines.

Anthocyanin extraction - Sixty skins (20 × 3 replicates) of berries belonging to S and H groups were used to study the extractability of anthocyanins. The berry skins, removed manually from the pulp and dried with absorbent paper, were quickly immersed in 75 mL of hydro-alcoholic buffer (pH 3.20), containing 200 mgL^{-1} of $Na_2S_2O_5$ to limit oxidation of phenolic compounds and 12% of ethanol.**[4]** The skins of other previously weighed berries (20 × 3 replicates), were introduced **to** the same volume (75 mL) of the described extractant solution and homogenised with Ultra-turrax T25 (IKA Labortechnik, Staufen, Germany).These homogenized solutions were then centrifuged (1126 *g*; 5 min; 20 °C) and the supernatant used to calculate the total anthocyanin concentration of the skins (TA, mg kg^{-1} grapes).

The total contents of anthocyanin of this supernatant, expressed in mgL^{-1} and defined as solution A, were further used to evaluate the rate of skin anthocyanin extractability during maceration. The kinetics of extraction were monitored at regular intervals: 10, 20, 30 minutes and 1, 2, 3, 4, 5, 24, 48 hours and the anthocyanin contents of these extracts (defined as solution B_{time}) were expressed as mgL^{-1}. The percentage of anthocyanin extraction at each extraction time was calculated as: (solution B_{time}/solution A)*100.

At the end of the maceration period, the berry skins from each trial were rinsed with a hydroalcoholic solution, dried with absorbent paper and introduced to other 75 mL of the same buffer solution, homogenised and the extract was centrifuged (see above); the anthocyanin concentration in the supernatant (defined as solution C and expressed in mgL^{-1}) corresponded to the amount of the non-extracted anthocyanins and it was used to estimate the percentage of anthocyanin recovered relative to the total content as: ((solution B_{48h} + solution C)/solution A)*100.

Spectrophotometry and HPLC Analysis - Anthocyanin concentrations in all the extracts were determined by spectrophotometry.[24] The analysis of individual anthocyanins was performed by HPLC after application of the berry skins extract to a SEP-PAK C18 cartridge (Waters Corporation, Milford, MA, USA) and elution with methanol.[24] The chromatograph consisted of a P100 pump, an AS3000 auto-sampler (Spectra Physics Analytical, Inc, San Jose, CA, USA) and a Reodyne injection valve equipped with a 20 µL sample loop. A LiChroCART column (25 cm x 0.4 cm i. d.) packed with LiChrosphere 100 RP-18 5-µm particles from Merck (Darmstadt, Germany) was used. A Spectra Focus Diode Array Detector (Spectra Physics Analytical, Inc, San Jose, CA, USA) operating at 520 nm was employed. The following solvents were used: solvent A = 10 % v/v formic acid in water; solvent B = 10 % v/v formic acid with 50 % v/v methyl alcohol in water. All solvents were filtered through a 0.20 µm filter. The solvent flow rate was 1 mL/min and the column temperature was 20 °C. The injection volume was 20 µL. The solvent gradient used was previously reported in the literature.[24,25] Data treatment was carried out using the ChromQuestTM chromatography data system (ThermoQuest, Inc, San Jose, CA, USA). The percentages of individual anthocyanins were calculated by comparing the area of the individual peak with the sum of the peak areas of all separated components.

Statistical analysis – The means of different parameters were studied by one-way analysis of variance (ANOVA). Means submitted to analysis of variance were separated with the Duncan test. Statistical analysis was performed using STATISTICA for Windows Release 7.1 (StatSoft Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

