Impact of grapes heterogeneity according to sugar level on both physical and mechanical berries properties and their anthocyanins extractability at harvest.

Published version:

DOI:10.1111/jtxs.12001

IMPACT OF GRAPES HETEROGENEITY ACCORDING TO SUGAR LEVEL ON BOTH PHYSICAL AND MECHANICAL BERRIES PROPERTIES AND THEIR ANTHOCYANINS EXTRACTABILITY AT HARVEST

ZOUID I.[1], SIRET R.[1,3], JOURJON F.[1], MEHINAGIC E.[1] AND ROLLE L.[2]

[1]: L'UNAM Université, Unité de Recherche GRAPPE, UMT VINITERA, Ecole Supérieure d'Agriculture, 55 rue Rabelais, B.P. 30748, F-49007 Angers Cedex 01, France.

[2]: DI.VA.P.R.A., University of Turin, *Dipartime*nto di *Scienze Agrarie, Forestali e Alimentari,* Via L. da Vinci 44, 10095 Grugliasco, Turin, Italy.

[3]: Corresponding author: Dr. Siret René

Email: r.siret@groupe-esa.com

Tel.: +33 2 41 23 55 55

Fax: +33 2 41 23 56 09

ABSTRACT

One of the major factors affecting wine quality is the degree of the grapes maturity at harvest time. Cabernet Franc grapes belonging to six different classes of sugar level content (from 176.5 to 258.5 gL^{-1}) at harvest were studied. Their physical proprieties and mechanical behavior were measured using compression and puncture tests. The evolution of anthocyanins extractability was determined. Significant differences were found in grape textural properties and anthocyanins content due to the sugar level content in the different samples of berries tested. It appears that sweetest berries are characterized by a higher extraction yield of anthocyanins (up to + 3.55%) respect to those with a more low sugar content. Furthermore, significant relationships exist between the extractability of anthocyanins and the mechanical and physical attributes of the grape berries. The best correlation coefficients were obtained with physical parameters such as the berry weight and the surface:volume ratio (r = -0.99 and 0.97 respectively) and with double compression test parameters such as hardness, chewiness and springiness (r = -0.90, -0.91 and 0.92 respectively).

PRACTICAL APPLICATIONS

The purpose and the originality of this work were to determine the real impact of the heterogeneity of grapes at harvest. Among the berries with different sugar contents, were assessed the real differences in anthocyanin contents and extractability at harvest. The results acquired, also in function of different distribution percentage of the berries after floatation, suggest that each class of berries may influence in unlike way the final quality of wine. Therefore, automatic densimetric berry-sorting machines recently developed and proposed for use in wineries, could be indeed useful to select the grapes after the harvest.

KEYWORDS

Grape heterogeneity, sugar level, anthocyanins extractability, mechanical and physical properties.

INTRODUCTION

One of the major factors affecting wine quality is the degree of the grapes maturity at harvest time (Kontoudakis et al. 2011). Furthermore red wine sensory attributes (color and taste) depend largely on the phenolic compounds extracted from grapes during the winemaking process. Therefore, the elaboration of high quality red wines requires the optimal level of maturity for wine grapes. Grape maturity is associated with physico-chemical changes in the skin and pulp cell walls. During ripening, changes in the composition and structure of the cell wall, as well as in the structure of the tissue, may determine the mechanical resistance and the texture of the fruit (Abbott 2004; Hertog et al. 2004; Brummel et al. 2004; Brummel 2006; Deytieux-Belleau et al. 2008). Determination of grape berry texture is complex and depends on geometrical, surface and mechanical attributes of the sample, tissue composition, turgidity and structure response to physical stresses. Very few studies have investigated the links between grape softening during grape maturity and the different changes in the structure and properties of the material composing the cell. It is difficult to identify the degradation mechanisms in the cell walls because of the complexity of plant tissues and the structure of the cell wall (Kunzek et al. 1999). Therefore, investigations employing objective techniques used to measure the physical proprieties and rheological characteristics of plant tissue are useful. The characterization of the mechanical proprieties of grape berries appears to be an important parameter for understanding grape ripening, owing to its key role regarding the main compounds responsible for wine quality such as anthocyanins. In fact, the diffusion during pomace contact of anthocyanins, which are the most important compounds responsible for the chromatic characteristics of red wine, depends on the tendency of the berry skin to yield them (Ortega-Regules et al. 2008; Río Segade et al. 2011a). Skin permeability is linked to the grape berry cell walls. The epidermal cells of the skin form a barrier to the diffusion of components such as aromas and phenols (Deytieux-Belleau et al. 2008) and one of the phenomena that permit the diffusion of phenolic compounds such as anthocyanins is the degradation of cell-wall polysaccharides. This change is believed to be a fundamental step in improving the release of phenols from the grape skin (Amrani Joutei and Glories 1995).

Several studies on wine grape instrumental texture parameters have been conducted (Rolle et al. 2012). Recently, published studies have analyzed the changes of certain grape textural properties during ripening and the influence of the terroir effect on mechanical behavior were considered (Le Moigne et al. 2008; Torchio et al. 2010; Río Segade et al. 2011b). However, more information about the contribution of the rheological behavior of grapevines to the anthocyanins diffusion process is still required. Differences in the easiness of anthocyanin extraction from grapes could be linked to differences in the mechanical behavior of berries and the extraction yield of anthocyanins from grapes could be predicted by their rheological properties (Zouid et al. 2010). However, this previous study provides the average value of a representative sample for berries belonging to the whole vineyard and does not consider any possible heterogeneity in their different degree of ripeness. It is known that variations in timing and the extent of ripening occur between berries on a cluster, between clusters on a vine and between different vines in a single cultivar vineyard.

