CHANGES IN TEXTURE ANALYSIS PARAMETERS OF WINE GRAPE BERRIES AT TWO RIPENESS STAGES: A STUDY ON VARIETAL EFFECT

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ABSTRACT

Ripening of grapes is associated with great modifications at both the chemical and physical level. The aim of this work was to describe the changes in physical-mechanical parameters associated to ripening of wine grape berries, as evaluated by texture analysis, in order to understand if these modifications are stable across cultivars, or they are cultivar-specific. Berries from 21 different cultivars were sorted by flotation in different saline solutions, separated in two ripening stages differentiated by the amount of sugars (183 and 217 g L^{-1}) and then analysed. Multivariate and univariate variations in texture analysis parameters were found, which were not constant across the studied grapevine varieties. However, a general behaviour was observed for skin weight, which had the largest variation between the two ripening stages. Other parameters showed significant differences between the ripening stages: skin thickness, berry gumminess, chewiness, and springiness, but the variation was not common to all cultivars. The work therefore evidenced the existence of cultivar-specific differences in the behaviour of physical-mechanical parameters between ripening stages.

Keywords: berry ripening, physical-mechanical properties, texture analysis, skin weight, wine grapes, *Vitis vinifera* L.

1. INTRODUCTION

Ripening of grape berries is a complex process, which happens according to a double sigmoid growth curve composed of three different phases (COOMBE, 1992). During the first period of growth, cell number per berry increases because of mitosis divisions, while cell expansion is limited. Generally, at the end of this period the grapevine reaches the phenological stage of bunch closure. This first growth period is followed by a lag phase during which enlargement slows and the seed develops. The phenological stage at the transition between this second and the third final phase is called *véraison*, which corresponds to the onset of ripening, when berries start to soften and change in colours because of anthocyanin synthesis (in red/black grapes). In the third stage, cells enlarge as a result of solutes (principally glucose and fructose) and water accumulation, and berries approximately double in size (CONDE *et al.*, 2007). This last step is crucial because important changes in secondary metabolites occur. These compounds are responsible for flavour, aroma, colour and mouth feel of grapes as well as wines. Modifications of pectins during this stage cause the progressive loss of firmness in ripe berries (NUNAN, 1998; NUNAN *et al.*, 2001). Such modifications are principally due to an increase in the enzymatic activity of pectin methylesterase, α-galactosidase and β-galactosidase, which has been registered after *véraison* (NUNAN *et al.*, 2001; DEYTIEUX-BELLAU *et al.*, 2008; ORTEGA-REGULES *et al.*, 2008). However, only in recent years, scientific studies have begun to instrumentally measure these visual and tactile changes, as summarized by ROLLE *et al.* (2012).

The structure and composition of skin cell walls directly impact textural characteristics and have been linked to phenol extractability (ORTEGA-REGULES *et al.*, 2006; BINDON *et al.*, 2012; HERNÁNDEZ-HIERRO *et al.*, 2014). Several studies have shown that the mechanical properties of whole berry and berry skin are significantly related to anthocyanin and flavanol extractability (ROLLE *et al.*, 2008; RÍO SEGADE *et al.*, 2011a, RÍO SEGADE *et al.*, 2011b). These studies are based on the use of Texture Analysis (TA) test, which is an effective instrumental texture analysis test for a quantitative evaluation of physical-mechanical characteristics of grape berries (LETAIEF *et al.*, 2008). The technique is rapid and cost-effective since it does not require long times for sample preparation and analysis.

However, literature describing the changes in physical-mechanical parameters according to different berry ripening stages is yet scarce and focuses on a very limited number of winegrape varieties (MAURY *et al.*, 2009; ZOUID *et al.*, 2010; RÍO SEGADE *et al.*, 2011c; ROLLE *et al.*, 2011a).

This study evaluates, on a heterogeneous dataset from 21 different grapevine cultivars, which texture analysis (TA) parameters change between two different sugar contents (i.e. stages of ripening). In particular, the aims of the work were *i*) to study if physical-mechanical parameters, as assessed by TA, can significantly vary between the two sampled ripening groups, *ii*) to evaluate if TA measurements allow to discriminate and classify the two ripening classes, therefore assessing the validity of the methods to describe variations in physical-chemical properties with ripening, and *iii*) to evaluate differences in physical-mechanical modifications with ripening across cultivars.

2. MATERIALS AND METHODS

2.1. Plant material and grape sampling

Vitis vinifera L. grapes from 21 red grapevine varieties were sampled in the CREA-VIT experimental collection (1.2 ha) located in Susegana (TV, Veneto, North-East Italy), in

2011. Sampled cultivars were Ancellotta, Barbera, Bonarda, Cabernet-Franc, Cannonau, Corvina, Croatina, Franconia, Gamay, Malbech, Malvasia Nera di Lecce, Marzemino, Merlot, Montepulciano, Negramaro, Pinot Noir, Primitivo, Raboso, Refosco, Schiava Gentile, and Teroldego. Vines were 15 years old, grafted on SO4 rootstock (interspecific cross between *Vitis riparia* Michx. and *Vitis berlandieri* Planch.), and planted at 3.0 m between rows and 1.5 m between vines. They were Sylvoz pruned and trained with a vertical shoot position system. For each cultivar, samples were composed of about 3 kg of grape berries, which were picked up randomly from ten vines.

The berries were sampled at two ripening stages (time lag of two weeks) of difference, and sorted using a densimetric method by berry flotation in different saline solutions (ROLLE *et al.*, 2011a). The two selected groups, called A (early harvest) and B (full ripeness), had respectively 183±8 and 217±8 g L^{-1} of sugars corresponding about to 11.0 ± 0.5% and 13.0 ± 0.5% potential alcohol content by volume, respectively.