The total amount of anthocyanin and relative profile of Nebbiolo grapes at harvest are reported in Table 1. The total concentration of anthocyanins of fresh berries (516 mg kg^{-1}), although low, was usual for this cultivar.[8,18] Peonidin 3-glucoside derivative forms are the main pigments and 3'-hydroxylated anthocyanins are present in high percentages (49.78%), although the values observed

in the CVT 71 clone were lower than those of other clones,[14,22] such as the percentages of acetylated and coumaroylated forms.[8,9] The kinetics of dissolution of anthocyanins in model hydroalcoholic solution, content (mgL^{-1}) and percentage of extraction (%), are reported in Table 2. On average, about 96.5 % of the total anthocyanin present in the entire skins were recovered in extractant media and residual solids parts, in agreement with literature data. [4]

The anthocyanin concentrations of the extracts at different times of maceration (solution B_{time}), from 5 hours onwards, were consistently different, depending upon the berry skin hardness (Table 2). Under our experimental conditions, at the end of the maceration (48 h), hard skins presented an extractive capacity of 76.6 % compared to the 67.2 % of soft skins. Thus, the toughest skins presented greater capacities for anthocyanin release (+ 9.4 %), confirming the results obtained for another variety even though, in the latter situation the model solution contained only 3 % of ethanol.[26] These results are also in accordance with the significant inverse correlation between break skin force and extractability indexes (EA) found in Cabernet franc grapes.[27] In general, more complete dissolution of phenols in the must corresponds to lower values of this index. **The** cell maturity index was found to be a satisfactory measure of the facility with which polyphenols are extracted during the first phases of maceration.[14] The chemical composition of the grape skin cell-walls may determine the mechanical resistance of berry skin to anthocyanin release;[28] nevertheless, correlation studies between skin physical-mechanical characteristics and chemical composition, to our knowledge, are not currently available in the literature.

Even if the influence of skin hardness on total anthocyanin extraction was observed, significant differences between the anthocyanin profile of extracts obtained from the S and H skins at different extraction times (Table 3) were only found in the first phases of dissolution. In the extracts of the hard skins at 10 minutes, higher percentages of petunidin 3-glucoside (+ 0.8 %), cyanidin 3-glucoside (+ 3.6 %) and peonidin 3-glucoside derivatives (+ 6.0 %) and lower percentages of malvidin 3-glucoside (- 9.6 %) were present in comparison to the soft skins. This aspect is particularly important for varieties rich in 3'-hydroxylated anthocyanins, because these pigments, extracted preferentially during the initial phase of the maceration, may be easily oxidised by the enzyme present in the juice of those cultivars containing an anthocyanin profile made up mainly of molecules tri-substituted in the B-ring, and therefore more protected against oxidation. [11,18,29] In fact, during wine-making using Nebbiolo grapes, a remarkable loss of peonidin 3-glucoside and cyanidin 3-glucoside was noticed.[30] Therefore, on the basis of these results, knowledge of skin hardness could provide interesting information for the oenologist during the planning and management of the maceration/fermentation step. Finally, no significant differences in anthocyanin

profile between hard and soft berry skin extracts at the end of the maceration (solutions B_{48h}) were observed (Table 3), even in those of the non–extracted skins (solutions C) (Table 4) when, as already mentioned, the total amounts were different.

The physical and morphological characteristics of the grape's skin play a critical role during the ripening process, regulating gas exchange between the berry and the surrounding environment, serving as a protective barrier against fungal disease and protecting the grape from UV light and climatic injuries.[31,32] Made possible by the use of a needle probe, the puncture test carried out in this study allowed the estimation of the changes in the physical-mechanical properties of the skin of Nebbiolo grapes during ripening (Figure 1), minimizing the possible interferences caused by pulp firmness on the results. From veraison to ripeness an increase of the skin hardness parameters (+ 0.052 N, + 17.3 % for F_{sk}; + 0.09 mJ, + 59.1 % for W_{sk}), and of the skin thickness Sp_{sk} (+ 26 μm, + 15.2 %) was observed above all in the first weeks. A peak in the value of the E_{sk} parameter was observed two weeks after veraison followed by a quick decrease; from the third week onwards no further change was detected. Therefore, even if the break skin force (F_{sk}) can be considered an important parameter to assess the total anthocyanins extractability, as already demonstrated on Cabernet franc grapes growing in different *terroirs* of the Loire Valley region[33] and on Barbera grapes from several Piedmont areas,[34] the constant value of the F_{sk} parameter close to harvest might indicate its applicability as an indicator of the maturity of grapes. Nevertheless, other studies indicated that texture parameters of whole berries can represent a good means to estimate grape maturity.[35,36] In fact, during ripening, the berries become softer and softer[37] as a result of significant changes in parietal constituent composition notably in pulp cells. Therefore, the compression test, which assesses parameters such as firmness, cohesiveness, gumminess, is presently favored to monitor ripeness;[35-37]. In this type of test, pulp and skin data are aggregated.

