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Mechanical tests and definition of new indexes of grape berry firmness. Evolution of berry skin hardness during alcoholic fermentation

P. ABBAL¹⁾, A. VERNHET²⁾, E. ABI-HABIB²⁾, S. CARRILLO¹⁾, M. A. DUCASSE³⁾ and C. PONCET-LEGRAND¹⁾

¹⁾ INRAE, UMR SPO, Montpellier, France

²⁾ L'Institut Agro-Montpellier SupAgro, Montpellier, France

³⁾ IFV, UE Pech Rouge, Gruissan, France

Summary

The mechanical strength or firmness of a fruit is considered an important parameter to characterise its state of ripeness or conservation, as well as other parameters such as sugar level or color. The mechanical hardness of grapes influences the integrity and sanitary quality of the harvest. In this study, the mechanical characteristics of grapevine berries were studied at harvest time in order to determine their rheological properties (firmness and hardness of the berry skin) during alcoholic fermentation. Special indexes were defined measuring the energy needed to crush the berries to 50 % of their initial diameter, and applied successively to two different varieties. The entire berry firmness and the skin hardness were both different.

Mechanical indexes linked to grape firmness were defined. Using these indexes, a significant effect on the firmness behavior due to variety was recorded: the skin of 'Grenache Noir' was found firmer and harder than 'Carignan Noir'. Furthermore, during the alcoholic fermentation, no change in skin hardness was observed for both varieties, despite changes in the composition of the must. These results give new information on mechanical properties of berries and could be used as an aid in the winemaking process. Indeed, they would probably help the winemaker to better choose the type of fermentation and maceration adapted to his grapes according to the type of wine he wishes to produce.

Key words: berry skin hardness; ABBAL indexes; fermentation; rheology; Penelaup rheometer.

Introduction

Softening is an important physiological change during the onset of ripening in fruits (ROBIN *et al.* 1997, CASTELLARIN *et al.* 2016). Thus, firmness is an essential quality parameter for wine grapes (*Vitis vinifera*) but also for table grapes (BALIC *et al.* 2014). The relationship between major events during softening in grapes (*Vitis vinifera* L.) have previously been investigated by quantifying elasticity in individual berries. On analysing the results provided by

the Penelaup rheometer, it is possible to discriminate varietal, vintage and even environmental effects (AGERON *et al.* 1995, ROBIN *et al.* 1997).

It was found that ripening processes commenced always at similarly low elasticity and turgor. Much of the softening occurred without the presence of other changes in berry physiology that have been investigated here. There was no decrease in solute potential, increase in sugar concentration, or color development until elasticity and turgor were near minimum values, and these processes were inhibited when berry growth was prevented (CASTELLARIN *et al.* 2016). During the onset of ripening, softening and increase in abscisic acid were some of the earliest events observed.

If we take into account the hardness of the grape skin, then changes in pectins and hemicelluloses in primary cell wall polysaccharides and alterations in the interactions of these polymers have been proposed as being the primary causes for texture changes that result in a decrease of firmness during the ripening of different fruit (BRUMMELL 2006). Changes in the homogalacturonan (HG) fraction associated with enzyme activity have been directly implicated in the softening of several fruits, including grapes (ROE and BRUEMMER 1981, PAULL and CHEN 1983, EL-ZOGHBI 1994, CABANNE and DONÈCHE 2001, QUESADA *et al.* 2009). Polygalacturonase (PG), which catalyzes the hydrolysis of galacturonic acids, has been associated with berry softening in *V. vinifera* 'Sauvignon blanc' (CABANNE and DONÈCHE 2001). Pectin hydrolysis has been associated with the degree of unesterified HG, which is catalyzed by pectin methyl esterase (PME, EC 3.1.1.11) (BONNIN *et al.* 2002). Earlier, the activities of PG and PME were considered as interconnected, since a decrease in HG methyl-esterification caused by PME generates accessible hydrolysis sites for PG and sites for Ca²⁺-bridge formation (FISCHER and BENNETT 1991).

