EVOLUTION OF GRAPE BERRIES DURING RIPENING: INVESTIGATIONS INTO THE LINKS BETWEEN THEIR MECHANICAL PROPERTIES AND THE EXTRACTABILITY OF THEIR SKIN ANTHOCYANINS

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Abstract

Aims: The aim of this work was to study the evolution of grape berries during ripening and investigate the possible relationship between the extractability of anthocyanins from grapes and their rheological properties.

Methods and results: Cabernet Franc grapes belonging to three different vineyards were harvested weekly from 10 September to 8 October 2007. Their mechanical behavior was measured by compression and puncture tests and the evolution of anthocyanin extractability was monitored during ripening. Significant differences were found in grape textural attributes and anthocyanin content due to the ripening stage and type of vineyard. A multivariate regression model was built to explain the evolution of anthocyanin extractability, by using the mechanical attributes of grape berries as variables.

Conclusion: Our results show that differences in the easiness of anthocyanin extraction from grapes could be linked to differences in the mechanical behavior of berries and that the extraction yield of anthocyanins from grapes could be predicted by their rheological properties. To confirm this first hypothesis, further studies with a larger number of vintages and vineyards would be necessary to link the mechanical properties of grape berries, established at macroscopic scale, to the susceptibility of anthocyanin extraction from grape skin.

Significance and impact of the study: The need to understand the evolution of the mechanical behavior of winegrapes during ripening and its impact on the release of anthocyanins is important for wine quality control. Understanding the evolution of the material properties of grapes is essential for developing better approaches to improve grape quality and could help winemakers to choose the best time of harvest and the process best adapted to the wine quality desired.

Keywords: anthocyanins extractability, ripening, compression, puncture test, grapes

Résumé

Objectifs : L'objectif de ce travail est d'étudier l'évolution des propriétés des baies de raisin pendant la maturation et les relations entre l'extractibilité des anthocyanes et les propriétés rhéologiques des raisins.

Méthodes et résultats : L'évolution des propriétés mécaniques et de l'extractibilité des anthocyanes des baies de Cabernet Franc de trois différentes parcelles ont été étudiées du 10 septembre au 8 octobre 2007. Les propriétés mécaniques des baies ont été déterminées par des mesures de double compression et de pénétrométrie. L'évolution de l'extractibilité des anthocyanes en milieu hydroalcoolique au cours de la maturation est déterminée. Les résultats ont montré des différences significatives en termes de propriétés mécaniques et d'extractibilité des anthocyanes des baies de raisin pendant la maturation. Un modèle de régression linéaire multiple a été établi pour expliquer l'évolution de l'extractibilité des anthocyanes, en utilisant les propriétés mécaniques des baies de raisin comme variables.

Conclusion : Nos résultats montrent que le potentiel d'extraction des anthocyanes peut être lié et prédit par les propriétés mécaniques des baies de raisin. Pour confirmer ce premier résultat, il semble donc important de travailler sur un réseau de parcelles plus important pendant plusieurs millésimes.

Signification et impact de l'étude : Ce travail a mis en évidence les relations entre les propriétés mécaniques des baies et l'extractibilité des anthocyanes de la pellicule. Ces informations permettraient aux professionnels de la filière de mieux piloter leurs itinéraires de vinification pour un vin de meilleure qualité.

Mots clés : extractibilité des anthocyanes, compression de la maturation, mesure de pénétrométrie, raisins

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INTRODUCTION

Previous studies have described grape composition at the moment of harvesting as an important determinant of their quality (BAUTISTA-ORTN *et al*., 2006; FERNANDEZ-LOPEZ *et al*., 1998; SURESH and ETHIRAJ, 1987; CARROLL and MARCY, 1982; FLORA and LANE, 1979; PIRIE and MULLINS, 1977). PEREZ-MAGARINO and GONZALEZ-SAN (2006) found that the degree of grape maturity influences the color of wines. For red wine, its color is mainly due to anthocyanins (PEREZ-MAGARINO and GONZALEZ-SAN JOSE, 2004; CHEYNIER *et al*., 1994), which are located in the cells of the grape skins of red varieties (AMRANI JOUTEI and GLORIES, 1995a; AMRANI JOUTEI and GLORIES, 1995b), inside the vacuoles, in a free, non-complex form. Increasing the amount of anthocyanins would be an advantage to the wine-maker (BOSS and DAVIES, 2009) since they play a key role in the sensory properties especially the colour of wine. Their concentration in wine relies on the maturity of the grape and also on maceration techniques implemented during the vinification process. Each grape variety has a distinctive set of anthocyanins. *Vitis vinifera* usually produces 3-monoglucoside, 3-acetylglucoside and 3-p-coumaroylglucoside of the anthocyanidins delphinidin, cyanidin, peonidin, petunidin and malvidin. Controlling the extractability of anthocyanins from grapes is an important task as grapes rich in phenols do not necessarily produce wines rich in anthocyanins. In order to understand how these compounds are extracted, several authors (ORTEGA REGULES *et al*., 2008; FOURNAND *et al*., 2006; CANALS *et al*., 2005) have studied the extractability of grape pigments, *i.e.* the extent to which anthocyanins are transferred from grape skin to wine (ROMERO-CASCALES *et al*., 2005a). Thus several extractability assays have been proposed in order to reproduce a similar situation to that occurring during maceration and winemaking. The diffusion of anthocyanins during pomace contact depends on the tendency of the berry skin to yield them (RIO SEGADE *et al*., 2008; ORTEGA-REGULES *et al*., 2008). Skin permeability is linked to cell and thus tissue structures. One of the phenomena that permit the diffusion of phenol 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 grape skin (AMRANI JOUTEI and GLORIES, 1995b). In essence, the extractability and the facility with which anthocyanins are released are very tightly linked to cell wall disassembly, cell separation and therefore cell rupture, tissue deterioration and thus grape berry softening (PINELO *et al*., 2006). Also the release of cell content such as anthocyanins is dependent on the forces holding the cells together. If these forces are stronger than the cell walls, then the latter collapse; if they are weaker than the cell walls, then the cells will separate (WALDRON *et al*., 1997). 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 fruit (HERTOG *et al*., 2004; ABBOT, 2004; BRUMMELL *et al*., 2004; BRUMMELL, 2006; DEYTIEUX-BELLEAU *et al*., 2008). However, very few studies have investigated the link between this softening, these transitions 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 essential parameter for understanding grape ripening, owing to its key role regarding the main compounds responsible for wine quality such as anthocyanins. Nevertheless, few studies of wine grape texture analyses have been conducted. Recently published studies have analyzed the modifications of certain grape textual properties during ripening and the influence of the terroir effect on mechanical behavior were considered (LE MOIGNE *et al*., 2008; TORCHIO *et al*., 2010). However, still more information about the contribution of the rheological behavior of grapevines to the anthocyanin diffusion process is required.

The aim of this work was to study the evolution of the mechanical properties of Cabernet Franc grapes belonging to three different vineyards during ripening by performing rheological tests (compression and puncture tests) and to correlate these changes with their degree of ripeness and with skin anthocyanin extractability. The process of anthocyanin extraction and diffusion from grape skins during maceration was monitored in a model hydroalcoholic solution in order to better control extraction and avoid the impact of seeds and yeasts present under real winemaking conditions. Such investigations could considerably increase our understanding of the material proprieties of grapes and thus help winemakers to choose the best time of harvest and the best process to make the wine desired.

MATERIALS AND METHODS

1. Grape samples

Cabernet Franc grapes, were harvested weekly from 10 September to 8 October 2007 (5 ripening stages coded C, D, E, F and G). The first stage (September 10) corresponds to 21 days after mid-veraison and the last stage (October 8) corresponds to the final ripeness stage. In order to cover the huge variability of plant material,

berries belonging to 3 different vineyards in the Loire Valley (France) (coded vineyard 1, vineyard 2 and vineyard 3) were picked. The vineyards were geographically close but had different soil types. Vineyard had argillaceous soil, the soil of vineyard 2 was composed of sands whereas that of vineyard 3 was green sandy soil with ochre. In order to minimize variation within samples and therefore to better reflect general berry development, 450 berries, with pedicels, were randomly picked up from each vineyard at each ripening stage. Then, the berries were divided into three batches: one, composed of 50 berries for the compression test; one of 30 berries for the puncture test; and the third one of 300 berries to measure anthocyanin extractability from grape skin.