Nevertheless, the skin hardness, defined by F_{sk} and W_{sk} parameters, at advanced stages of ripening, is an effective tool to discriminate among different vineyards,[23,33,34] although F_{sk} values can be strongly affected by climate trends of the vintages.[23] In particular, in the year 2005, Nebbiolo grapes growing in mountainous vineyards, were characterized by a higher berry skin firmness, with higher mean values of break skin force (+ 28.7 %) and of break skin energy (+ 47.3 %) compared to the grapes of vineyards growing in a hilly area.[38] Nevertheless in the alpine environment, the high berry skin resistance to rupture (splitting) is important from the agronomical and phytopathological point of view,[39] and it could likewise be the consequence of the higher berry skin thickness (+ 20.4 %) detected in mountainous Nebbiolo grapes.[38] Furthermore, significant modifications of berry

skin mechanical characteristics were found in Mondeuse grapes in the phases of over ripeness and on-vine drying process, where increases of F_{sk}, W_{sk} and Sp_{sk} values were observed.[40]

Finally, high variability among studied clones was found in the W_{sk} parameter (0.048 mJ, 22.3 %) (Table 5). CVT 141 clone was characterized by higher skin break force values (F_{sk}) in comparison to CVT 185, with mean differences of 0.044 N (11.9 %). Also the Sp_{sk} value showed an about 12 % variability among clones with CVT 180 grapes characterized by the thinnest skins (179 µm) and CVT 141 characterized by those having the thickest skins (203 µm). However, no correlations were found between F_{sk} and Sp_{sk} parameters, in accordance with those already reported.[21,41] Histological studies on skin tissues are therefore required to explain these mechanical behaviours. On the base of the knowledge already acquired, the physical-mechanical properties of wine grape berry skin appear to be influenced by weather conditions, meteorological events during ripening, area of production, stage of ripeness and variety. Within each cultivar, the clone also assumes importance in the characterization of skin hardness and, consequently, in the anthocyanin extractability.

CONCLUSION

Skin textural characterization can be an efficient method to easily assess anthocyanin extractability. In this work, extracts from skins of higher hardness did, indeed, show the highest content of total anthocyanin (+9.4 %). On the other hand, the anthocyanin profile of extracts obtained at different extraction times showed significant differences in the distribution of different anthocyanins only in the first phases of dissolution with the extracts of hard skins characterized by higher percentages of cyanidin and peonidin derivatives. Therefore, hard skins seem to be characterized by increased fragility of the cell walls, which allows easier release of coloured pigments. Thus, although the evolution of F_{sk} values during the grape ripening could represent a limit for the choice of this parameter as an indicator of maturity, it can be used as an extractability marker for grapes from different vineyards. Break skin force can be considered as a new index of grape quality, applicable by winemakers interested in optimizing the anthocyanin extraction process during the maceration phase. Further, in comparison to other methods for evaluating the extractability of phenols, the texture analysis tests are rapid and inexpensive, showing promise as routine tools in monitoring vineyards. However, further studies on different grape varieties will be necessary to confirm the observed relationship between skin hardness and anthocyanin extractability.