The aims of this work were to study the impact of the grapes heterogeneity according to sugar level on the physical and mechanical properties of Cabernet Franc grapes and to select the best instrumental parameters of the whole berries or of the skin linked with anthocyanins extractability. For this last goal, the process of anthocyanins extraction and diffusion from grape skins during maceration was carried out at the individual berry level in a model hydroalcoholic solution in order to better control extraction and avoid the impact of seeds and yeasts present in actual winemaking conditions. Such investigations could considerably increase the understanding of the material proprieties of grapes and thus help winemakers to choose the best time to harvest and the best process to produce the wine desired.

MATERIALS AND METHODS

Grape samples

In 2008, 2000 Cabernet Franc berries were sampled from a vineyard located at Chinon in the Loire Valley (France) that belongs to a network established in 1999 by the Institut Français de la Vigne et du Vin (IFV).

Berries, with pedicels, were randomly picked according to the method of IFV (Vinsonneau and Anneraud 2008). This method extracts fractions of the bunches in the middle zone of the spurs. Each fraction of a bunch had 3 to 5 berries, randomly selected alternatively from the upper and lower parts of the bunches, until 2000 berries were collected. The berries were then sorted according to density. This was performed by flotation of the berries in different salt solutions (from 130 to 180 gL^{-1} NaCl) so that the difference in total soluble solids of two consecutive batches of berries was ~17 gL^{-1} or 1% vol in potential alcohol (Fournand et al. 2006). Six batches (coded from F1 to F6) composed of 15 berries were retained according to their total soluble sugar content (Table 1).

Physical parameters

Measurements of berry and skin weight for each grape berry were performed. The length between top and bottom sides (L) and length between both lateral sides at middle of berry height (l) were measured using a calliper with an accuracy of 0.1 mm. For Cabernet Franc grapes, surface and volume were also calculated comparing the berry form to an ellipsoid about spherical, following the equations (Khojastehnazhand et al. 2010):

$$\text{Volume (mm}^3\text{)} = 4\,\pi\, a\, b\, c/3$$

$$\text{Surface (mm}^2\text{)} = 4\,\pi\, ((a^p b^p + a^p c^p + b^p c^p) / 3)^{1/p}$$

where a = b = l/2; c = L/2; for surface assessment, p = 1.607 (1.061% maximum error) (Río Segade et al. 2011a).

Texture analysis procedure

Skin mechanical properties were evaluated using a puncture test carried out on two positions: the bottom and equatorial side (Letaief et al. 2008a). Tests were performed with a universal testing machine TAxT2i Texture Analyzer (Stable Micro Systems-SMS, Surrey, UK) equipped with a HDP/90 platform and a 5 kg load cell was used. Tests were performed with a P/2N needle probe to a depth of 3 mm with a speed test of 1 $mm.s^{-1}$. Force/time curves were analyzed and three parameters were studied: the berry skin break force (F_{sk}) in N, the berry skin break energy (W_{sk}) in mJ and the slope associated with the break force ($Grad_{sk}$) in Nmm^{-1} corresponding to the Young modulus of the berry.

Whole berry mechanical properties were assessed using a double compression test (Letaief et al. 2008b; Maury et al. 2009). Berries were compressed in the equatorial position twice with 25% deformation of their height at 1 mms^{-1}. Force / time curves (Fig. 1) were analyzed and seven parameters were calculated using the software: hardness (N, as P_1), cohesiveness (adimensional, as $(A_2+A_{2W})/(A_1+A_{1W})$), gumminess (N, as hardness x cohesiveness), springiness (mm, as d_2), chewiness (mJ, as gumminess x springiness) and resilience (adimensional, as (A_{1w}/A_1)) (Deng et al. 2005; Letaief et al. 2008b). Berry firmness expressed in terms of millimeters of deformations under a fixed force of 1 N was calculated using the same curve from the first compression (Bellincontro et al. 2009).

Anthocyanin extraction in hydroalcoholic solution

Berries (batches of 15) belonging to each of the six classes of berries grouped according to their total soluble sugar content were used for studying the extractability of anthocyanins. Skins from the berries were separated from the pulp and seeds using a laboratory spatula. Each berry skin (15 replicates for group), was placed in 5 mL of an hydroalcoholic solution containing 12% ethanol, 100 mgL^{-1} $Na_2S_2O_5$, 5 gL^{-1} tartaric acid. The pH was adjusted to 3.20 with sodium hydroxide (Fournand et al. 2006; Rolle et al. 2009). Flasks were placed at 25°C in darkness for 2 days. At the end of extraction, the extract media (A1) and the residual solid material were collected separately. The residual materials (A2) were quickly immersed in new flasks with 5 ml of hydroalcoholic solution (same buffer above mentioned) and homogenized for 1 min at 8000 rpm with an Ultra Turrax T25 (IKA Labortechnik, Staufen, Germany) (Río Segade et al. 2011a). The extract medium (A1) and (A2) were centrifuged for 10 min at 3000 x g at 20°C. The two supernatants were then immediately used for spectrophotometric analysis.

Total anthocyanin content (TA) in both extract media, (A1) and (A2), was determined using the methodology described by Di Stefano and Cravero (1991). The relative standard deviations (RSD), measured by the laboratory, based on repeated analysis (n =20) of the same sample were 1.14% (Torchio et al. 2010). Total anthocyanin content (TA) was expressed as malvidin equivalent against the fresh weight of the berries (mgg^{-1}). The anthocyanins extractability (EA%) was calculated as follows:

$$EA\% = [TA_{A1}/(TA_{A1} + TA_{A2})] \times 100.$$

Statistical analysis

Analysis of variance was applied to all variables studied. Mean values obtained in the different measurements were compared by one-way ANOVA, also a PCA was applied and the confidence ellipses were determined. Pearson's correlation coefficients were calculated in order to study the relationship between all variables. Statistical analysis was performed with the statistical software Statgraphics® Plus 5.0 (StatPoint, Inc., Virginia, USA).

RESULTS AND DISCUSSIONS

Separating berries by flotation in different salt solutions and counting them made it possible to determinate the density distribution of berries. Fig. 2 shows a similar Gaussian bell-shaped distribution confirming that an important heterogeneity of berries is present at harvest according to the sugar level content.