2. Texture analysis procedure

Compression of the berries in equatorial position was performed with a universal testing machine by using a two-parallel plate geometry (MTS, Synergie 200H, Minnesota, USA). Berries were compressed twice with 20% deformation of their height at 50 mm.min^{-1} (MAURY *et al*., 2009). Force/deformation curves were analyzed (Figure 1A) and eight parameters studied:

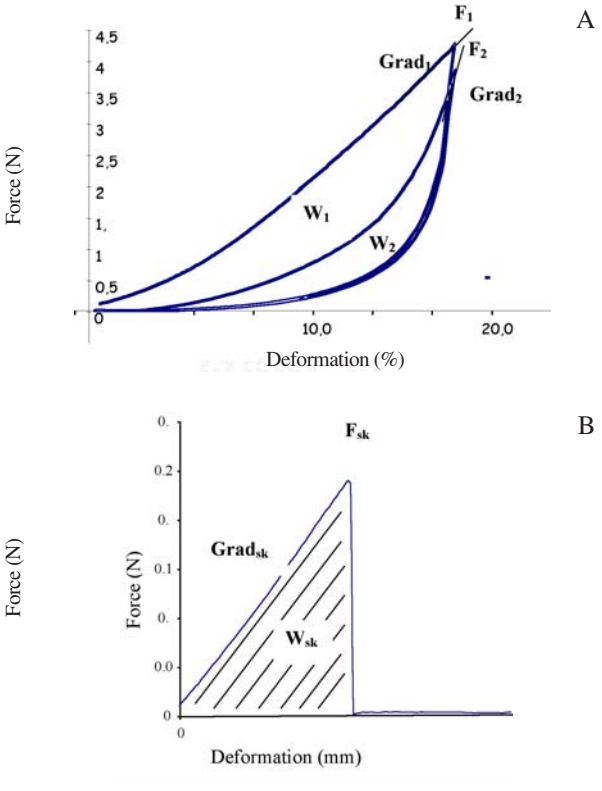

Figure 1 - A: Typical double compression Force/deformation curve obtained on grape berries using an MTS (Synergy 200H) traction machine (50 mm.min^{-1}20 %). B: Typical Penetrometric by using an MTS (Synergy 200H) traction machine (8 mm.min^{-1}/ depth 2 mm).

hardness corresponding to the maximal force in (N) associated with the first compression (F1) and the second compression (F2), energy in (mJ) associated with the first and second compressions (WF1 and WF2, respectively) and the global firmness of the first and second compressions (Grad1 and Grad2, respectively) (in N/mm). Two secondary parameters were computed (BOURNE, 2002): cohesiveness, which is the ratio between WF2 and WF1, and gumminess, which is the product of cohesiveness with F1.

A puncture test was carried out on the equatorial side of the berries according to MAURY *et al*. (2009). Tests were performed with a 0.16 mm diameter probe to a depth of 2 mm at 8 mm.min^{-1}. Force/deformation curves (Figure 1B) were analyzed and three parameters were studied: the berry skin break force (Fsk) in (N), the berry skin break energy (Wsk) in (mJ) and the slope associated with the break force (Gradsk) in (N/mm) corresponding to the Young modulus of the berry (LETAIEF *et al*., 2008).

3. Sample preparations for HPLC analysis and anthocyanin extraction in a model solution

To evaluate the yield of anthocyanin extraction, skins from 100 (50 x 2) berries were manually separated from pulp and seeds. 50 skins were pooled and immediately frozen at -20 °C to determine their anthocyanin content. The second batch of skins was placed in 150 mL of a hydroalcoholic solution containing 12 vol% ethanol, 3 g/L tartaric acid, 100 mg/l SO_2. pH was adjusted to 3.5 with sodium hydroxide (FOURNAND *et al*., 2006). Flasks were placed under nitrogen at 20 °C in darkness for 7 days. At the end of extraction, the extraction media and the residual solid material were stored at -20 °C until analysis. All the experiments were carried out in triplicate.

Powders obtained from both the freeze-dried skins and the residual solid material from the hydroalcoholic extraction (40 mg) were first mixed separately with 2 mL of Methanol and then twice with 3 mL of acetone/ water/ TFA (70:30:0.05, v/v/v). The supernatants were collected 10 minutes after each extraction. All 3 supernatants were pooled and dried under vacuum at 30° C for 3h. Samples were then dissolved in 1.5 mL of methanol/ water/ HCl (10:89:1, v/v/v) (SALAS *et al*., 2003). The extracts were finally filtered (0.45 µm) before injection.

Samples of extraction liquid media taken at the end of maceration were filtered through 0.45 µm and directly analyzed by HPLC. All the experiments were carried out in triplicate.

4. Identification and quantification of anthocyanins

The equipment used was an Agilent HPLC System (Agilent Technologies, Waldbronn, Germany). Separation

was performed on a reverse phase Nucleosyl LC-18 column. Column temperature was maintained at 30 °C. Eluent (A) was composed of water and formic acid (95:5, v/v) while eluent (B) was composed of acetonitrile, water and formic acid, (80:15:5, v/v/v) (SOUQUET *et al*., 2006) at a flow rate of 1 mL/min. The elution program of the solvent (B) used was as follows: 0-7 min, 3 %; 7-20 min, 13 %; 20-23 min, 14 %; 23-34 min, 20 %; 34-38 min, 20 %; 38-45 min, 24 %; and 45-54 min, 35 %. Samples (5 µL) were injected. Spectra were recorded between 250 and 600 nm with a bandwidth of 2 nm. Anthocyanins were identified according to their retention time at 520 nm and the concentration of each anthocyanin was calculated by using a calibration curve with malvidin 3-O-glucoside (SIGMA).

5. Statistical data processing

Statistical analyses of the data (least significant differences among samples and for each variable, together with a two-way ANOVA and multivariable regression analysis) were performed by using Statgraphics® Plus 5.0 (StatPoint, Inc., Virginia, USA).

RESULTS AND DISCUSSION

Table 1 shows the physical and chemical properties of Cabernet Franc grapes collected throughout ripening from three different vineyards. The weight of 50 berries, the sugar content (as °Brix) and skin weight per berry show that the berries developed normally during ripening, confirming that the degree of ripeness of the samples selected differed significantly from one vineyard to another. Grapes from vineyard 1 had the highest sugar content, while vineyard 2 produced heavier berries (Table 1). As for skin/berry ratios, the differences observed at the early ripening stages were not significant at the final stage. As maturation advanced, the sugar content kept increasing and, at the moment of harvest, the grapes from the three vineyards reached high sugar levels (21.7 °Brix for vineyard 1 and 20.8 °Brix for vineyards 2 and 3). The criteria traditionally used to determine grape maturity are based on sugar content. However, it is known that sugar alone may not be a fully adequate criterion for deciding harvesting time, and other factors such as berry weight, total phenols, anthocyanins and acidity are also important factors for assessing ripeness.

1. The mechanical behaviour of berries during maturation

The mechanical responses to the rheological tests are reported in this section. The typical force-deformation curves during both compression and puncture tests were similar to those found by MAURY *et al.* (2009) (Figure 1).

Two-way ANOVAs (ripening and vineyard effects, with interactions) were performed on the raw data of the compression parameters (Table 2). The effect of the ripening stage was highly significant, whatever the compression parameter measured, as was the vineyard effect. Indeed, only the interaction observed between the ripening stage and vineyard type for the «Grad2» parameter was non significant. This means that the vineyard effect on the rheological properties of grapes depends on the ripening stage while the effect of the ripening stage on these properties differs between vineyards. All the compression parameters are represented for each vineyard separately in table 3. Hardness (F1) ranged from 3.38 to 2.97 N; 3.52-2.99 N and 3.53-3.29 N respectively for vineyards 1, 2 and 3. Energy (WF1) ranged from 3.94-3.27 mJ; 4.37-3.63 mJ and 4.27-3.88 mJ respectively for vineyards 1, 2 and 3. This is consistent with the results previously reported by MAURY *et al*. (2009).

The mechanical behavior relative to Cabernet Franc berries determined by compression tests showed slight but significant changes during ripening (Table 3). All the values of the parameters (F1, F2, WF1 ,WF2, Grad1, Grad2 and gumminess), except «cohesiveness», generally decreased through the ripening stages and barely increased

Table 1. Physicochemical characteristics of Cabernet Franc grapes during ripening.