REFERENCES

1. Gonzáles-Neves, G.; Franco, J.; Barreiro, L.; Gil, G.; Moutounet, M.; Carbonneau, A. Varietal differentiation of Tannat, Cabernet sauvignon and Merlot grapes and wines

according to their anthocyanin composition. European Food Research and Technology **2007**, *225*, 111-117.

2. Revilla. E.; García-Beneytez, E.; Cabello, F. Anthocyanin fingerprint of clones of Tempranillo grapes and wines made with them. Australian Journal of Grape and Wine Research **2009**, *15*, 70-78.
3. Boulton, R. The copigmentation of anthocyanins and its role in the color of red wine: a critical review. American Journal of Enology and Viticicolture **2001**, *52*, 67-87.
4. Fournand, D.; Vicens, A.; Sidhoum, L.; Souquet, J.M.; Moutounet, M.; Cheynier, V. Accumulation and extractability of grape skin tannins and anthocyanins at different advanced physiological stages. Journal of Agriculture and Food Chemistry **2006**, *54*, 7331-7338.
5. **Mattivi, F.; Guzzon, R.; Vrhovsek, U.; Stefanini, M.; Velasco, R. Metabolite profiling of grape: flavonols and anthocyanins. Journal of Agriculture and Food Chemistry 2006, *54*, 7692-7702.**
6. **Rababah, T. M.; Ereifej, K. I.; Al-Mahasneh, M. A.; Ismaeal, K.; Hidar, A. G.; Yang, W. Total phenolics, antioxidant activities, and anthocyanins of different grape seed cultivars grown in Jordan. International Journal of Food Properties 2008, *11*, 472-479.**
7. Downey, M.; Dokoozlian, N.K.; Krstic, M.P. Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. American Journal of Enology and Viticulture **2006**, *57*, 257-268.
8. Guidoni, S.; Ferrandino, A.; Novello, V. Climate and agronomical practice effects on anthocyanin accumulation in cv. 'Nebbiolo' (Vitis vinifera L.) berries. American Journal of Enology and Viticulture **2008**, *59*, 22-29.
9. Chorti, E.; Guidoni, S.; Ferrandino, A.; Novello, V. Effects of different cluster sunlight exposure levels on ripening and anthocyanin accumulation in Nebbiolo grapes. American Journal of Enology and Viticulture **2010,** *61*, **23-30**.
10. **Guidoni, S., P. Allara, and A. Schubert. Effect of cluster thinning on berry skin anthocyanin composition of *Vitis vinifera* cv. Nebbiolo. American Journal of Enology and Viticulture 2002, *53*, 224- 226.**
11. Gonzáles-Neves, G.; Gil, G.; Barreiro, L. Influence of grape variety on the extraction of anthocyanins during the fermentation on skins. European Food Research and Technology **2008**, *226*, 1349-1355.
12. Pardo, F.; Salinas, M.R.; Alonso, G.L.; Navarro, G.; Huerta, M.D. Effect of diverse enzyme preparations on the extractions and evolution of phenolic compounds in red wines. Food Chemistry **1999**, *67*, 135-142.
13. Canals, S.; Llaudy, M.C.; Valls, J.; Canals, J.M.; Zamora, F. Influence of ethanol concentration on the extraction of color and phenolic compounds from the skin and seeds of Tempranillo grapes at different stages of ripening. Journal of Agriculture and Food Chemistry **2005**, *53*, 4019-4025.
14. Jensen, S.J.; Blachez, B.; Egebo, M.; Meyer, A.S. Rapid extraction of polyphenols from red grape. American Journal of Enology and Viticicolture **2007**, *58*, 451-461.
15. Gómez-Míguez, M.; Heredia, F.J. Effect of the maceration technique on the relationships between anthocyanin composition and objective color of Syrah wines. Journal of Agriculture and Food Chemistry **2004**, *52*, 5117-5123.