Physical parameters

Table 2 shows the physical properties of Cabernet Franc grapes for each soluble solids class. The berry weight, the skin weight per berry and both the surface area and volume of the berry confirm that the samples selected differed significantly according to their levels of sugar. It is widely acknowledged that berry size is a determining factor in wine grape quality. The implicit mechanism of this conception is that surface area/volume ratio of the approximately spherical berries decreases with the increase of berry size as shown in Table 2. Smaller berries have a relatively higher solute to solvent ratio than larger berries (Coombe et al. 1987). Relative skin mass ([total skin mass/whole berry mass] × 100) varied between 12.26 and 15.42% of berry mass. Significant positive correlations were found among berry size (Berry weight, Volume, Surface area) and skin weight.

The variation in sugar concentration and thus the heterogeneity of ripe grapes harvested at the same stage of ripening may be associated with a loss of water from berries, without change in the weight of solutes per berry. There is also evidence that berries can gain solutes with no change in water per berry (Coombe 1992). The berry size depends essentially on the number of seeds and the level of maturity. The variation in developmental kinetics occurs between berries and finally on the accumulation of water by the berry (Cadot et al. 2008).

Compression test

The mechanical behavior relative to Cabernet Franc berries determined by compression test showed significant changes among different sugar classes (Table 3). The effect of the ripening stage was highly significant using all compression parameters measured except for firmness. This means that the rheological properties of grapes depend on the level of sugar content of berries. Value ranges obtained for firmness, hardness, cohesiveness, gumminess, springiness, chewiness and resilience were 1.63 to 1.46 mm; 2.23 to 1.71 N; 0.76 to 0.70; 1.66 to 1.20 N; 2.07 to 1.39 mm; 3.43 to 1.71 mJ and 0.38 to 0.335 respectively.

All the values of the compression parameters generally decreased with the increase in sugar content of berries. The grapes of F6 (258.5 gL^{-1} sugar) had lower compression parameters values than those of the other sugar classes. These results agree with those of Río Segade et al. (2011b) who found that a decreasing tendency was found for the compression parameters determined in Mencia, Brancellao and Merenzao varieties when ripeness increased and thus causing the sugar level to increase as well. All these textural differences are believed to involve modifications in the cell wall structure also loss in turgor pressure (Goulao and Oliveira 2008).

Based on our results, the density of the grapes greatly affects the softening of berries measured by compression tests. Overall, double compression measurements showed that at harvest the rheological properties of Cabernet Franc berries differ and are sugar content dependant.

Puncture test

Skin puncture tests and relative instrumental texture parameters have already been used to examine differences between varieties and ripening stages (Lee and Bourne 1980; Letaief et al. 2008a), as well as used as phenol extractability markers (Río Segade et al. 2011a). Table 4 provides the puncture parameters values for each level of sugar content of the Cabernet Franc berries on two positions: the bottom (F_{sk1}, $Grad_{sk1}$,W_{sk1}) and equatorial (F_{sk2}, $Grad_{sk2}$,W_{sk2}) side.

For this variety, all the parameters are not significant at a probability level of 5%, except for berry skin Young's modulus ($Grad_{sk1}$ and $Grad_{sk2}$). No modifications for both of the berry skin break force (F_{sk}) and the berry skin break energy (W_{sk}) were noted for the different class of berries levels of sugar. For F_{sk}, no large variations were noted. A slight difference between the values of this parameter was noted for the different levels of sugar content. No significant changes in the parameters that characterize the skin hardness of berries belonging to Barbera grapes containing different levels of soluble solids were observed (Torchio et al. 2010). The authors suggested that the behavior of the F_{sk} values close to harvest could limit the choice of this parameter as a berries heterogeneity indicator of ripeness. For the $Grad_{sk}$ parameter (Young's modulus), differences between the sugar level content were significant according to the Newman-Keuls test but the evolution of this parameter was not clear (Torchio et al. 2010).This parameter is used to measure the rigidity or stiffness of material in which low values correspond to springier and elastic tissues. Elasticity is controlled by the network bending formed by the cell wells (Roudot 2006). It is important to consider that puncture test parameters did not decrease with the increasing of total sugar content and show no clear evolution. This may be due to the complexity of the berries and the number of mechanisms which can initiate softness.

The mechanical behavior of berries can be approached in several ways which should be considered complementary rather than mutually restricting. The compression test could be more appropriate than the puncture test to explain differences between berries with different sugar levels. The lack of knowledge of cell tissue mechanics makes predicting and interpreting the evolution of such physical modifications very difficult, hence the importance of defining a mechanical theory to explain the changes that occur to the tissue in order to improve texture analysis.

Changes on the extractability of total anthocyanins from skins

Grapes with high phenol content do not necessarily produce wines that are also rich in phenolic compounds. In addition to phenolic composition, phenolic extractability is also an important concern. A method for evaluating this extractability is therefore proposed. Extraction media and residual skins were analyzed and the extraction yield of anthocyanin (EA%) was calculated (Table 5). EA%, under our experimental conditions, ranged from 91.07 to 94.55%. Since the extraction of anthocyanins from the grape skin is incomplete, it has been suggested that an equilibrium based on absorption is established between anthocyanin concentration in grapes and in wine. When this equilibrium is reached, no more anthocyanins can be extracted from grape skins (Boulton 2001).

One-way ANOVA was performed on the extraction yield of anthocyanin compounds. Significant differences with $p<0.001$ were observed between different levels of sugar content on berries for both the total anthocyanin content of the extraction media (A1) (TA_{A1}), the total anthocyanin content in the residual material (A2) (TA_{A2}) and the anthocyanin extractability (EA%). Regarding the extraction yield of total anthocyanins, a significant increase of this parameter was noted in concordance with the increase of the level of sugar content in berries except for berries belonging to F5 (242.1 gL^{-1} sugar) and F6 (258.5 gL^{-1} sugar) which have the same highest value of anthocyanin extractability (94.62%). It appears that sweeter berries are characterized by a higher extraction yield of anthocyanins. Since anthocyanins accumulate in the skin, smaller berries followed by sweeter berries in this study have a relatively higher solute to solvent ratio than larger berries (Conde et al. 2007). However, the rise of EA% in concordance with the increase of sugar content on berries was not notable. If a real path of the maturation and fermentation process occurs, there will be an effect of gradual alcohol accumulation, temperature increase and duration of fermentation. In the model, these were controlled.