After ripening, and during wine processing, polyphenols, among other molecules, are extracted from the grape skin. During fermentation, several parameters are modified in the must/wine matrix: the ethanol concentration increases as the sugar concentration decreases, but very little is known about the parietal structures during the winemaking. Skin hardness has already been studied during maceration for both 'Shiraz' and 'Nebbiolo' berries and seemed

Correspondence to: Dr. P. ABBAL, INRAE, UMR SPO, 2, Place Viala, 34060 Montpellier Cedex, France. E-mail: philabbal@gmail.com

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to be well correlated with the extractability respectively of anthocyanins for the first (ROLLE *et al.* 2009, GIACOSA *et al.* 2015) and the second (ROLLE *et al.* 2012) variety. The aim of this study was to verify this hypothesis for other varieties, investigate possible changes in the mechanical behavior of grape skins during fermentation and assess whether they can be linked to possible structural changes.

Material and Methods

Grape varieties: The study used 'Grenache Noir' and 'Carignan Noir' grape varieties. They were harvested on the experimental unit of INRA Pech Rouge (Gruissan, France) on August 22nd and September 5th 2018. The berries of the same cultivar were sorted according to their natural heterogeneity, on the basis of size (volume: V) and density (degree of maturity: D). Variability was determined the day before harvest by measuring the diameter of 1,000 berries of each variety and estimating their density by flotation in different saline solutions, corresponding to a total difference in soluble solids between two successive baths of 17 g·L⁻¹ (1 % vol. potential alcohol). The berries were separated from the stalk by cutting them at the pedicel and were sorted, firstly according to their size using a grading machine (V-, V+) and then according to their density (D-, D+) using an aqueous solution of concentrated rectified grape must of the adequate density. Thus, four different modalities or combinations for each variety were obtained (V-D-, V-D+, V+D- and V+D+).

The same experiments were carried out in 2019 (harvests on August 21st and September 3rd), but berries were sorted only on the basis of their density (results shown in suppl. data).

Fermentations: Fermentations were carried out at 21 °C in a thermostatically controlled chamber, in low volume tanks (< 1 kg) using "French Press" coffee plungers with similar and standard conditions. 1 kg of berries were crushed and placed in the plunger. Lalvin ICV OKAY yeast (20 g·hL⁻¹) and SO₂ (250 µL of a 8 % solution) were added simultaneously. Cap management was carried out daily during alcoholic fermentation (AF) by submerging the pomace with the plunger. The decrease in sugar concentration was monitored by measuring Brix degree and density (Easydens, Anton Paar). Fermentations were considered finished when the density remained stable (7 to 8 d) with values below 995 g·L⁻¹. At the end of AF the classical chemical parameters of the wine (pH, % vol, glucose/fructose and volatile acidity) were determined.

Total pigments (TP) and total polyphenol Index (TPI) were measured as absorbency at 520 nm and 280 nm after a 100-fold dilution in 1 M hydrochloric acid, measured in a 10 mm cuvette at the beginning (T0) and at the end (T3) of AF. A UV-visible spectrophotometer was used with three replicates at each time of estimation (T).

Skin hardness measurements were carried out at the beginning (T0) and at the end (T3) of AF plus at two points in between, corresponding to approximately half (T1) and ¾ (T2) of the fermentation process.

Physical measurements on berries: There are two different types of devices for assessing grape firmness: portable devices that provide field measurements and more accurate and sophisticated laboratory tools.

The device used to collect the measurements for this trial, the Penelaup robot (trade mark), was developed by INRA and CTIFL (Abbal and Planton, 1990). This device makes it possible to automatically determine the physical characteristics such as the dimensions and/or the firmness of a product. It consists of a support, a measuring rod whose end is designed to support a part intended to come into contact with the sample (Fig. 1). This tool is either a

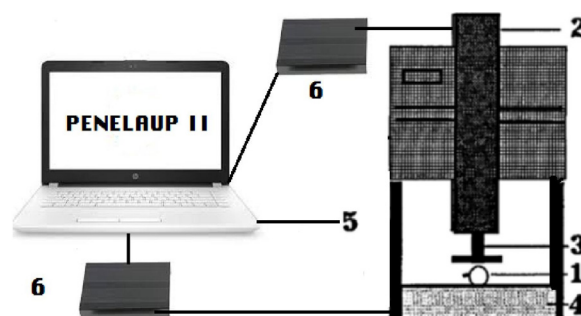


Fig. 1: Scheme of the Penelaup rheometer (patented and licensed to Serisud company, serisud@yahoo.fr). 1. berry; 2. column and servo screw moving system; 3. mandrel and crushing tool; 4. precision sensor; 5. computer; 6. electronic control device.

flat tool able to measure the berry firmness or a needle-like tool able to measure the berry skin hardness (Penelaup II).