16. Glories, Y. ; Augustin, M. Maturité phénolique du raisin, consèquences technologiques: applications aux millésimes 1991 et 1992. In *Actes du Colloque Journée Technique du CIVB*, Bordeaux, France ept 56, **1993**.
17. Romero-Cascales, I.; Ortega-Regules, A.; López-Roca, J.M.; Fernández-Fernández, J.I.; Gómez-Plaza, E. Differences in anthocyanin extractability from grapes to wines according to variety. American Journal of Enology and Viticulture **2005**, *56*, 212-219.
18. Cagnasso, E.; Rolle, L.; Caudana, A.; Gerbi, V. Relations between grape phenolic maturity and red wine phenolic composition. Italian Journal of Food Science **2008**, *20*, 365-381.
19. Kontoudakis, N.; Esteruelas, M.; Fort, F.; Canals, J.M.; Zamora, F. Comparison of methods for estimating phenolic maturity in grapes: correlations between predicted and obtained parameters. Analytica Chimica Acta **2010**, *660*, 127-133.
20. Roudot, A.C. Some considerations for a theory of plant tissue mechanics. Science of Aliments **2006**, *26*, 409-426.
21. Río Segade, S.; Rolle, L.; Gerbi, V.; Orriols, I. Phenolic ripeness assessment of grape skin by texture analysis. Journal of Food Composition and Analysis **2008**, *21*, 644-649.
22. Letaief, H.; Rolle, L.; Zeppa, G.; Gerbi, V. Assessment of grape skin hardness by a puncture test. Journal of the Science of Food and Agriculture **2008**, *88*, 1567-1575.
23. Letaief, H.; Rolle, L; Gerbi, V. Mechanical behavior of winegrapes under compression tests. American Journal of Enology and Viticulture **2008**, *59*, 323–329.
24. Di Stefano, R.; Cravero, M.C. Metodi per lo studio dei polifenoli dell'uva. Rivista di Viticoltura e di Enologia **1991**, *44***,** 37-45.
25. Rolle, L. ; Guidoni, S. Color and anthocyanin evaluation of red winegrapes by CIE L*, a*, b* parameters. Journal International des Sciences de la Vigne et du Vin **2007**, *41*, 193-201.
26. Rolle. L.; Torchio, F.; Zeppa, G.; Gerbi, V. Relations between break skin force and anthocyanin extractability at different stages of ripening. American Journal of Enology and Viticulture **2009**, *60*, 93-97.
27. Zouid, I.; Siret, R.; Maury, C.; Letaief, H.; Jourjon, F. (2008) Variation de la textura du raisin Cabernet franc au cours de la maturation. Corrélation avec l'extractibilité des composes phénolique. In *Proceedings of the 31st World Congress of Vine and Wine – OIV*. Verona, Italy, CD-R.
28. Ortega-Regules, A.; Romero-Cascales, I.; Ros-García, J.M.; López-Roca, J.M.; Gómez-Plaza, E. A first approach towards the relationship between grape skin cell-wall composition and anthocyanin extractability. Analytica Chimica Acta **2006**, *563*, 26-32.
29. Di Stefano, R.; Borsa, D.; Gentilini, N. Estrazione degli antociani dalle bucce dell'uva durante la fermentazione. L'Enotecnico **1994**, *30*,76-83.
30. Gerbi, V.; Zeppa, G.; Rolle, L. Evoluzione delle antocianine nel corso della vinificazione delle uve Nebbiolo. In *Ricerche e innovazioni nell'industria alimentare, Vol. 5*; Porretta, S., Eds.; Chiriotti Editori, Pinerolo (TO), **2002**; 420-427.
31. Gabler, F.M.; Smilanick, J.L.; Mansour, M.; Ramming, D.W.; Mackey, B.E. Correlations of morphological, anatomical, and chemical features of grape berries with resistance to Botrytis cinerea. Phytopathology **2003**, *93*, 1263-1273.
32. Rosenquist, J.K.; Morrison, J.C. Some factors affecting cuticle and wax accumulation on grape berries. American Journal of Enology and Viticulture **1989**, *40*, 241-244.