Relationships between the physical and mechanical proprieties of berries and the extractability of anthocyanins from skins

For a better understanding of the real influence of the berries heterogeneity at harvest in the mechanical proprieties of berries and extractability of anthocyanins from skins, a principal component analysis (PCA) was carried out. PCA was performed on the physical parameters (berry and skin weight and the surface:volume ratio) and was applied on mechanical parameters (firmness, hardness, cohesiveness, gumminess, springiness, chewiness, resilience, Fsk_1, Wsk_1, $Gradsk_1$, Fsk_2, Wsk_2 and $Gradsk_2$) and on the extractability of anthocyanins (EA%). Principal component analysis explained 83.6% of the variation in the data in the first two dimensions, with 62.20% and 21.40% explained by factor 1 and factor 2, respectively (Fig. 3).

The ellipses present the confidence intervals of the barycenter at a probability of 0.05 for one group of berries (Fig. 3A). If two ellipses corresponding to two different groups of berries do not overlap, then both groups are different. Almost four groups of berries (F1, F2, F4 and F6) can be clearly distinguished. Only grapes from F3 and F5 were not clearly differentiated and they were mixed with those of F2 and F4 regarding to F3 and to F6 regarding to F5. The first factorial plot of PCA showed that compression parameters and both surface area and volume decreased from F1 to F6 (Fig. 3B). On the contrary, the extraction yields of total anthocyanins and the surface:volume ratio increased. Sweetest grapes (F6) were less firm and have highest value of extraction yield of total anthocyanins.

To determine the extent and the impact of the berries heterogeneity on compression and puncture parameters and extraction yield of anthocyanins from skin's berries, Pearson's correlation coefficients between parameters were calculated and presented in Table 6. These coefficients are significant at the 0.05 level if their absolute value is greater than 0.81 (O'Mahony 1986). The extraction yield of anthocyanins from berry skins (EA%) correlated positively with the parameter surface: volume ratio (r = 0.97). Similar correlations were noted for berry weight, skin weight, surface area and volume. Overall, an excellent relationship between the physical parameters and the extraction yield of anthocyanins was noted.

No significant correlations were found between (EA%) and puncture parameters ($r < 0.74$) except for the parameter Fsk_2 ($r = 0.83$), although, under other extraction conditions using Brachetto and Nebbiolo wine grape varieties with anthocyanin profile characterized by a prevalent content of peonidin and cyanidin 3-glucoside derivative forms, Rolle et al. (2009) suggested that harder skins allow a greater release of pigments.

Significant correlations were found between (EA%) and most of the compression variables (r = -0,90; -0,84; -0,92; -0,91 respectively for hardness, gumminess, springiness and chewiness). There is evidence that phenols are contained or associated with cell vacuoles or linked to the cell walls and that the degradation of cell-wall polysaccharides is a fundamental step to improve the release of phenols from grape skin (Deytieux-Belleau et al. 2008), resulting in grape softness. In this research, berries heterogeneity according to sugar level influences both the physical and mechanical grapes properties and the skin anthocyanin extractability. It is also possible to directly connect mechanical properties, especially those conducted by compression test, to the susceptibility of phenols' release from the grape berry skin.

CONCLUSION

The purpose and the originality of this work were to determine the real impact of the heterogeneity of grapes at harvest according to their sugar content on the mechanical and physical proprieties of berries and the extractability of the grape skin anthocyanins. A general trend could be observed in this study. The smallest and sweetest berries had the highest yield of extraction of anthocyanins from skins. In addition, the less soft berries and thus the biggest berries had the lowest extraction yield of anthocyanins from grapes. It can be concluded that the heterogeneity of berries could explain the differences observed in the extraction of anthocyanins from Cabernet Franc grapes. To confirm this approach, further studies are needed to determine how this heterogeneity can be measured quickly and simply. The next step is understanding the influences of this heterogeneity in berry tissue behavior by the cell's geometry, size, position or physical parameter.

Finally, among the correlation coefficients between anthocyanins extractability and instrumental texture parameters, the better were obtained with some double compression test parameters (hardness, chewiness and springiness) in comparison with puncture test parameters.

ACKNOWLEDGMENT

We would like to thank S. Giacosa for technical assistance and M. J. Cohen for revising the English version of the manuscript.

REFERENCES

ABBOTT, J.A. 2004. Quality of fresh and processed foods. Fresh quality assessment for fresh fruit and vegetables. Kluwer Academic, New York, U.S.A.

AMRANI JOUTEI, K., and GLORIES, Y. 1995. Tanins et anthocyanes: localisation dans la baie de raisin et mode d'extraction. Rev. Franc. Oenol. 153, 28-31.

BELLINCONTRO, A., NICOLETTI, I., VALENTINI, M., TOMAS, A., DE SANTIS, D., CORRADINI, D., and MENCARELLI, F. 2009. Integration of nondestructive techniques with destructive analyses to study postharvest water stress of winegrapes. Am. J. Enol. Vitic. 60, 57-65.

BOULTON, R. 2001. The copigmentation of anthocyanins and its role in the color of red wine: a critical review. Am. J. Enol. Vitic. 52, 67-87.

BRUMMELL, D.A. 2006. Cell wall disassembly in ripening fruit. Func Plant Biol 33, 103-119.

BRUMMELL, D.A., DAL CIN, V., CRISOSTO, C.H. and LABAVITHCH, J.M. 2004. Cell wall metabolism during maturation, ripening and senescence of peach fruit. J. Exp. Bot. 55, 2029-2039.