Ripening dates	Vineyards								
	Vineyard 1			Vineyard 2			Vineyard 3		
	TSS (°Brix)[1]	weight of 50 berries (g)[1]	mg skin/berry[1]	TSS (°Brix)[1]	weight of 50 berries (g)[1]	mg skin/berry[1]	TSS (°Brix)[1]	weight of 50 berries (g)[1]	mg skin/berry[1]
C	19.20 (±0.02) ^{a}	66.90 (±2.10) ^{b}	0.21 (±0.03) ^{a}	18.20 (±0.01) ^{a}	85.65 (±2.65) ^{c}	0.26 (±0.01) ^{a}	18.20 (±0.02) ^{a}	76.19 (±0.64) ^{cd}	0.34 (±0.07) ^{c}
D	20.30 (± 0.02) ^{c}	63.03 (±1.24) ^{a}	0.26 (±0.09) ^{c}	19.80 (±0.02) ^{d}	71.38 (±2.06) ^{a}	0.34 (±0.10) ^{a}	18.80 (±0.02) ^{c}	78.20 (±0.34) ^{d}	0.30 (±0.04) ^{bc}
E	20.00 (±0.01) ^{b}	77.87 (±1.14) ^{c}	0.31 (±0.01) ^{bc}	19.30 (±0.02) ^{b}	91.02 (±3.87) ^{c}	0.29 (±0.05) ^{a}	18.30 (±0.01) ^{b}	72.48 (±3.07) ^{bc}	0.24 (±0.03) ^{ab}
F	20.40 (±0.01) ^{c}	65.51 (±1.56) ^{ab}	0.25 (±0.01) ^{ab}	19.50 (±0.01) ^{c}	78.37 (±3.87) ^{b}	0.26 (±0.01) ^{a}	20.40 (±0.02) ^{d}	67.94 (±3.00) ^{a}	0.23 (±0.04) ^{a}
G	21.70 (±0.01) ^{d}	63.39 (±0.76) ^{a}	0.27 (±0.00) ^{bc}	20.80 (±0.02) ^{e}	79.76 (±2.56) ^{b}	0.31 (±0.02) ^{a}	20.80 (±0.01) ^{e}	71.76 (±2.93) ^{ab}	0.30 (±0.00) ^{bc}

[1]All measurements are recorded as means (standard error, n=3). For each column any two values not followed by the same letter were significantly different following an LSD test at $p<0.05$.

again until the harvest date (stage G) even if the trend of these parameters was rather irregular. Regarding grapes, COOMBE and PHILLIPS, (1980) hypothesized that softening is due to the decrease of the elastic modulus of pericarp cells. The grapes of vineyard 1 had lower firmness values than those of the other vineyards. According to DU PLESSIS (2008), berry firmness is determined basically by vacuole turgidity, although this turgidity can be affected by possibly related physiological and water factors. At the final ripening stage, we noted that greater force was required to carry out the compression test. According to De BAERDEMAEKER *et al*. (1978), compressive stress at failure of potato and apple tissue increases with decreasing water potential, and hence with decreased turgor potential which corresponds to the osmotic pressure generated within the cell sap (ROUDOT, 2006). Turgor decrease in tissue may be attributed to the degradation of biological membranes upon ripening. An increase in membrane permeability allows water to leave the cell, thereby resulting in a decrease in cellular and therefore tissue turgor. However cells with high turgor require higher rupture force (DE BELIE *et al*., 1999).

The « cohesiveness » parameter remained constant whatever the ripening stage for vineyard 3, while it decreased for vineyard 1 and increased slightly for vineyard 2. The grapes of vineyard 3 had higher cohesiveness values than those of vineyards 2 and 1. This parameter showed that tissue failure can differ from one vineyard to another during ripening. « Cohesiveness » is considered as it relates to the disaggregation of the tissue cells after the first compression. The closer this parameter was to zero, the more the fruit cell walls were ruptured during the first compression (BOURNE, 2002). All these textural changes are believed to involve loss in turgor

Table 2. Two-way ANOVA results for compression parameters.

Parameters	Ripening effect		Vineyard effect		Ripening x vineyard effect	
	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
F_1	9.88	<0.001	7.38	<0.001	2.28	<0.05
F_2	8.98	<0.001	7.09	<0.001	2.52	<0.05
$Grad_1$	4.29	<0.01	5.41	<0.01	2.48	<0.05
$Grad_2$	6.30	<0.001	3.95	<0.05	1.77	ns
W_{F1}	7.70	<0.001	13.33	<0.001	2.32	<0.05
W_{F2}	7.05	<0.001	10.95	<0.001	2.25	<0.05
Cohesiveness	3.35	<0.01	9.47	<0.001	3.88	<0.001
Gumminess	7.98	<0.001	6.71	<0.01	2.52	<0.05

Table 3. Evolution of berry mechanical characteristics during ripening.

Vineyard	Ripening dates	F_1 [1] (N)	F_2 [1] (N)	$Grad_1$[1] (N/mm)	$Grad_2$[1] (N/mm)	W_{F1} [1] (mJ)	W_{F2} [1] (mJ)	Cohesiveness[1](-)	gumminess[1](-)
1	C	3.38 (±0.53) $^{c.A}$	3.13 (±0.48) $^{c.A}$	1.52 (±0.28) $^{b.B}$	2.71 (±0.51) $^{c.A}$	3.94 (±0.77) $^{c.A}$	2.23 (±0.39) $^{c.A}$	0.57 (±0.04) $^{bc.B}$	1.92 (±0.26) $^{b.B}$
	D	3.21 (±0.48) $^{bc.A}$	2.98 (±0.43) $^{bc.A}$	1.42 (±0.22) $^{a.A}$	2.65 (±0.39) $^{bc.A}$	3.73 (±0.76) $^{bc.A}$	2.05 (±0.34) $^{b.A}$	0.55 (±0.04) $^{a.AB}$	1.77 (±0.20) $^{a.A}$
	E	2.97 (±0.41) $^{a.A}$	2.76 (±0.37) $^{a.A}$	1.40 (±0.25) $^{a.A}$	2.49 (±0.38) $^{ab.A}$	3.27 (±0.73) $^{b.A}$	1.90 (±0.40) $^{a.B}$	0.58 (±0.05) $^{c.B}$	1.73 (±0.22) $^{a.A}$
	F	3.07 (±0.49) $^{ab.AB}$	2.86 (±0.44) $^{ab.AB}$	1.37 (±0.18) $^{a.A}$	2.48 (±0.37) $^{a.AB}$	3.60 (±0.84) $^{bc.A}$	2.03 (±0.38) $^{ab.A}$	0.57 (±0.04) $^{ab.A}$	1.73 (±0.21) $^{a.AB}$
	G	3.28 (±0.43) $^{c.A}$	3.05 (±0.40) $^{c.A}$	1.42 (±0.20) $^{a.A}$	2.66 (±0.38) $^{c.AB}$	3.89 (±0.73) $^{a.A}$	2.14 (±0.37) $^{bc.A}$	0.55 (±0.03) $^{a.A}$	1.81 (±0.23) $^{a.A}$
Average		3.18 (±0.16)	2.96 (±0.15)	1.43 (±0.06)	2.60(±0.11)	3.69 (±0.73)	2.07 (±0.12)	0.56 (±0.01)	1.79 (±0.08)
2	C	3.38 (±0.59) $^{bc.A}$	3.11 (±0.57) $^{bc.A}$	1.36 (±0.37) $^{ab.A}$	2.65 (±0.47) $^{bc.A}$	4.24 (±1.00) $^{bc.A}$	2.25 (±0.50) $^{bc.A}$	0.53 (±0.04) $^{a.A}$	1.80 (±0.32) $^{ab.A}$
	D	3.52 (±0.56) $^{c.B}$	3.27 (±0.50) $^{c.B}$	1.50 (±0.23) $^{c.A}$	2.73 (±0.38) $^{c.A}$	4.37 (±1.04) $^{c.B}$	2.38 (±0.52) $^{c.B}$	0.55 (±0.02) $^{b.A}$	1.92 (±0.27) $^{c.B}$
	E	3.27 (±0.46) $^{b.B}$	3.04 (±0.42) $^{b.B}$	1.41 (±0.24) $^{bc.A}$	2.60 (±0.37) $^{bc.A}$	3.97 (±0.76) $^{ab.B}$	2.19 (±0.38) $^{ab.A}$	0.55 (±0.04) $^{bc.A}$	1.81 (±0.24) $^{b.AB}$
	F	2.99 (±0.41) $^{a.A}$	2.79 (±0.38) $^{a.A}$	1.29 (±0.24) $^{a.A}$	2.35 (±0.29) $^{a.A}$	3.63 (±0.69) $^{a.A}$	2.04 (±0.37) $^{a.A}$	0.56 (±0.04) $^{c.A}$	1.69 (±0.24) $^{a.A}$
	G	3.29 (±0.62) $^{b.A}$	3.07 (±0.58) $^{bc.A}$	1.47 (±0.25) $^{c.A}$	2.57 (±0.40) $^{b.A}$	4.01 (±1.04) $^{bc.A}$	2.22 (±0.55) $^{abc.A}$	0.56 (±0.05) $^{bc.A}$	1.83 (±0.35) $^{bc.A}$
Average		3.29 (±0.16)	3.06 (±0.17)	1.41 (±0.08)	2.58(±0.14)	4.04 (±0.73)	2.22 (±0.12)	0.55 (±0.01)	1.81 (±0.08)
3	C	3.53 (±0.51) $^{b.A}$	3.25 (±0.48) $^{b.A}$	1.51 (±0.31) $^{a.B}$	2.71 (±0.40) $^{a.A}$	4.27 (±0.81) $^{b.A}$	2.40 (±0.46) $^{b.A}$	0.56 (±0.05) $^{a.B}$	1.99 (±0.32) $^{b.B}$
	D	3.30 (±0.53) $^{a.A}$	3.05 (±0.45) $^{a.A}$	1.49 (±0.23) $^{a.A}$	2.65 (±0.45) $^{a.A}$	3.88 (±0.77) $^{a.A}$	2.17 (±0.34) $^{a.A}$	0.57 (±0.05) $^{a.B}$	1.85 (±0.23) $^{a.AB}$
	E	3.29 (±0.57) $^{a.B}$	3.07 (±0.50) $^{ab.B}$	1.45 (±0.21) $^{a.A}$	2.64 (±0.47) $^{a.A}$	3.96 (±0.96) $^{ab.B}$	2.20 (±0.45) $^{a.A}$	0.56 (±0.04) $^{a.A}$	1.84 (±0.26) $^{a.B}$
	F	3.25 (±0.54) $^{a.B}$	3.01 (±0.49) $^{a.B}$	1.46 (±0.20) $^{a.B}$	2.63 (±0.44) $^{a.B}$	3.84 (±0.95) $^{a.A}$	2.13 (±0.42) $^{a.A}$	0.56 (±0.04) $^{a.A}$	1.81 (±0.23) $^{a.B}$
	G	3.43 (±0.59) $^{ab.A}$	3.19 (±0.54) $^{ab.A}$	1.48 (±0.32) $^{a.A}$	2.76 (±0.45) $^{a.B}$	4.11 (±0.89) $^{ab.A}$	2.25 (±0.46) $^{ab.A}$	0.55 (±0.03) $^{a.A}$	1.88 (±0.32) $^{ab.A}$
Average		3.36 (±0.14)	3.11 (±0.10)	1.48 (±0.02)	2.68(±0.06)	4.01 (±0.73)	2.23 (±0.10)	0.56 (±0.01)	1.87 (±0.07)