33. Le Moigne, M.; Maury, Ch.; Bertrand, D.; Jourjon, F. Sensory and instrumental characterisation of Cabernet Franc grapes according to ripening stages and growing location. Food Quality and Preference **2008**, *19*, 220-231.
34. Torchio, F.; Cagnasso, E.; Gerbi, V.; Rolle, L. Mechanical properties, phenolic composition and extractability indexes of Barbera grapes of different soluble solids contents from several growing areas. Analytica Chimica Acta **2010**, *660*, 183-189.
35. Maury, C.; Madieta, E.; Le Moigne, M.; Mehinagic, E.; Siret, R.; Jourjon, F. Development of a mechanical texture test to evaluate the ripening process of Cabernet Franc grapes. Journal of Texture Studies **2009**, *40*, 511–535.
36. Río Segade, S.; Orriols, I.; Giacosa, S.; Rolle, L. Texture analysis parameters as winegrapes varietal markers and ripeness predictors. International Journal of Food Properties **2010**, in press
37. Robin, J.P.; Abbal, P.; Salmon, J.M. Fermeté et maturation du raisin. Définition et évolution de différents paramètres rhéologiques au cours de la maturation. Journal International de la Science de la Vigne et du Vin **1997**, *31*, 127-138.
38. Rolle, L.; Letaief, H.; Cagnasso, E.; Ghirardello, D.; Zeppa, G.; Gerbi, V. Studio delle proprietà meccaniche di uve Nebbiolo coltivate in ambienti diversi. Quaderni di Scienze Viticole ed Enologiche **2006**, *28*, 185-194.
39. Lang, A.; During, H. Grape berry splitting and some mechanical properties of the skin. Vitis, **1990**, *29*, 61-70.
40. Rolle, L.; Torchio, F.; Giacosa, S.; Gerbi, V. Modification of mechanical characteristic and phenolic composition in berry skins and seeds of Mondeuse winegrapes throughout the on-vine drying process. Journal of the Science of Food and Agriculture **2009**, *89*, 1973-1980.
41. Rolle, L.; Letaief, H.; Gerbi, V. Application of Texture Analysis for the evaluation of the wine grape quality. Bulletin de l'OIV **2008**, *81*, 221-229.

Table 1 Total anthocyanin content (TA) and profile of Nebbiolo CVT 71 berries containing 242 ± 8 g L^{-1} of sugars (2007 vintage). Means of three replicates (average value ± standard deviation). Cinnamoyl-glucosides included both p-coumaroyl and caffeoyl anthocyanin forms.

TA (mg kg^{-1}grapes)	Free Glucosides (%)	Acetyl-glucosides (%)	Cinnamoyl-glucosides (%)	∑ of delphinidin derivatives (%)	∑ of cyanidin derivatives (%)	∑ of petunidin derivatives (%)	∑ of peonidin derivatives (%)	∑ of malvidin derivatives (%)
516 ± 7	82.10±0.52	5.17±0.11	12.73±0.45	5.88±0.31	7.37±0.30	6.61±0.30	42.20±0.65	37.94±0.72

Table 2 Kinetic of extraction of anthocyanins, content (mgL^{-1}) and percentage of extraction (%), during maceration in a model solution, from the skins of the two groups of Nebbiolo CVT 71 berries. S = soft skin 0.26±0.04N; H = hard skin 0.47±0.05N). Sign = Significance: ns not significant, * significant at $p \leq 0.05$.