CADOT, Y., CHEVALIER, M., MINANA CASTELLO, M.T. and MOUTOUNET, M. 2008. Le potentiel phénolique du Cabernet Franc. INRA Angers, France.

CONDE, C., SILVA, P., FONTES, N., DIAS, A.C,P., TAVARES, R.M., SOUSA, M.J., AGASSE, A., DELROT, S. and GEROS, H. 2007. Biochemical throughout grape berry development and fruit and wine quality. Food 1, 1-22.

COOMBE, B.G., BOVIO, M. and SCHNEIDER, A. 1987. Solute accumulation by grape pericarp cells. V. Relationship to berry size and the effects of defoliation. J. Exp. Bot. 38, 1789-1798.

COOMBE, B.G. 1992. Research on development and ripening of the grape berry. Am. J. Enol. Vitic. 43, 101-110.

DENG, Y., WU, Y. and LI, Y. 2005. Effects of high O_2 levels on post-harvest quality and shelf life of table grapes during long-term storage. Eur. Food. Res. Technol. 221, 392-397.

DEYTIEUX-BELLEAU, C., VAILLET, A., DONECHE, B. and GENY, L. 2008. Pectin methylesterase and polygalactorunase in the developing grape skin. Plant Physiol. Biochem. 46, 638-646.

DI STEFANO, R. and CRAVERO, M.C. 1991. Metodi per lo studio dei polifenoli dell'uva. Riv. Vitic. Enol. 44, 37-45.

FOURNAND, D., VINCENS, A., SIDHOUM, L., SOUQUET, J.M., MOUTOUNET, M. and CHEYNIER, V. 2006. Accumulation and extractability of grape skin tannins and anthocyanins at different advanced physiological stages. J. Agric. Food Chem. 54, 7331-7338.

GOULAO, L.F. and OLIVEIRA, C.M. 2008. Cell wall modifications during fruit ripening: when a fruit is not the fruit. Trends Food Sci. Technol. 19, 4-25.

HERTOG, M.L.A.T.M., BEN-ARIE, R., ROTH, E. and NICOLAI, B.M. 2004. Humidity and temperature effects on invasive and non-invasive measures. Postharvest Biol. Technol. 33, 79-91.

KHOJASTEHNAZHAND, M., OMID, M. and TABATABAEEFAR, A. 2010. Determination of Tangerine Volume Using Image Processing Methods. Int. J. Food Prop. 13, 760-770.

KONTOUDAKIS, N., ESTERUELAS, M., FORT, F., CANALS, J.M., DE FREITAS, V. and ZAMORA, F. 2011. Influence of the heterogeneity of grape phenolic maturity on wine composition and quality. Food Chem. 124, 767-774.

KUNZEK, H., KABBERT, R. and GLOYNA, R. 1999. Aspects of material science in food processing: changes in plant cell walls of fruits and vegetables. Z. Lebensm Unters Forsch A 208, 233-250.

LE MOIGNE, M., MAURY, C., BERTRAND, D. and JOURJON, F. 2008. Sensory and instrumental characterisation of Cabernet Franc grapes according to ripening stages and growing location. Food Qual. Pref. 19, 220-331.

LEE, C.Y. and BOURNE, M.C. Changes in grape firmness during maturation. 1980. J. Texture Studies 11, 163-171.

LETAIEF, H., ROLLE, L., ZEPPA, G. and GERBI, V. 2008a. Assessment of the grape skin hardness by a puncture test. J. Sci. Food. Agric. 88, 1567-1575.

LETAIEF, H., ROLLE, L. and GERBI, V. 2008b. Mechanical behaviour of winegrapes under compression tests. Am. J. Enol. Vitic. 59, 323-329.

MAURY, C., MADIETA, E., LE MOIGNE, M., MEHINAGIC, E., SIRET, R. and JOURJON, F. 2009. Development of a mechanical texture test to evaluate the ripening process of Cabernet Franc. J. Texture Studies 40, 511-535.

O'MAHONY, M. 1986. Sensory Evaluation of Food: Statistical Methods and Procedure. Marcel Dekker, New York, U.S.A.

ORTEGA-REGULES, A., ROS-GARCIA, J.M., BAUTISTA-ORTIN, A.B., LOPEZ-ROCA, J.M. and GOMEZ-PLAZA, E. 2008. Changes in skin cell wall composition during the maturation of four premium wine grape varieties. J. Sci. Food Agric. 88, 420-428.

RÍO SEGADE, S., GIACOSA, S., GERBI, V. and ROLLE, L. 2011a. Berry skin thickness as main texture parameter to predict anthocyanin extractability in winegrapes. LWT – Food Sci. Technol. 44, 392-398.

RÍO SEGADE, S., ORRIOLS, I., GIACOSA, S. and ROLLE, L. 2011b. Instrumental texture analysis parameters as winegrapes varietal markers and ripeness predictors. Int. J. Food Prop. 14, 1318-1329.

ROLLE, L., SIRET, R., RÍO SEGADE, S., MAURY, C., GERBI, V. and JOURJON, F. 2012. Instrumental texture analysis parameters as markers of table-grape and winegrape quality: a review. Am. J. Enol. Vitic. 63, 11-28.

ROLLE, L., TORCHIO, F., ZEPPA, G. and GERBI, V. 2009. Relationship between skin break force and anthocyanin extractability at different ripening stages. Am. J. Enol. Vitic. 60, 93-97.

ROUDOT, A.C. 2006. Some considerations for a theory of plant tissue mechanics. Sci. Aliment 20, 409-426.

TORCHIO, F., CAGNASSO, E., GERBI, V. and ROLLE, L. 2010. Mechanical properties, phenolic composition and extractability indices of Barbera grapes of different solids contents from several growing areas. Anal. Chim. Acta 660, 183-189.

VINSONNEAU, E. and ANNERAUD, C. 2008. Estimation de la maturité : des outils simples de mise en œuvre. Matevi 36, 1-11.