[1] All measurements are recorded as means (standard error, n=50). For each column any two values not followed by the same letter were significantly different following an LSD test at $p<0.05$. Lower case letters concern ripening stages. Upper case letters concern only vineyards.

pressure and also modifications in the cell wall structure (GOULAO and OLIVEIRA, 2008).

Based on our results, the softening of berries measured by compression tests may occur via different mechanisms: not all the firmer berries have the same value for the « cohesiveness » parameter or require the same energy « WF1 and WF2 » to be compressed. As observed, the results obtained vary from one vineyard to another and cannot be extrapolated accurately to every vineyard planted with Cabernet Franc. Overall, double compression measurements showed that the rheological properties of Cabernet Franc berries differ during ripening and that they are also vineyard dependant. However, due to the complexity of the berry cell wall structure and to the different ways in which these properties are related, changes occur during fruit ripening, especially in the case of winegrapes, still remain unclear.

2. Puncture test

Puncture tests have already been used to examine differences between varieties and ripening stages (LETAIEF *et al*., 2008; ROLLE *et al*., 2008; LEE and BOURNE, 1980). Table 4 provides the puncture parameters values for each vineyard separately at each stage of ripening. A two-way ANOVA was performed on these data to investigate the effects of the ripening stage, the vineyard and of their interaction in puncture parameters (Table 5). Concerning the ripening stage, all the parameters are significant at a probability level of 5%, except for berry skin energy (Wsk). Concerning the vineyard effect, only the berry skin break force (Fsk) is not significant, with a probability of 5%; also no interaction effect was observed. No modifications for the berry skin break energy Wsk were noted for ripening for the different vineyards. Also, for Fsk, no large variations were noted except for vineyard 2, for which a slight difference between the values of this parameter was noted at different ripening stages. It has been reported that puncture force decreases rapidly at veraison and then continues to decrease at a slower rate later in stage III of berry development (LEE and BOURNE, 1980). In a recent study performed by LETAIEF (2008), differences between ripening stages were noted according to Fsk and Wsk in the case of two out of a total of three studied. Nevertheless the evolution of these two parameters during the study period was not clear. In Nebbiolo grapes, an increase of Fsk was noted from veraison to ripeness, but above all in the first phases, with a steady or slight decrease close to technological maturity and a renewed increase in the over-ripeness phase (ROLLE *et al*., 2008). Moreover, TORCHIO *et al*. (2010) observed no significant changes in the parameters that characterize the skin hardness of berries belonging to Barbera grapes containing different levels of soluble solids. The above studies suggested that the behavior of the Fsk values close to harvest could limit the choice of this parameter as a maturity indicator. By contrast, the Gradsk parameter (Young's modulus), decreased throughout the study period (0.38-0.33; 0.37-0.28 and 0.38-0.30) (N/mm) respectively

Table 4. Berry skin mechanical behaviour during ripening.

Vineyard	Ripening dates	F_{sk} [1] (N)	W_{sk} [1] (mJ)	$Grad_{sk}$ [1] (N/mm)
1	C	0.30 (±0.07) $^{a.A}$	0.12 (±0.05) $^{a.A}$	0.38 (±0.07) $^{b.A}$
	D	0.29 (±0.06) $^{a.A}$	0.12 (±0.06) $^{a.A}$	0.35 (±0.06) $^{ab.B}$
	E	0.30 (±0.07) $^{a.A}$	0.12 (±0.04) $^{a.A}$	0.37 (±0.10) $^{b.B}$
	F	0.29 (±0.07) $^{a.A}$	0.13 (±0.06) $^{a.A}$	0.33 (±0.06) $^{a.A}$
	G	0.28 (±0.05) $^{a.A}$	0.12 (±0.04) $^{a.A}$	0.33 (±0.06) $^{a.B}$
Average		0.29 (±0.01)	0.12 (±0.00)	0.35 (±0.02)
2	C	0.33 (±0.08) $^{c.A}$	0.14 (±0.06) $^{a.A}$	0.37 (±0.07) $^{b.A}$
	D	0.32 (±0.08) $^{bc.A}$	0.16 (±0.07) $^{a.B}$	0.32 (±0.06) $^{ab.A}$
	E	0.28 (±0.07) $^{a.A}$	0.12 (±0.07) $^{a.A}$	0.31 (±0.06) $^{ab.A}$
	F	0.31 (±0.09) $^{abc.A}$	0.15 (±0.07) $^{a.A}$	0.32 (±0.05) $^{ab.A}$
	G	0.28 (±0.07) $^{ab.A}$	0.14 (±0.07) $^{a.A}$	0.28 (±0.06) $^{a.A}$
Average		0.30 (±0.02)	0.14 (±0.01)	0.32 (±0.03)
3	C	0.33 (±0.08) $^{a.A}$	0.14 (±0.07) $^{a.A}$	0.38 (±0.06) $^{b.A}$
	D	0.31 (±0.08) $^{a.A}$	0.15 (±0.07) $^{a.AB}$	0.32 (±0.08) $^{a.AB}$
	E	0.30 (±0.06) $^{a.A}$	0.14 (±0.05) $^{a.A}$	0.32 (±0.07) $^{a.A}$
	F	0.29 (±0.07) $^{a.A}$	0.13 (±0.07) $^{a.A}$	0.31 (±0.06) $^{a.A}$
	G	0.29 (±0.06) $^{a.A}$	0.14 (±0.05) $^{a.A}$	0.30 (±0.05) $^{a.A}$
Average		0.30 (±0.01)	0.14 (±0.00)	0.33 (±0.03)

[1] All measurements are recorded as mean (standard error, n=30). For each column any two values not followed by the same letter significantly different following an LSD test at $p<0.05$. Lower case letters concern ripening stages. Upper case letters concern only vineyards.

for vineyards (1; 2 and 3) and differences between the ripening stages were significant according to the LSD test. This parameter is used to measure the rigidity or stiffness of material in which low values correspond to springier tissues. These results agree with those of VARGAS *et al*. (2001), who concluded that the gradient or elasticity coefficient can be considered as a good berry flesh firmness index for Thompson seedless grapes. Finally, at the same ripening stage, the mechanical proprieties of berry skin show significant differences for the different vineyards according to parameters (Wsk) and (Gradsk).

The mechanical behavior of berries can be approached in several ways which should be considered complementary rather than mutually restricting. The complexity of the berries and the number of mechanisms possibly involved in initiating softness and loss of firmness are such that no single body of theory can perfectly describe the evolution and final stages of ripening. Consequently, 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 analyses.

3. Evolution of anthocyanin composition in berry skins during maturartion

Fifteen different anthocyanins (3-monoglucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin, and their acetate and coumarate forms) were detected in skins (Figure 2), but only fourteen were quantified. Concentrations of petunidin 3-monoglucoside-coumarate were not taken into account as its content was too low to be successfully determined. For each anthocyanin, the sum of the derivative forms was calculated in addition to the sum of the total non-acylated glucosides, total coumarates and total acetates (Table 7).