	10 min	20 min	30 min	1h	2h	3h	4h	5h	24h	48h	non-extracted skins
						mgL^{-1}					
S	26±2	31±1	51±4	57±2	107±3	134±9	150±7	159±4	174±8	178±8	78±4
H	29±1	39±1	58±3	67±3	116±3	147±7	167±7	184±4	199±2	203±3	53±6
Sign	ns	*	ns	ns	ns	ns	ns	*	*	*	*
						%					
S	9.7±0.7	11.9±0.5	19.1±1.3	21.6±0.9	40.2±1.2	50.7±3.6	56.6±2.7	60.0±1.6	65.7±2.9	67.2±3.2	29.3±1.7
H	11.1±0.3	14.7±0.5	21.9±1.1	25.4±1.1	43.9±1.2	55.6±2.6	62.9±2.5	69.2±1.3	75.2±0.9	76.6±1.1	19.9±0.9
Sign	ns	*	ns	ns	ns	ns	ns	*	*	*	*

Table 3 Nebbiolo clone CVT 71: anthocyanin profile (expressed in %) of the extracts (solutions B_{time}) as influenced by different extraction times and skin hardness. S = soft skin 0.26±0.04N; H = hard skin 0.47±0.05N. Average value ± standard deviation (n = 3). Sign = Significance: ns not significant, * p ≤ 0.05, ** p ≤ 0.01, *** p≤ 0.001,.nd = not detected. Cinnamoyl-glucosides included both *p*-coumaroyl and caffeoyl anthocyanin forms.

		10 min	20 min	30 min	1h	2h	3h	4h	5h	24h	48h
Free glucosides	S	97.21±2.16	97.90±0.60	97.54±0.26	96.98±0.36	91.87±0.28	89.93±1.96	88.13±0.34	88.21±0.45	88.77±2.73	89.39±2.04
	H	97.51±0.50	96.76±0.43	96.84±0.14	96.51±0.47	91.98±1.12	89.81±0.35	89.62±0.53	88.48±0.27	88.63±4.77	87.97±0.31
Sign		ns	ns	*	ns	ns	ns	ns	ns	ns	ns
Acetyl-glucosides	S	2.79±2.16	1.55±0.21	1.73±0.33	2.32±0.41	3.93±0.26	4.65±0.50	4.87±0.10	4.77±0.07	4.88±0.03	4.48±0.14
	H	1.77±0.22	2.78±0.45	2.26±0.15	2.86±0.28	4.26±0.21	4.82±0.11	4.49±0.18	4.66±0.22	4.77±1.00	4.62±0.22
Sign		ns	*	ns	ns	ns	ns	ns	ns	ns	ns
Cinnamoyl-glucosides	S	nd	0.55±0.50	0.73±0.24	0.70±0.12	4.20±0.06	5.42±1.45	7.00±0.24	7.02±0.40	6.35±2.74	6.13±2.02
	H	0.72±0.34	0.46±0.02	0.91±0.07	0.63±0.31	4.80±0.53	5.37±0.24	5.89±0.71	6.86±0.16	6.60±0.78	7.20±0.46
Sign		-	ns	ns	ns	ns	ns	ns	ns	ns	ns
∑ of delphinidin derivatives	S	5.78±0.67	5.49±0.62	6.47±0.51	7.04±0.62	7.18±0.20	7.43±0.50	6.78±0.36	6.96±0.17	6.97±0.54	6.95±0.19
	H	4.96±0.19	5.29±0.02	5.99±0.29	6.98±0.76	7.07±0.10	7.06±0.01	6.83±0.25	6.68±0.18	7.41±0.48	6.70±0.15
Sign		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
∑ of cyanidin	S	7.10±0.77	8.46±2.86	9.14±0.42	8.21±0.64	8.00±0.21	7.84±0.38	7.53±0.25	7.73±0.41	7.61±0.16	7.52±0.43
	H	10.77±0.91	8.17±0.52	9.37±0.56	8.83±0.67	8.75±0.46	8.35±0.69	8.46±0.60	8.36±0.45	8.30±0.59	8.05±0.47
Sign		**	ns	ns	ns	ns	ns	ns	ns	ns	ns
∑ of petunidin derivatives	S	3.60±0.20	4.31±1.21	5.17±0.11	5.28±0.79	6.17±0.23	6.50±0.17	6.47±0.17	6.55±0.04	6.52±0.27	6.41±0.16
	H	4.43±0.29	3.70±1.12	4.81±0.43	5.24±0.38	6.26±0.14	6.38±0.08	6.35±0.29	6.20±0.14	5.92±0.49	6.23±0.13
Sign		*	ns	ns	ns	ns	ns	ns	ns	ns	ns
∑ of peonidin	S	46.67±0.48	47.18±0.49	46.28±1.71	42.09±1.69	40.86±1.50	39.05±0.62	40.44±1.65	40.31±1.18	39.55±2.66	39.68±1.63
	H	52.67±0.55	49.29±1.42	48.59±1.14	44.52±1.16	43.65±0.60	42.32±1.03	42.04±0.40	42.28±0.89	40.86±1.14	41.41±0.84
Sign		***	ns	ns	ns	*	ns	ns	ns	ns	ns
∑ of malvidin	S	36.84±0.68	34.56±5.17	32.94±0.74	37.38±1.06	37.79±1.25	39.19±0.09	38.78±1.25	38.45±1.70	39.35±2.01	39.43±1.72
	H	27.17±1.39	33.54±3.07	31.24±1.74	34.43±1.83	35.33±2.09	35.88±1.82	36.32±0.93	36.47±1.59	37.51±1.66	37.41±1.12
Sign		***	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 4 Nebbiolo clone CVT 71: anthocyanin profile, expressed in %, of the non-extracted skins (solutions C). S = soft skin 0.26±0.04N; H = hard skin 0.47±0.05N. Average value ± standard deviation (n=3); Sign = Significance: ns not significant. Cinnamoyl-glucosides included both p-coumaroyl and caffeoyl anthocyanin forms.