The measuring rod is mounted in translation relative to a worm extending parallel to the measuring rod, which is driven in rotation by a high-precision stepper motor. The sample is placed on a measuring sensor support and set up perpendicular to the measuring rod. The system control consists of a computer (PC Windows 10tm) connected to several microcontroller modules specifically developed for executing commands and processing analog signals.

For each measurement the robot supplies the mass, the diameter as well as the stress curve (expressed in Newton) of the product as a function of the user-selected % of crushing. Given the very high accuracy of this device, the user can work in the superficial areas of the berry (a few %) or otherwise seek to the bursting of the berry. The recorded measurement curve can be directly interfaced to Microsoft Excel (suppl. data S1). The new version of the robot has a second specific device to study the skin of the berry, and measures its hardness by penetrometry.

In the present research we were able to define and calculate specific indexes to quantify the firmness of berries and hardness of their skin. These indexes could be used for other fruits like apples, cherries, or tomatoes to study firmness and skin hardness with the same methodology as for grapes. Consider the following variables:

- p: percentage of desired deformation
- D: berry diameter (mm)
- M: fruit weight (g)
- x: the displacement of the tool (mm)
- f(x): force (Newton) applied at location x

Processing the test, the deformation curve of the grape has the equation $y = f(x)$, x going from the contact of the tool with the grape up to the location programmed by the operator. The integration of $f(x)$ gives a graphical representation of the total energy A (joule) of the process. A is the sum of the force $f(x)$ multiplied by the displacement x of the tool for every position x .

$$A = \int_a^b f(x)dx$$

A is the energy, represented by the area under the curve $f(x)$. If $a = 0$ (initial contact of the tool with the grape) and $b = D * p$. (b is the final location of the tool: p is the percentage of the grape diameter D , programmed by the operator)

$$AF1(p) = A = \int_0^{D*p} f(x)dx \quad (1)$$

$AF1(p)$ is called the first Abbal index (joule). It is an index of absolute firmness for p % deformation of the initial diameter D of the fruit. Using equation (1) if we calculate:

$$A/M = \frac{\int_a^b f(x)dx}{M}$$

with $a = 0$ and $b = D * p$ we can define:

$$AF2(p) = \frac{\int_0^{D*p} f(x)dx}{M} \quad (2)$$

$AF2(p)$ is called the second Abbal index ($\text{joule} \cdot \text{kg}^{-1}$). $AF2(p)$ takes into account fruit weight for a p % deformation of the initial berry diameter (D).

For penetrometry, the test consists of measuring the energy needed to push a needle through a berry skin for a displacement of $L = 10$ mm.

Unlike previous tests, penetrometry test does not take into account the diameter or the weight of the grape. So we defined $AP(x=10) = dx$

$$AP(x = 10) = \int_0^{x=10} f(x)dx \quad (3)$$

$AP(x = 10)$ is called the third Abbal index (joule). The needle is 35 mm long and 2,5 mm thick and the bottom has a V shape (Fig. 2).

In the experiments described below, we used $AF1(p)$ index with $x = 10$ mm for berry skins (penetrometry) and $AF2$ with $p = 50$ % for the entire berries (firmness).