Two-way ANOVAs (ripening and vineyard effects, with interactions) were performed on the amount of anthocyanin compounds (Table 6). Significant differences with $p<0.01$ were observed between different stages of ripening for all variables. On the other hand, significant qualitative differences between vineyards were detected for only 5 variables. According to the F ratios, variations from vineyard to vineyard particularly affected peonidin, cyanidin and acetate derivatives. In general, the proportion of peonidin was higher in vineyard 3 than in vineyards 1 and 2 (average of all ripening stages). The same phenomenon was observed for cyanidin. Nevertheless, these differences between vineyards were not verified at all stages of ripening, as was highlighted by the significant interaction between vineyard and ripening stage listed in Table 6.

The changes in anthocyanin concentrations in Cabernet Franc are presented in table 8. Anthocyanin content in grape skin was 4.53-6.04 µg/g for grapes belonging to vineyard 1, 4.63-6.24 µg/g for vineyard 2 and 4.16-6.27 µg/g for vineyard 3 (Table 7). This is consistent with results previously reported by LE MOIGNE (2008). The monoglucoside forms are the predominant anthocyanins. The concentration of both acetylated (1.13-0.87; 0.93-1.16 and 0.73-1.23) (µg/g) respectively for vineyards (1; 2 and 3) and coumarylated glucosides (0.53-0.66; 0.60-0.81 and 0.42-0.68) (µg/g) respectively for vineyards (1; 2 and 3) is lower than that of their corresponding non-acylated glucosides for all the vineyards throughout ripening. The degradation rate observed at the final stage of ripeness of all derivatives was higher for vineyard 2 than the two others. The sum of free anthocyanins decreased slightly at the end of ripening. Similar results were obtained by FOURNAND *et al*. (2006) who found that free anthocyanin content decreased as sugar content increased, suggesting a conversion of free anthocyanins to derived pigments. DELGADO *et al*. (2004) reported that the anthocyanin accumulation in the Tempranillo variety showed an irregular trend throughout the grape ripening process. Concentrations increased initially, then fluctuated, reaching a maximum a few days before harvest and then fell until harvest. Also MATEUS *et al*. (2002) reported that the evolution of anthocyanin monoglocuside concentrations in Touriga National and Touriga Francesa varieties fluctuated during the last month of ripening. Their levels reached a maximum concentration between 40 and 60 days after veraison, and then started to decrease until harvest. Along the same lines, BERT VIAN *et al*. (2006) stated that the content of total anthocyanins in Syrah grape skins reached a maximum 28 days after veraison and then decreased until harvest whereas MAZZA *et al*. (1999), reported that phenolic contents, mainly anthocyanins in Cabernet Franc grape skins,

Table 5. Two-way ANOVA results for puncture parameters.

Parameters	Ripening effect		Vineyard effect		Ripening x vineyard effect	
	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
F_{sk}	2.81	< 0.05	1.11	ns	0.71	ns
$Grad_{sk}$	16.21	<0.001	10.49	<0.001	1.16	ns
W_{sk}	0.85	ns	6.22	<0.01	0.37	ns

increased steadily before stabilizing during ripening. Under our conditions, the evolution throughout the ripening stages showed that the synthesis of anthocyanins in the berries had already reached an advanced phase as the first sample was taken around 21 days after mid-veraison.

4. Evolution of the extractability of free anthocyanins from skin during maturation

In additional to phenolic maturity, phenolic extractability is also an important issue. Grapes with high phenol content do not necessarily produce wines that are also rich in phenolic compounds. A method for evaluating this extractability is therefore proposed. Extraction media and residual skins (crushed) were analyzed at the end of hydroalcoholic maceration (lasted 7 days). About 79% of the anthocyanins present in the skins were recovered in extraction media and residual solid parts. The extraction yield calculated on the basis of the amounts of anthocyanins recovered in hydroalcoholic solution was therefore lower (67.18 ± 6.26) than that calculated on the basis of the amounts recovered from residual solid materials (95.73 ± 0.88).

These data are consistent with the findings of ROMERO-CASCALES *et al.* (2005b) who studied the rate of anthocyanin extraction from Monastrell grape skins during the maceration process, by observing anthocyanin accumulation in wine and in crushed grape skins. According to the initial content in grape skins at harvest (8366 µg/g skin) and its content in crushed skins after 7 days maceration (2474 µg/g skin), only 67% of initial skin anthocyanin was found in the must and therefore only a slight decrease in monomeric anthocyanins was detected from day 10 to day 14 (end of

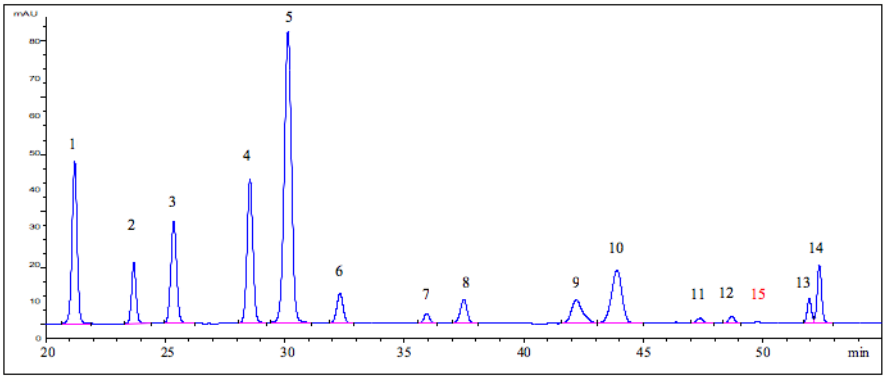

Figure 2 - A HPLC chromatogram of an extracted medium (7 days skin maceration) recorded at 520 nm.
1: celphinidin-3-glucoside; 2: cyanidin-3-glucoside; 4: peonidin-3-glucoside; 5: malvidin-3-glucoside; 6: delphinidin-3-glucoside-acetate; 7: cyanidin-3-glucoside-acetate; 8: petunidin-3-glucoside; 9: peonidin-3-glucoside-acetate; 10: malvidin-3-glucoside-acetate; 11: delphinidin-3-glucoside-coumarate; 12: cyanidin-3-glucoside-coumarate; 13: peonidin-3-glucoside-coumarate; 14: malvidin-3-glucoside-coumarate; 15: petunidin-3-glucoside-coumarate.

Table 6. Two-way ANOVA results for anthocyanin accumulation

Parameters	Ripening effect		Vineyard effect		Ripeping x vineyard effect	
	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
Delphinidin	5.86	<0.01	0.88	ns	2.46	<0.05
Cyanidin	5.44	<0.01	18.85	<0.001	2.62	<0.05
Petunidin	7.8	<0.001	1.94	ns	2.91	<0.05
Peonidin	14.21	<0.001	27.59	<0.001	2.67	<0.05
malvidin	6.18	<0.01	3.96	<0.05	3.44	<0.01
Non-acylated	9.23	<0.001	3.24	ns	2.73	<0.05
Coumarates	7.26	<0.001	3.37	ns	3.43	<0.01
Acetates	8.29	<0.001	27.53	<0.001	2.11	ns
Sum of anthocyanins	8.89	<0.001	5.29	<0.05	2.77	<0.05

maceration). Since the extraction of anthocyanins from grape skins is clearly incomplete, it has been suggested that an equilibrium based on adsorption-desorption is established between the anthocyanin concentration in grapes and in wine and that once this equilibrium is reached, it is no longer possible to extract anthocyanins from grape skins (BOULTON, 2001).

In an analogous study carried out on the extractability of phenolic compounds of Cabernet-Sauvignon grapes, FOURNAND *et al*. (2006), found that the extraction yield of red pigments remained constant whatever the pulp sugar content. Under our experimental conditions, the effect of the ripening stage is generally significant whatever the extraction yield measured except for delphinin. Concerning the vineyard effect, only the extraction yields of delphinin, cyanidin, petunidin and peonidin were non significant (data not shown). Regarding the extraction yield of free anthocyanins (calculated on the basis of the amounts of anthocyanins recovered in hydroalcoholic solution), an increase of this parameter was noted in the first week of the study (stages C and D). It reached its maximum at stage D whatever the vineyard, then decreased for the next two weeks and finally increased again close to harvesting, which takes place at technological maturity (Figure 3). The extracted anthocyanins present in the extraction media were exclusively composed of monoglocuside forms. Anthocyanin forms displayed different behaviors during extraction. In fact, malvidin derivatives were much easier to extract than the other forms of anthocyanins whatever the ripening stage and vineyard (71,10% ± 7,45 average) while the contrary was observed for cyanidin derivates (59,11% ± 9.33 average) (Table 8). VICENS (2007) who studied the rate of anthocyanin extraction from Shiraz grape skins by observing anthocyanin accumulation in model solutions detected the contrary, probably due to the differences between the variety (Cabernet Franc in our case instead of Shiraz).

The easiness with which these anthocyanins can be extracted and the high yield of their extractability are believed to be related to the failure that occurs in the cell wall at advanced stages of ripening (BOSS and DAVIES, 2009).