	Free glucosides	Acetyl-glucosides	Cinnamoyl-glucosides	∑ of delphinidin derivatives	∑ of cyanidin derivatives	∑ of petunidin derivatives	∑ of peonidin derivatives	∑ of malvidin derivatives
S	84.90±0.54	4.26±0.14	10.84±0.43	5.91±0.60	7.17±0.24	6.09±0.27	41.69±2.49	39.14±1.72
H	84.42±1.24	4.58±0.70	11.00±0.63	5.74±0.27	7.73±0.59	6.02±0.16	42.95±0.62	37.56±1.45
Sign	ns	ns	ns	ns	ns	ns	ns	ns

Table 5 Berry skin mechanical characteristics from different Nebbiolo clone grapes grown in the same vineyard, at harvest in 2007. F_{sk} = Berry skin break force; W_{sk} = Berry skin break energy; E_{sk} = Skin Young's modulus; Sp_{sk} = Berry skin thickness. Average value ± standard deviation (*n* = 30). Mean values followed by the same letter are not significantly different for $p \leq 0.05$).

	F_{sk} (N)	W_{sk} (mJ)	E_{sk} (Nmm^{-1})	Sp_{sk} (µm)
CVT 71	0.36±0.06 [a]	0.20±0.07 [ab]	0.30±0.05 [ab]	197±26 [ab]
CVT 141	0.37±0.09 [a]	0.22±0.09 [a]	0.29±0.04 [b]	203±36 [a]
CVT 180	0.36±0.08 [ab]	0.19±0.08 [ab]	0.31±0.06 [a]	179±32 [c]
CVT 185	0.32±0.08 [b]	0.17±0.07 [b]	0.29±0.04 [b]	186±36 [bc]
CVT 308	0.35±0.07 [ab]	0.18±0.07 [ab]	0.31±0.04 [ab]	199±32 [ab]

Figure 1 Trends in the berry skin mechanical properties of Nebbiolo clone CVT 71 grapes during ripening (vintage 2007). A) F_{sk} = Berry skin break force; B) W_{sk} = Berry skin break energy; C) E_{sk} = Skin Young's modulus; D) Sp_{sk} = Berry skin thickness. Average values ± standard error (*n*=30). Mean values followed by the same letter are not significantly different for $p \leq 0.05$.