To illustrate the use of these indexes, the two grapevine varieties described above, 'Grenache Noir' and 'Carignan Noir', were used. The firmness of the whole berry was measured at harvest time using a sample of 50 berries for each variety. At the same time, always for each separate variety, samples composed of 20 randomly chosen berries

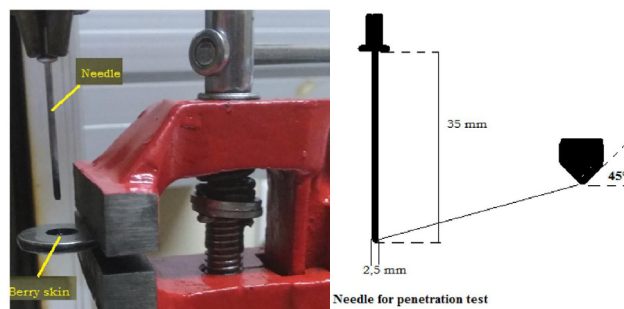


Fig. 2: Details of the device and of the needle used.

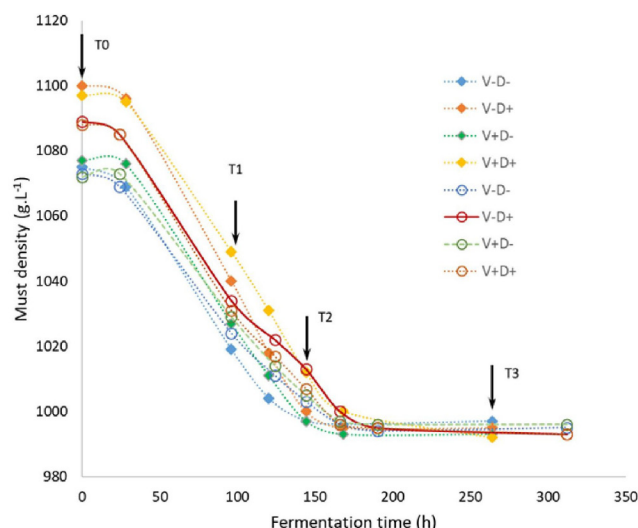


Fig. 3: Comparison of fermentation of the 'Grenache' (diamonds) versus 'Carignan' grapes (circles) in the french press coffee plunger. All the fermentations were considered finished after 200 h. The arrows (T0, T1, T2, T3) indicate the skin hardness measurement times.

were prepared for skin hardness assessments. Skin hardness measurements were carried out at the beginning (T0) and at the end of AF (T3) plus at two intervals (T1, T2) in between using samples previously prepared for each estimation (Fig. 3).

Statistical analysis: The data are expressed as the average of twenty independent measurements for mechanical properties, three independent measurements for TPI, TP, wine density on each fermentation triplicate. ANalysis Of VAriance (ANOVA) was performed using Minitab17 Statistical Software. The difference between the mean values was determined using the Tukey and Dunnett test, and significant results were considered at $p < 0.05$.

Results

Fermentations were monitored by measuring Brix and density values (Fig. 3), with three replicates for each estimation time. As expected, the fermentation curves of the most mature grapes V-D+ and V+D+ were similar, as were those of the less mature ones (V-D- and V+D-). All fermentations were considered finished after 200 hours, with those of D- grapes being slightly shorter than the other combinations.

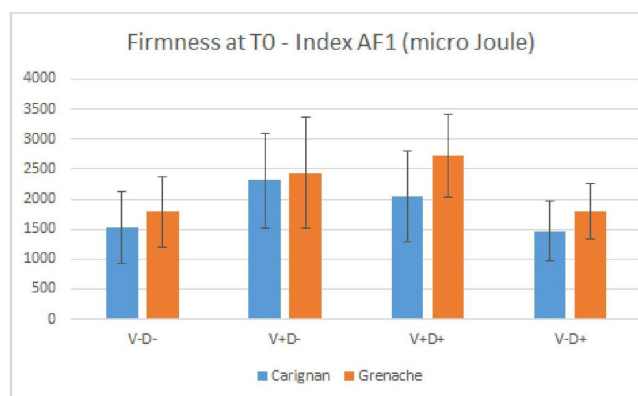


Fig. 4: Firmness of 'Carignan' versus 'Grenache' calculated with AF1 index (micro joule) with $p = 50$ % of berry diameter.