5. Relationships between the mechanical properties and the extractability o anthocyanins from skins

Due to the large number of parameters capable of characterizing the mechanical characteristics of grape berries, each variable may give little information, independently, on its importance in the rheological behavior of grape berries, thereby making it necessary to perform a multivariate statistical analysis. To do this, a multiple regression analysis was carried out on the average texture data, °Brix and the anthocyanin extraction yield (n= 15). The best predictive model for the anthocyanin extraction yield (Figure 4) gave statistically significant results ($P< 0.01$) with $R^2 = 91\%$; R^2 (adjusted for degree

Table 7. Content of anthocyanins in Cabernet Franc grapes during ripening

Vineyard	Ripening stage	Delphinin [a, 1]	Cyanidin [a,1]	Petunidin [a].1	Peonidin [a ,1]	Malvidin [a,1]	Non-acylated [1]	Coumarates [1]	Acetates [1]	Sum of [1] anthocyanins
1	C	0.93 (±0.10) $^{c.A}$	0.29 (±0.03) $^{b.A}$	0.66 (±0.07) $^{c.A}$	1.08 (±0.10) $^{b.A}$	2.78 (±0.26) $^{abc.B}$	4.01(±0.41) $^{bc.A}$	0.59 (±0.05) $^{a.B}$	1.13 (±0.09) $^{b.B}$	5.73 (±0.55) $^{ab.AB}$
	D	0.72 (±0.11) $^{ab.A}$	0.20 (±0.03) $^{a.A}$	0.54 (±0.06) $^{ab.A}$	0.82 (±0.10) $^{a.A}$	2.56 (±0.28) $^{ab.B}$	3.35 (±0.40) $^{ab.AB}$	0.56 (±0.06) $^{a.B}$	0.94 (±0.12) $^{ab.AB}$	4.84 (±0.58) $^{ab.AB}$
	E	0.92 (±0.19) $^{bc.A}$	0.27 (±0.06) $^{ab.A}$	0.67 (±0.12) $^{bc.A}$	1.06 (±0.16) $^{b.A}$	3.13 (±0.43) $^{c.AB}$	4.25 (±0.74) $^{c.A}$	0.66 (±0.07) $^{a.AB}$	1.12 (±0.15) $^{b.A}$	6.04 (±0.97) $^{b.A}$
	F	0.71 (±0.05) $^{a.A}$	0.21 (±0.01) $^{a.A}$	0.53 (±0.03) $^{ab.A}$	0.87 (±0.13) $^{ab.A}$	2.93 (±0.19) $^{bc.A}$	3.64 (±0.22) $^{ab.A}$	0.61 (±0.11) $^{a.A}$	1.01 (±0.09) $^{ab.A}$	5.25 (±0.40) $^{ab.A}$
	G	0.63 (±0.05) $^{a.A}$	0.21 (±0.01) $^{a.A}$	0.46 (±0.04) $^{a.A}$	0.89 (±0.05) $^{ab.B}$	2.33 (±0.15) $^{a.A}$	3.13 (±0.31) $^{a.A}$	0.53 (±0.06) $^{a.A}$	0.87 (±0.22) $^{a.A}$	4.53 (±0.31) $^{a.A}$
Average		0.78 (±0.10)	0.23 (±0.03)	0.57 (±0.07)	0.94 (±0.11)	2.75 (±0.26)	3.68 (±0.42)	0.59 (±0.07)	1.01 (±0.13)	5.28 (±0.56)
2	C	0.85 (±0.06) $^{b.A}$	0.24 (±0.02) $^{c.A}$	0.63 (±0.04) $^{b.A}$	0.99 (±0.04) $^{b.A}$	3.21 (±0.12) bc.B	4.11 (±0.22) $^{b.A}$	0.71 (±0.01) $^{b.B}$	1.11 (±0.04) $^{bc.B}$	5.92 (±0.27) $^{bc.B}$
	D	0.75(±0.09) $^{ab.A}$	0.19 (±0.02) $^{a.A}$	0.53 (±0.03) $^{a.A}$	0.81 (±0.07) $^{a.A}$	2.98 (±0.22) ab.C	3.58 (±0.20) $^{a.B}$	0.69 (±0.06) $^{ab.C}$	0.98 (±0.10) $^{ab.B}$	5.25 (±0.36) $^{ab.B}$
	E	0.82 (±0.08) $^{b.A}$	0.23 (±0.01) $^{bc.A}$	0.63 (±0.06) $^{b.A}$	1.01 (±0.10) $^{b.A}$	3.55 (±0.39) $^{cd.B}$	4.29 (±0.40) $^{b.A}$	0.79 (±0.10) $^{bc.B}$	1.16 (±0.15) $^{c.A}$	6.24 (±0.65) $^{c.A}$
	F	0.79 (±0.08) $^{b.A}$	0.21 (±0.02) $^{abc.A}$	0.62 (±0.06) $^{b.A}$	0.94 (±0.05) $^{b.A}$	3.66 (±0.20) $^{d.B}$	4.27 (±0.36) $^{b.A}$	0.81 (±0.01) $^{c.B}$	1.14 (±0.05) $^{c.A}$	6.23 (±0.41) $^{c.A}$
	G	0.62 (±0.01) $^{a.A}$	0.20 (±0.01) $^{ab.A}$	0.46 (±0.01) $^{a.A}$	0.77 (±0.02) $^{a.A}$	2.58 (±0.01) $^{a.A}$	3.11 (±0.01) $^{a.A}$	0.60 (±0.02) $^{a.A}$	0.93 (±0.01) $^{a.A}$	4.63 (±0.04) $^{a.A}$
Average		0.76 (±0.06)	0.21 (±0.02)	0.57 (±0.04)	0.91 (±0.06)	3.20 (±0.19)	3.87 (±0.24)	0.72 (±0.04)	1.06 (±0.07)	5.65 (±0.35)
3	C	0.72 (±0.15) $^{ab.A}$	0.27 (±0.05) $^{ab.A}$	0.50 (±0.11) $^{a.A}$	0.91 (±0.18) $^{a.A}$	1.99 (±0.47) $^{a.A}$	3.25 (±0.65) $^{a.A}$	0.42 (±0.11) $^{a.A}$	0.73 (±0.20) $^{a.A}$	4.39 (±0.95) $^{a.A}$
	D	0.65 (±0.02) $^{a.A}$	0.25 (±0.01) $^{a.B}$	0.46 (±0.02) $^{a.A}$	0.86 (±0.04) $^{a.A}$	1.94 (±0.05) $^{a.A}$	2.93 (±0.07) $^{a.A}$	0.42 (±0.01) $^{a.A}$	0.81 (±0.03) $^{ab.A}$	4.16 (±0.11) $^{a.A}$
	E	0.74 (±0.06) $^{ab.A}$	0.27 (±0.02) $^{a.A}$	0.53 (±0.04) $^{ab.A}$	1.02 (±0.07) $^{a.A}$	2.51 (±0.18) $^{ab.A}$	3.48 (±0.23) $^{a.A}$	0.55 (±0.04) $^{ab.A}$	1.04 (±0.10) $^{bc.A}$	5.07 (±0.36) $^{a.A}$
	F	0.88 (±0.11) $^{b.A}$	0.33 (±0.04) $^{b.B}$	0.65 (±0.09) $^{b.A}$	1.27 (±0.19) $^{b.B}$	3.15 (±0.48) $^{b.AB}$	4.36 (±0.58) $^{b.A}$	0.68 (±0.12) $^{b.AB}$	1.23 (±0.19) $^{c.A}$	6.27 (±0.89) $^{b.A}$
	G	0.71 (±0.01) $^{ab.A}$	0.27 (±0.01) $^{a.B}$	0.52 (±0.00) $^{a.A}$	1.03 (±0.02) $^{ab.C}$	2.47 (±0.04) $^{ab.A}$	3.49 (±0.03) $^{a.A}$	0.53 (±0.01) $^{ab.A}$	0.96 (±0.02) $^{abc.A}$	4.99 (±0.00) $^{a.A}$
Average		0.74 (±0.07)	0.28 (±0.03)	0.53 (±0.05)	1.02 (±0.10)	2.41 (±0.24)	3.50 (±0.31)	0.52 (±0.06)	0.95 (±0.11)	4.98 (±0.46)

[a]: sum of derivative forms in µg of maldivin-3-monoglucoside/mg fresh skin
[1]All measurements are recorded as mean (standard error, n=30). For each column any two values not followed by the same letter significantly different following an LSD test at $p<0.05$. Lower case letters concern ripening stages. Upper case letters concern only vineyards.

of freedom) = 82 % and the RMSEC (Root Mean Squared Error Calibration)= 1.58:

EY(AT) =902.68 x Wsk - 900.86 x Fsk + 317.57 x Gradsk + 91.39 x W1 -223.98 x W2 +103.74 x Grad1 - 6.17 x °Brix + 211.46