ZOUID, I., SIRET, R., MEHINAGIC, E., MAURY, C., CHEVALIER, M. and JOURJON, F. 2010. Evolution of grape berries during ripening: Investigations into the links between their mechanical properties and the extractability of their skin anthocyanins. J. Int. Sci. Vigne Vin 44, 87-90.

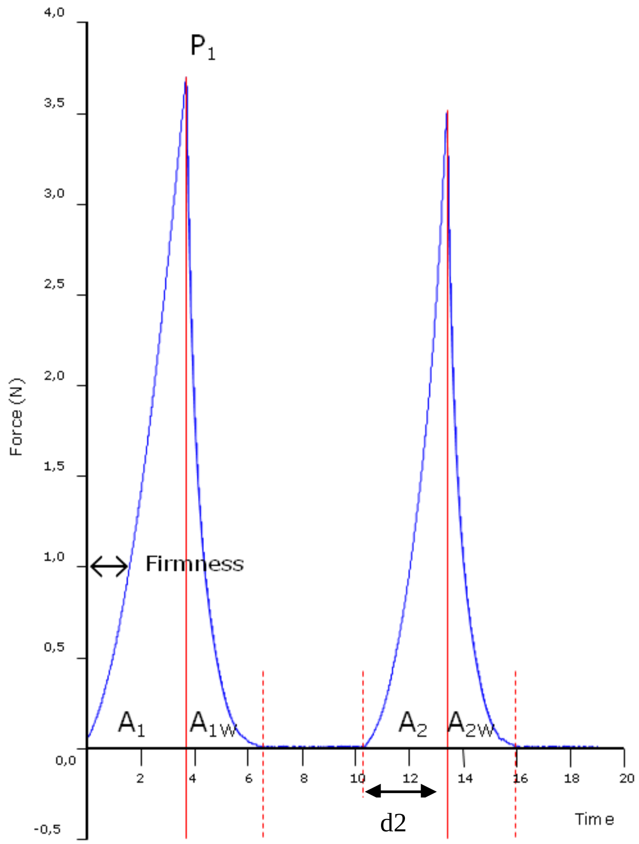

FIG. 1. TYPICAL FORCE-TIME (DEFORMATION) CURVES CORRESPONDING TO DOUBLE COMPRESSION TEST
A_1, A_{1w}, A_2, A_{2w}: areas under compression and withdrawal portions of the first bite and the second bite curve; P_1: peak of first compression; d2: crosshead travel corresponding to second compression.