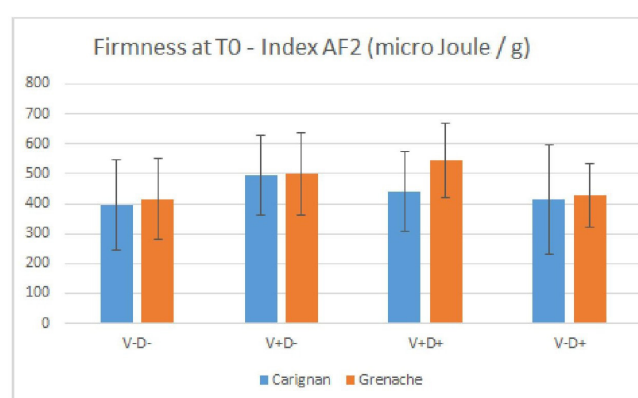


Fig. 5: Firmness of 'Carignan' versus 'Grenache' calculated with AF2 index (micro joule by gram) with $p = 50$ % of berry diameter.

At T0, firmness was measured with the Penelaup robot on 20 berries of each modality, for both varieties. Indexes AF1 and AF2 were calculated as previously described, and presented in Figs 4 and 5. Surprisingly, no clear relationship was observed between concentration of sugar and berry firmness: in particular, values of firmness of D- berries were not higher than D+, due to the fact that berry volume played a more important role with respect to sugar accumulation. It should be noted that these experiments were previously conducted in 2017 and later in 2019 leading to the same conclusions (suppl. data). Based on the results obtained, once AF1 was divided by berry weight to calculate AF2, the differences decreased.

The results regarding the evolution of skin hardness during AF in relation to combinations (modalities, in which 20 berries/combination at each time of estimation were measured) are presented in Fig. 6. As the differences between the modalities are insignificant compared to those related to varietal effect, we decided to group all modalities and therefore consider the average of 80 measurements. Differences between varieties were much higher than those observed throughout the process of fermentation: in fact, skin hardness was not affected by the fermentation process, showing similar rheological performances. Furthermore, on visual and tactile examination, the skins did not appear to be damaged.

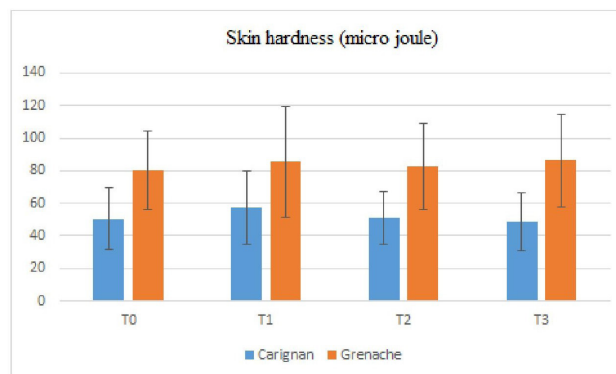


Fig. 6: Comparison of skin hardness during fermentation in relation to variety with AF1 index (micro joule).

Discussion

The use of the previously defined firmness indexes, combined with the use of a high-precision robot that calculates them automatically, showed that the differences recorded are firstly related to grape variety, 'Grenache Noir' and 'Carignan Noir'. It should be noted that we worked with ripe berries that were sorted according to their potential level of alcohol, which ranged between 10 and 16 %. Our results are similar to those reported by some authors (ROBIN *et al.* 1997), who observed a sharp decrease in firmness at ripening, with a behaviour characterized by stable values during the three weeks prior to harvest and one week after. Other authors (ROLLE *et al.* 2009) observed the same behavior on another grapevine variety ('Brachetto'), at two ripening stages, and noted a high variability of skin break force values.

Concerning skin hardness during fermentation, until now it was common to think that the skins of fermenting grapes deteriorated according to the length of maceration time and the simultaneous increase in alcohol content, but also due to enzymatic activities which could modify the structure of pectic polysaccharides. These phenomena would enable the diffusion of phenolics through the skins. A recent work (RIO SEGADE *et al.* 2015) showed that the addition of enzymes increases the extraction of anthocyanins and decreases the mechanical properties of the skins after 192 h of maceration, for Cabernet Sauvignon and 'Nebbiolo'. A study done with the 'Shiraz' variety (GIACOSA *et al.* 2015) showed that intact berries and macerated skins had similar values of skin break energy in a range of 60 µJ, but also noted that samples of macerated skins were very heterogeneous.