According to the previous equation, it seems that the extractability of anthocyanins is mainly correlated with the skin mechanical parameter tests (Fsk, Gradsk, Wsk) and with several of the mechanical parameters related to the compression test (W1, W2, Grad1) and the amount of soluble solids (°Brix). This suggests that grape skin properties influence the extraction of anthocyanins. It also seems that grapes with a high Young modulus (Gradsk), and thus springier and elastic tissue, will produce higher anthocyanin contents during extraction in a model hydroalcoholic solution. RIO SEGADE *et al*. (2008) evaluated the ability of grape texture analysis to assess phenolic ripeness. A multiple linear regression between the cellular maturity index (determined according to SAINT CRICQ *et al*., 1998), berry skin break force and skin thickness was demonstrated. The authors suggested that assessing grape textural ripeness proved to be an efficient method for assessing phenolic ripeness. However this study was performed at only one ripening stage, *i.e.* harvesting, so it is thus reasonable to assume that more than one ripening stage should be considered to confirm these findings. ROLLE *et al.* (2008), suggested that harder skins allow a greater release of pigments. In fact in each of the cultivars examined (Brachetto and Nebbiolo), the grapes with higher skin break forces produced extracts with higher total anthocyanin contents. More research is needed to understand the relationship between the components of the grape skin cell-wall and the rheological behavior of grapes. The histological theory of plant mechanisms provides a new path for understanding the behavior of plant tissue as it focuses on the link between cell mechanisms and tissue behavior.

CONCLUSION

The purpose of this work was to fill a gap in knowledge of how the loss of texture during ripening can influence the extractability of skin anthocyanin. This study examined the evolution of the rheological behavior of grapes belonging to different vineyards during the last four-weeks of the ripening period of Cabernet Franc grapes. A general trend could be observed in the present study and we conclude that the extraction yield of anthocyanins from grapes can be predicted by instrumental techniques such as texture measurements. However, the relationships between mechanical parameters and the extraction yield of anthocyanins from grapes may change from one variety to another and also for the same variety; what is more, changes may occur between vineyards. To confirm this initial approach, further studies with larger numbers of vintages and vineyards would be necessary to link the mechanical properties established at macroscopic scale to the susceptibility of grape berry skins to release anthocyanin and to improve understanding of the biomechanical mechanisms involved. This will lead to the identification of key factors for each textural

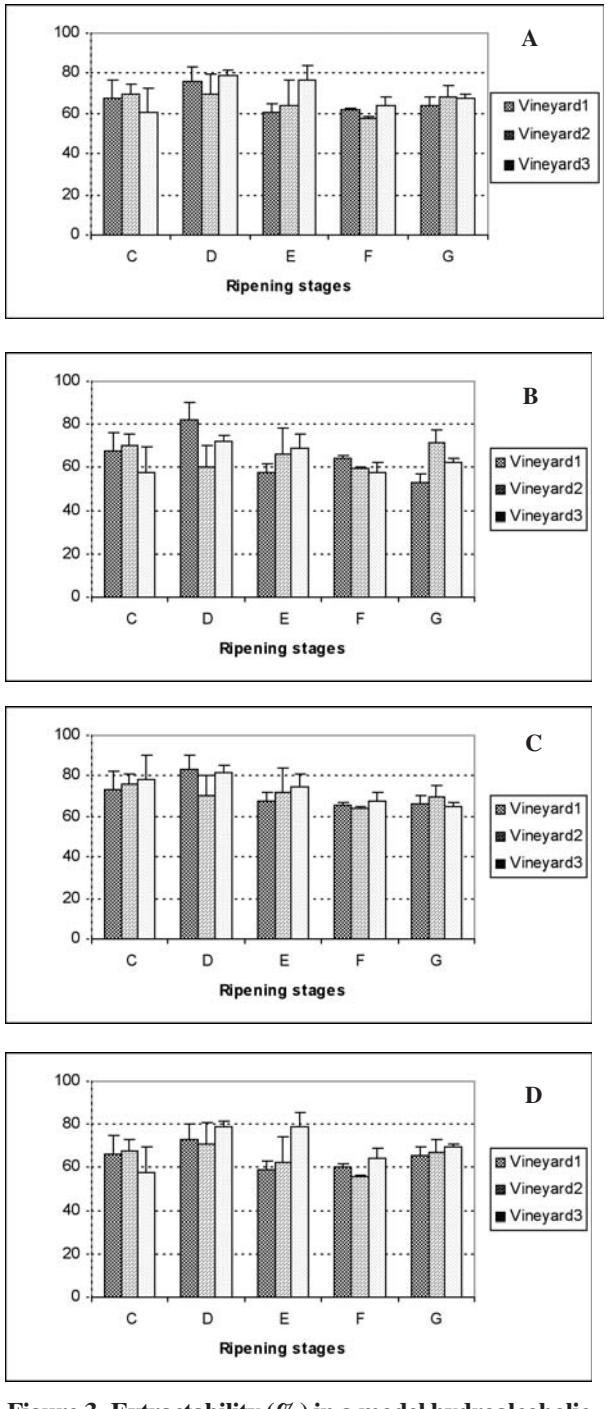

Figure 3- Extractability (%) in a model hydroalcoholic solution of total free anthocyanins (A), non acylated glucosides (B). acetylglucosides (C), and coumaroylglucosides (D) for the three vineyards at 5 ripening stages.

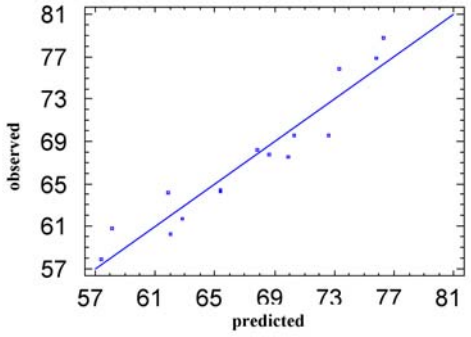

Figure 4 - Multiple regression analysis for extractability of anthocyanins (EY(AT)).

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.

BOURNE M.C. 2002. *Food texture and viscosity, concept and measurement*, 2nd ed. London Academic Press: Elsevier. 487 p..

BRUMMELL D.A., 2006. Cell wall disassembly in ripening fruit. *Functional Plant Biology*, **33**, 103-119.

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

CANALS R., LLAUDY M.C., VALLS J., CANALS J.M. and ZAMORA F., 2005. 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. *J. Agric. Food Chem*., **53**, 4019-4025.

CARROLL D.E and MARCY J.E., 1982. Chemical and physical changes during maturation of Muscadine grapes (*Vitis rotundifolia*). *Am. J. Enol. Vitic*., **33**, 168-172.

modification, and may facilitate the prediction of anthocyanin extractability yields during ripening.

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REFERENCES

ABBOTT J.A., 2004. Fresh quality assessment for fresh fruit and vegetables. *In: Quality of fresh and processed foods*. Ed. Kluwer academic, New York 265 p..

AMRANI JOUTEI K. and GLORIES Y., 1995a. Étude de localisation et de l'extractibilité des tannins et des anthocyanines de la pellicule de raisin. *In: Œnologie 95. 5e Symp. Int. Œnol.*, Bordeaux, France.119-123.

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

BAUTISTA-ORTINI A.B., FERNANDEZ-FERNANDEZ J.I., LOPEZ-ROCA J.M. and GOMEZ-PLAZA E., 2006. The effect of grape ripening stage on red wine color. *J. Int. Sci. Vigne Vin*, **40**, 15-24.

BERT VIAN M., TOMAO V., COULOMB P.O., LACOMBE J.M. and DANGLES O., 2006. Comparaison of the anthocyanin composition during ripening of Syrah grapes grown using organic or conventional agricultural practices. *J. Agric. Food Chem*., **54**, 5230-5235.

BOSS P.K. and DAVIES C., 2009. Molecular biology of anthocyanin accumulation in grapes berries. *In: Grape molecular physiology of biotechnology*. Ed. Springer Netherlands. 263-292.