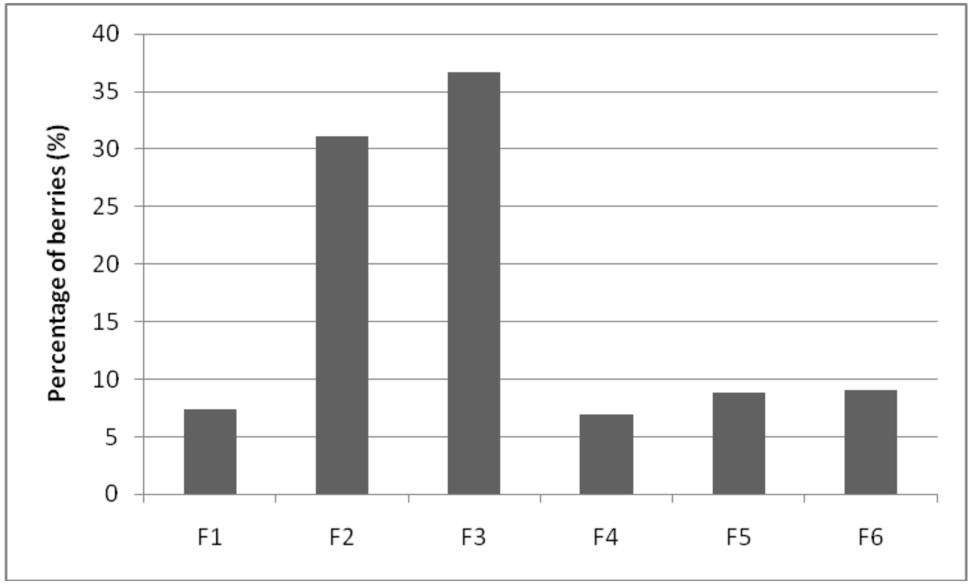

FIG. 2. DISTRIBUTION PERCENTAGE OF CABERNET FRANC GRAPE BERRIES IN DIFFERENT DENSITY CLASSES AT HARVEST

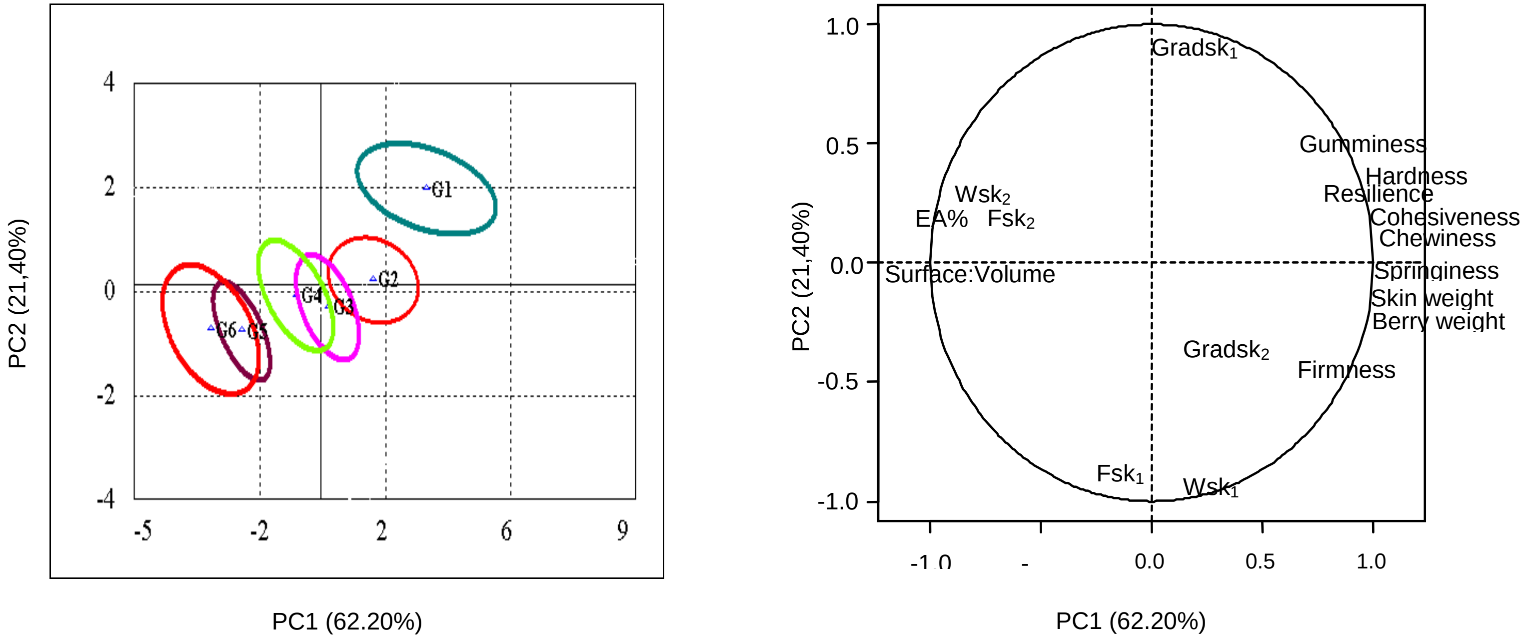

FIG. 3. (A): PCA MAP OF THE 6 GROUPS OF SUGAR LEVELS CONTENT OF GRAPES IN THE PLANE DEFINED BY PRINCIPAL COMPONENTS 1 AND 2. G1 TO G6 ARE THE CONFIDENCE ELLIPSES FOR THE GROUPS OF GRAPES F1 TO F6 RESPECTIVELY. (B): PCA CORRELATION PLOT OF THE PHYSICAL AND MECHANICAL PARAMETERS AND THE EXTRACTION YIELD OF ANTHOCYANINS PARAMETERS IN THE PLANE DEFINED BY PRINCIPAL COMPONENTS 1 AND 2

TABLE 1. SALT SOLUTIONS CONCENTRATION USED FOR GRAPE FLOTATION AND THE EQUIVALENCE IN SUGAR OF THE CLASSES STUDIED

Code	**NaCl concentration in saline solutions (gL^{-1})**	**Sugar equivalent (gL^{-1})**
F1	130	176.5
F2	140	192.6
F3	150	209.3
F4	160	225.0
F5	170	242.1
F6	180	258.5

TABLE 2. PHYSICAL CHARACTERISTICS OF CABERNET FRANC GRAPES WITH DIFFERENT TOTAL SOLUBLE SUGAR CONTENT

	Berry weight (g)		Skin weight (g)		Surface area (mm^2)		Volume (mm^3)		Surface:Volume	
Signif[β]	***		***		***		***		***	
code	**Mean[α]**	**SD**	**Mean[α]**	**SD**	**Mean[α]**	**SD**	**Mean[α]**	**SD**	**Mean[α]**	**SD**
F1	1.88[d]	0.42	0.24[c]	0.05	589.83[d]	93.05	1358.03[d]	323.44	0.44[a]	0.03
F2	1.52[c]	0.29	0.19[b]	0.04	450.42[c]	63.66	904.67[c]	196.99	0.50[b]	0.03
F3	1.27[b]	0.15	0.16[b]	0.03	376.32[b]	33.18	688.03[b]	90.58	0.55[c]	0.02
F4	1.11[b]	0.19	0.17[b]	0.03	362.73[b]	46.64	652.54[b]	125.93	0.56[c]	0.04
F5	0.91[a]	0.09	0.13[a]	0.02	297.26[a]	19.90	482.36[a]	48.35	0.62[d]	0.02
F6	0.83[a]	0.11	0.12[a]	0.04	259.80[a]	38.13	395.93[a]	87.98	0.67[e]	0.05

All measurements are recorded as means (SD: standard deviation. n=15).

[α] For each column any two values not followed by the same letter were significantly different following Newman-Keuls test at p<0,05.

[β] *** indicates significance at p< 0,001.

TABLE 3. COMPRESSION PARAMETERS OF THE WHOLE BERRIES OF CABERNET FRANC GRAPES WITH DIFFERENT TOTAL SOLUBLE SUGAR CONTENT

	Firmness (mm)		**Hardness (N)**		**Cohesiveness (-)**		**Gumminess (N)**		**Springiness (mm)**		**Chewiness (mJ)**		**Resilience (-)**	
Signif$^{\beta}$	ns		***		**		***		***		***		***	
code	**Mean$^{\alpha}$**	**SD**	**Mean$^{\alpha}$**	**SD**	**Mean$^{\alpha}$**	**SD**	**Mean$^{\alpha}$**	**SD**	**Mean$^{\alpha}$**	**SD**	**Mean$^{\alpha}$**	**SD**	**Mean$^{\alpha}$**	**SD**
F1	1.63^{a}	0.14	2.23^{b}	0.50	0.73^{ab}	0.05	1.62^{b}	0.38	2.07^{d}	0.29	3.43^{c}	1.29	0.38^{ab}	0.03
F2	1.52^{a}	0.11	2.19^{b}	0.27	0.76^{b}	0.06	1.66^{b}	0.23	2.03^{d}	0.17	3.41^{c}	0.71	0.40^{b}	0.04
F3	1.51^{a}	0.22	2.13^{ab}	0.47	0.75^{ab}	0.03	1.59^{b}	0.34	1.84^{c}	0.18	2.98^{c}	0.86	0.40^{b}	0.03
F4	1.47^{a}	0.24	2.05^{ab}	0.57	0.72^{ab}	0.07	1.48^{ab}	0.46	1.73^{bc}	0.34	2.71^{bc}	1.20	0.37^{ab}	0.05
F5	1.53^{a}	0.18	1.75^{a}	0.39	0.72^{ab}	0.05	1.27^{a}	0.31	1.55^{ab}	0.17	2.01^{ab}	0.70	0.37^{ab}	0.04
F6	1.46^{a}	0.15	1.71^{a}	0.30	0.70^{a}	0.06	1.20^{a}	0.25	1.39^{a}	0.21	1.71^{a}	0.57	0.35^{a}	0.04

All measurements are recorded as means (SD: standard deviation. n = 15).