Our results, obtained from skin hardness measurements performed throughout the fermentation process but without the addition of pectolytic enzymes, show that the mechanical properties of the skins do not evolve significantly during fermentation, pointing out that the greatest variability is due to the varietal effect. In fact, the main factor of difference recorded in mechanical properties of berry skins is related to variety, with respective values of 50 µJ for 'Carignan' and 80 µJ for 'Grenache'. We also

found a high degree of heterogeneity and therefore carried out 80 measurements for each maceration time. These experiments were also carried out in 2019 and led to the same results (suppl. data S2-S4).

Studies carried out in parallel in our group, focusing on how phenolics diffuse through the skins to the must during fermentation, showed that the main differences in composition between these two varieties are related to their composition in anthocyanins (ABI HABIB *et al.* 2020),

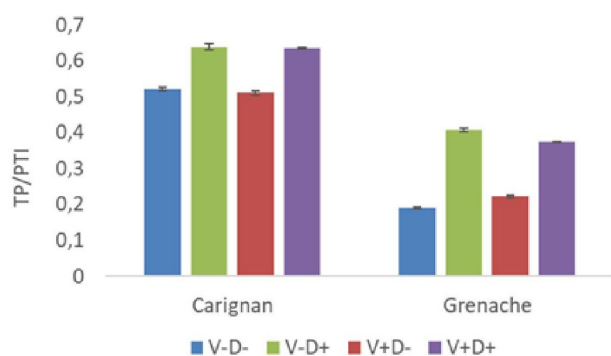


Fig. 7: Ratio total pigments / total polyphenol index for 'Grenache' and 'Carignan' wines.

'Carignan' being roughly 4 times richer in anthocyanins than 'Grenache'. These results have been observed when measuring total pigments/total polyphenol index on the final wines (Fig. 7), with 'Carignan' wines having the highest values of absorbance at 520 nm. However, it should be reminded that other parameters may play an important role in final content of wine, such as the polysaccharide and protein composition of insoluble cell material (ABI HABIB *et al.* 2020).

These two varieties were chosen because their phenolic composition and their assumed skin hardness are very different. Indeed, they produced wines with different phenolic compositions. There seems to be a correlation between phenolic composition, skin hardness and final wine composition. As the causality is not clear, further work is needed to understand whether 'Carignan' gives wines richer in anthocyanins because its grapes are richer in anthocyanins than 'Grenache', or because their skin is softer and easier to extract compared to 'Grenache'. Indeed, the percentage of total polyphenols extracted in relation to what is present in the skins varies from 16 to 20 % for 'Carignan' and from 30 to 45 % for 'Grenache' according to recently proposed methods (ABI-HABIB, unpubl. data 2020). This result may not be consistent with the conclusions of some authors (ROLLE *et al.* 2009 and 2012). Indeed, they reported that hard skins seem to be characterized by an increased fragility of the cell walls, which allows an easier release of colored pigments for the same variety. However, the softening of skins induced by enzymatic treatments led to increased extractability of anthocyanins (RÍO SEGADÉ *et al.* 2015). Regarding the role of sugar, our experimental research center performed some diffusion experiments by putting "unripe" skins in model solutions with as much sugar as if they were ripe. The results obtained showed that

slightly more anthocyanins were extracted, especially at the beginning of the fermentation period, when ethanol is not present.

Conclusion

Mechanical tests carried out for several years on 'Grenache' and 'Carignan' using previously defined indexes have shown that differences in skin hardness are significant depending on the variety. In the future, other tests will have to be carried out by studying other varieties in order to compare them using the proposed indexes. More precise analyses of polysaccharides and proteins are being carried out in an attempt to relate the composition of alcohol-insoluble material to skin hardness.

Current work has also shown that skin hardness is constant during alcoholic fermentation, which seemed unlikely at first glance. A new research objective would be to try to explain precisely why and what consequences these indications may have on the finished product, especially for phenolic compounds and the extractability of polyphenols.

Finally, the oenological consequences of these measures could be important for the winemaker: choice of harvest date or choice of a specific maceration technique depending on the hardness of the berry skin. This is already evident in the case of carbonic maceration.

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