Table 8. Extraction yield (%) in a model hydroalcoholic solution of free anthocyanins grouped as a function of their B-ring substitution pattern during ripening

Vineyard	Ripening dates	Delphinidin[1]	Cyanidin[1]	Petunidin[1]	Peonidin[1]	Malvidin[1]
1	C	58.76 (±7.94)	54.54 (±5.21)	62.27 (±8.64)	63.07 (±9.08)	74.85 (±9.84)
	D	60.20 (±7.02)	59.91 (±8.91)	65.84 (±7.08)	71.09 (±11.03)	85.17 (±6.60)
	E	53.56 (±4.71)	55.99 (±5.87)	59.78 (±6.42)	59.21 (±4.20)	63.08 (±4.33)
	F	56.26 (±0.77)	52.92 (±1.64)	57.89 (±0.80)	58.96 (±4.22)	65.31 (±0.16)
	G	64.24 (±4.13)	60.76 (±2.53)	66.71 (±4.38)	57.83 (±4.54)	66.32 (±3.98)
Average		58 (±5.96)	56.56 (±5.30)	62.25 (±6.20)	61.88 (±7.42)	70.61 (±9.68)
2	C	61.66 (±3.53)	60.53 (±4.29)	63.74 (±3.88)	65.40 (±4.38)	74.82 (±5.93)
	D	62.73 (±9.38)	72.82 (±10.33)	68.80 (±9.90)	73.94 (±9.80)	70.03 (±10.52)
	E	54.24 (±11.00)	57.95 (±10.47)	56.72 (±11.33)	59.00 (±10.00)	69.82 (±13.41)
	F	52.18 (±2.42)	41.84 (±3.35)	53.23 (±0.93)	55.76 (±3.16)	61.31 (±2.11)
	G	57.41 (±5.86)	42.15 (± 3.66)	61.21 (±5.94)	57.61 (±4.69)	77.27(±6.34)
Average		57.65 (±7.43)	55.06 (±13.64)	60.74 (±8.43)	62.34 (±9.09)	70.65 (±9.22)
3	C	58.59 (±12.24)	57.46 (±9.83)	61.23 (±12.46)	58.37 (±10.81)	63.13 (±12.76)
	D	72.15 (±1.36)	74.60 (± 2.93)	76.97 (± 1.96)	82.48 (±2.53)	80.49 (±3.76)
	E	71.96 (±7.92)	67.66 (±11.18)	76.01 (±7.51)	72.46 (±9.64)	81.13 (±4.99)
	F	64.70 (±4.12)	61.12 (±4.11)	65.39 (±4.34)	62.59 (±4.28)	65.07 (±4.31)
	G	66.15 (±2.86)	66.51 (±2.23)	66.98 (±2.80)	67.71 (±2.22)	68.65 (±1.50)
Average		61.04 (±7.84)	59.20 (±8.52)	69.32 (±8.67)	68.72 (±10.44)	71.69 (±9.72)

CHEYNIER V., SOUQUET J., KONTEK A. and MOUTOUNET M.,1994. Anthocyanin degradation in oxidising grape musts. *J. Sci. Food Agric*., **66**, 283-288.

COOMBE B.G. and PHILLIPS P.E., 1980. Development of the grape berry. III. Compositional changes during veraison measured by sequential hypodermic sampling. UCD Grape and Wine Continental Symposium. University of California, Davis.

DE BELIE N., TU K., JANCSOK P. and DE BAERDEMAEKER J., 1999. Preliminary study on the influence of turgor pressure on body reflectance of red laser light as a ripeness indicator for apples. *Postharvest Biol. Technol.*, **16**, 279-284.

DELGADO R., MARTIN P., DEL ALAMO M. and GONZALEZ M.R., 2004. Changes in the phenolic composition of grape berries during ripening in relation to vineyard nitrogen and potassium fertilisation rates. *J. Sci. Food Agric*., **84**, 623-630.

DE BAERDEMAEKER J., SEDERLIND L.J., MURASE H. and MERVA G.E., 1978. Water potential effect on tensile and compressive failure stresses of apple and potato tissue. ASAE paper No. 78-3057.

DEYTIEUX-BELLEAU C., VAILLET A., DONECHE B. and GENY L., 2008. Pectin methylesteras and polygalactorunase in the developing grape skin. *Plant. Physiol. Biochem*., **46**, 638-6466.

DU PLESSIS B.W., 2008. Cellular factors that affect table grape berry firmness. Master Thesis, Stellenbosch University.

grapes harvested at different ripeness grade. *Food Chem*., **96**, 197-208.

FERNANDEZ-LOPEZ J.A., ALMELA L., MUNOZ J.A., HIDALGO V. and CARRENO J., 1998. Dependence between colour and individual anthocyanin in ripening grapes. *Food Res. Int*., **9**, 667-672.

FLORA L.F. and LANE R.P., 1979. Effects of ripeness and harvest date on several physical and compositional factors of Cowart Muscadine grapes. *Am. J. Enol. Vitic*., **30**, 241-246.

FOURNAND D., VICENS A., SIDHOUM L., SOUQUET J.M., MOUTENET 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. and NICOLAI E., 2004. Humidity and temperature effects on invasive and non-invasive measures. *Postharvest Biol. Technol.*, **33**, 79-91.

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

LE MOIGNE M., 2008. Recherche de mesures innovantes pour suivre la qualité du raisin de Cabernet Franc pendant la maturation. *Thèse Doctorat*, Université d'Angers.

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. Prefer.*, **19**, 220-331.

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

LETAIEF H., 2008. Application of chemical-physical and mechanical tests for the definition of wine grape quality. *Thèse Doctorat*, Università Degli Studi Di Torino.

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

MATEUS N., MACHADO J.M. and FREITAS V., 2002. Development changes of anthocyanins in Vitis vinifera grapes grown in the Douro Valley and concentration in respective wines. *J. Sci. Food Agric*., **82**, 1689-1695.

MAURY C., MADETIA 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.

MAZZA G., FUKUMOTO, P., DELAQUIS B., GIRARD B. and EWERT B., 1999. Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia. *J. Agric. Food Chem*., **47**, 4009-4017.

ORTEGA-REGULES A., ROS-MARIA 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.

PEREZ-MAGARINO S. and GONZALEZ-SAN J., 2006. Polyphenols and colour variability of red wines made from grapes harvested at different ripeness grade. *Food Chem*., **96**, 197-208.

PEREZ-MAGARINO S. and GONZALEZ-SAN JOSE M.L., 2004. Evolution of flavanols, anthocyanins, and their derivates during the aging of red wines elaborated from grapes harvested at different stages of ripening. *J. Agric. Food Chem*., **52**, 1181-1189.

PINELO M., ARNOUS A. and MEYER A.S., 2006. Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release. *Trends Food Sci. Technol*., **17**, 579-590.

PIRIE A. and MULLINS M.G., 1977. Interrelationships of sugars, anthocyanins, total phenolics and dry weight in the skin of grape during ripening. *Am. J. Enol. Vitic*., **28**, 204-209.

RIO SEGADE S., ROLLE L., GERBI V. and ORRIOlS I., 2008. Phenolic ripeness assessment of grape skin by texture analysis. *J. Food Comp. Anal*., **21**, 644-649.

ROLLE L., TORCHIO F., ZEPPA G. and GERBI V., 2008. Anthocyanin extractability assessment of grape skins by texture analysis. *J. Int. Sci. Vigne Vin*, **42**, 157-162.

ROMERO-CASCALES I., FERNANDEZ-FERNANDEZ J.I., LOPEZ-ROCA J.M. and GOMEZ-PLAZA E., 2005a. The maceration process during winemaking extraction of anthocyanins from grape skins into wine. *Eur Food Res Technol*., **221**, 163-167.

ROMERO-CASCALES I., ORTEGA-REGULES A., LOPEZ-ROCA J.M., FERNANDEZ-FERNANDEZ J.I. and

GOMEZ-PLAZA E., 2005b. Differences in anthocyanin extractability from grapes to wines according to variety. *Am. J. Enol. Vitic*., **56**, 212-219.

ROUDOT A.C., 2006. Some considerations for a theory of plant tissue mechanics. *Sci. Aliments*, **26**, 409-426.

SAINT CRICQ N., VIVAS N. and GLORIES Y., 1988. Maturité phénolique: définition et côntrole. *Rev. Franc. Œnol*., **173**, 22-25.

SALAS E., ATANASOVA V., PONCET-LEGRAND C., MEUDEC E., MAZAURIC, J.P. and CHEYNIER, V., 2003. Demonstration of the occurrence of flavanols-anthocyanin adducts in wine and in model solutions. *Anal. Chim. Acta*., **513**, 325-332.

SOUQUET J.M., VERAN F., MANE C. and CHEYNIER V., 2006. Optimization of extraction conditions on phenolic yields from the different parts of grape clusters. Quantitative *distribution of their proanthocyanidins, In: XXIII International Conference on Polyphenols Winipeg Manitoba*., Canada, 245-246.

SURESH E.R. and ETHIRAJ.S., 1987. Effect of grape maturity on the composition and quality of wines made in India. *Am. J. Enol. Vitic*., **38**, 329-331.

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.

VARGAS A., PEREZ J., ZOFFOLI J.P. and PEREZ A., 2001. Comparación de variables de textura en la mediación de firmeza de bayas de uva Thompson seedless. *Cien. Inv. Agr*., **28**, 37-42.

VICENS A., 2007. Étude de l'évolution des composés phénoliques et des polysaccharides pariétaux de la pellicule de raisin pendant la maturation. Impact sur leur extractibilité en milieu hydroalcoolique. *Thèse Doctorat*, Université Montpellier II.

WALDRON K.W., SMITH A.C., PARR, A.J., NG A. and PARKER M.L. 1997. New approaches to understanding and controlling cell separation in relation to fruit and vegetable texture. *Trends Food Sci. Technol*., **8**, 213-221.