$^{\alpha}$ For each column any two values not followed by the same letter were significantly different following Newman-Keuls test at p<0.05.

$^{\beta}$ ***, ** and ns indicate significance at p< 0.001; p<0.01; and not significant, respectively.

TABLE 4. PUNCTURE PARAMETERS OF THE SKIN OF CABERNET FRANC GRAPES WITH DIFFERENT TOTAL SOLUBLE SUGAR CONTENT

	Fsk_1 (N)		Wsk_1 (mJ)		$Gradsk_1$ (Nmm^{-1})		Fsk_2 (N)		Wsk_2 (mJ)		$Gradsk_2$ (Nmm^{-1})	
Signif$^{\beta}$	**ns**		**ns**		**ns**		**ns**		**ns**		*****	
code	**Mean$^{\alpha}$**	**SD**	**Mean$^{\alpha}$**	**SD**	**Mean$^{\alpha}$**	**SD**	**Mean$^{\alpha}$**	**SD**	**Mean$^{\alpha}$**	**SD**	**Mean$^{\alpha}$**	**SD**
F1	0.56^{a}	0.18	0.63^{a}	0.22	0.07^{a}	0.21	0.37^{a}	0.11	0.29^{a}	0.19	0.24^{ab}	0.05
F2	0.55^{a}	0.11	0.56^{a}	0.16	0.03^{a}	0.23	0.43^{a}	0.12	0.35^{a}	0.16	0.24^{ab}	0.05
F3	0.53^{a}	0.06	0.49^{a}	0.10	0.04^{a}	0.25	0.39^{a}	0.14	0.28^{a}	0.17	0.27^{b}	0.08
F4	0.50^{a}	0.15	0.43^{a}	0.18	0.06^{a}	0.26	0.46^{a}	0.21	0.45^{a}	0.24	0.19^{a}	0.08
F5	0.57^{a}	0.15	0.56^{a}	0.19	0.04^{a}	0.21	0.50^{a}	0.16	0.42^{a}	0.20	0.27^{b}	0.07
F6	0.60^{a}	0.21	0.57^{a}	0.22	0.07^{a}	0.22	0.45^{a}	0.17	0.41^{a}	0.26	0.23^{ab}	0.06

All measurements are recorded as means (SD: standard deviation. n = 15).

$^{\alpha}$ For each column any two values not followed by the same letter were significantly different following Newman-Keuls test at p<0,05.

$^{\beta}$ * and ns indicate significance at p< 0,05; and not significant, respectively.

TABLE 5. ANTHOCYANIN EXTRACTABILITY OF CABERNET FRANC GRAPES WITH DIFFERENT TOTAL SOLUBLE SUGAR CONTENT

	TA_{A1} (mg/g)		TA_{A2} (mg/g)		EA%	
Signif[β]	***		***		***	
code	**Mean**[α]	**SD**	**Mean**[α]	**SD**	**Mean**[α]	**SD**
F1	0.54[a]	0.17	0.05[a]	0.01	91.07[a]	1.56
F2	0.78[a]	0.22	0.06[ab]	0.02	92.64[b]	2.01
F3	1.09[b]	0.20	0.08[b]	0.02	93.23[bc]	1.18
F4	1.46[c]	0.28	0.10[c]	0.02	93.39[bc]	1.25
F5	1.91[d]	0.35	0.11[c]	0.03	94.62[c]	1.16
F6	2.29[e]	0.70	0.13[d]	0.05	94.62[c]	1.18

All measurements are recorded as means (SD: standard deviation. n=15).

[α] For each column any two values not followed by the same letter were significantly different following Newman-Keuls test at $p<0.05$.

[β] *** indicate significance at $p< 0.001$.

TABLE 6. PEARSON'S CORRELATION COEFFICIENTS (R) BETWEEN PHYSICAL, COMPRESSION AND PUNCTURE PARAMETERS AND THE EXTRACTION YIELD OF ANTHOCYANINS

	EA%	**Berry weight**	**Skin weight**	**Surface**	**Volume**	**Surface:Volume**
EA%	-	**-0.99**	**-0.99**	**-0.99**	**-0.98**	**0.97**
Fsk_1	0.23	-0.16	-0.25	-0.17	-0.12	0.34
Wsk_1	-0.30	0.39	0.30	0.39	0.43	-0.23
$Gradsk_1$	0.05	-0.15	-0.08	-0.17	-0.21	0.03
Fsk_2	**0.83**	**-0.81**	-0.74	-0.77	-0.77	0.74
Wsk_2	0.68	-0.72	-0.58	-0.66	-0.66	0.65
$Gradsk_2$	0.07	0.05	-0.14	-0.01	-0.02	-0.01
Firmness	-0.78	**0.82**	0.79	**0.85**	**0.87**	-0.79
Hardness	**-0.90**	**0.89**	**0.87**	**0.86**	**0.83**	**-0.93**
Cohesiveness	-0.45	0.54	0.41	0.46	0.41	-0.61
Gumminess	**-0.84**	**0.86**	**0.81**	**0.81**	0.77	**-0.90**
Springiness	**-0.92**	**0.95**	**0.91**	**0.92**	**0.89**	**-0.97**
Chewiness	**-0.91**	**0.92**	**0.89**	**0.89**	**0.86**	**-0.96**
Resilience	-0.54	0.62	0.49	0.54	0.49	-0.67