The sorted berries were visually inspected before analysis; those with damaged skins were discarded. For each variety studied, a sub-sample of 36 sorted berries (therefore a total of 756 berries for all cultivars together) was randomly selected for the determination of the physical-mechanical properties.

2.2. Physical-mechanical measurements

Grape berries were singularly weighed (g, BW parameter), with an analytical laboratory balance (Radwag AS 220/X, Radwag, Radom, Poland), and a Texture Profile Analysis (TPA) non-destructive mechanical test was then performed for each of them as described by LETAIEF *et al.* (2008). Analyses were made using a Universal Testing Machine (UTM) TAxT2i texture analyzer (Stable Micro Systems, Godalming, Surrey, UK) equipped with a 5 kg load cell and a HDP/90 platform. A SMS P/35 flat cylindrical probe was used, and the test was carried out on each berry in the equatorial position under 25% deformation, with a waiting period of 2 s between the two compressions and a test speed of 1 mm s^{-1}. All force-deformation curves were acquired at 400 Hz and evaluated using the Texture Expert Exceed software package (Stable Micro Systems). TPA parameters calculated were berry hardness (N, as H), cohesiveness (adimensional, as Co), gumminess (N, as G), springiness (mm, as S), chewiness (mJ, as Ch) and resilience (adimensional, as R). After the TPA test, each berry was manually peeled with a razor blade, and skin weight (g, as SW) and skin thickness (μm, as Sp_{sk}) were singularly measured. For the latter test, the same UTM texture analyzer was used, equipped with a SMS P/2 flat cylindrical probe, and setting a compression test at 0.2 mm s^{-1} test speed (BATTISTA *et al.*, 2015).

2.3. Statistical analysis

Analysis of covariance (ANCOVA) was performed to evaluate the univariate differences in each physical-mechanical parameter between the two ripening classes, treating BW as a nuisance factor. The TA data were also normalised by BW as described in SANTINI *et al.* (2011), and the resulting data were analysed by one-way ANOVA. Robust multivariate analysis of variance (robust MANOVA) was used to investigate multivariate differences for physical-mechanical parameters between the ripening groups.

Multiple logistic regression was used to understand if texture analysis (TA, including TPA and Sp_{sk}) measurements can differentiate the two ripening classes, and results were interpreted to assess if relationships between mechanical parameters and ripening classification are stable across cultivars. The choice of the TA parameters in the model (independent variables) was based on an exhaustive search in order to minimise the Bayesian Information Criterion (BIC) and the Akaike Information Criterion (AIC). Finally,

a cross-validation procedure was used to choose between the minimal AIC and BIC proposed models.
The statistical analysis presented in this work was performed in R v.3.2.0 (R CORE TEAM, 2015). Robust multivariate analysis of variance was performed using the rrcov package (TODOROV and FILZMOSER, 2009), and best subset logistic regression with the 'bestglm' package(McLEOD and XU, 2014).

3. RESULTS

3.1. Differences in physical-mechanical parameters between ripeness levels

To observe differences in physical-mechanical parameters with ripening, the first step was to exclude possible differences in BW between classes because this parameter is correlated to other berry physical-mechanical parameters. As an example, lighter berries have lower S and Ch values than heavier ones ($r = 0.88$ between BW and S, $r = 0.44$ between BW and Ch), but also lower skin weight (SW) ($r = 0.76$). It would be logical to assume that the ripe A group (early harvest) could have different BW than the ripe B group (full ripeness), for some reason such as sugar accumulation, water accumulation or loss, etc. This hypothesis is rejected by an ANOVA test, which excludes a significant difference in BW means between the two groups (p-value = 0.19). The same results were obtained when performing the ANOVA test individually by cultivars: the difference in BW was not significant (p-value > 0.05) between ripe A and B groups for each cultivar sample in the experiment. Analysis was performed by taking into account the multiplicity problem by using the Bonferroni correction.
However, in order to increase the robustness of the statistical analysis, BW was treated as a nuisance parameter and then an ANCOVA test was performed to adjust, for any treatment, group differences in BW before assessing the impact of the ripening stage. The inclusion of BW in the model permits to exclude the effect of berry weight on the observed differences in physical-mechanical parameters.
The test was first performed including the interaction between BW and ripening class in order to assess if the assumption of slope equality is respected, which is necessary to the analysis. Because the interaction term was never significant, the assumption was respected and the interaction term excluded. This also means that the relation between BW and all tested mechanical parameters did not depend on the level of ripening.
In all cultivars pooled, the only significant difference between ripe A and B berries for all studied physical-mechanical parameters were found in SW (p-value < 5^{-04}). The difference remained significant even after a Bonferroni correction accounting for the increase in risk because of the multiple comparisons (p-value < 0.01 after correction).
While BW was not different between the two ripening stages considered, a difference in SW exists and berries in the B group had skins 20 mg (approx. 10% over the mean) heavier than those in the A group. The ANCOVA analysis for SW is presented in Fig. 1.
The analysis was also performed by normalising each TA parameter by BW, as already made in SANTINI *et al.* (2011). However, even when normalising data by BW, these results were confirmed. When TA parameters were analysed singularly in a univariate way, differences between the classes were too little to be significantly noticed, with the exception of SW. Nevertheless, considering the variations in all TA parameters as a whole in a multivariate way (robust MANOVA), significant differences were found in the texture parameters between the two ripening groups, even when SW was removed from the dataset (Wilks' $\lambda = 0.82$, p-value < 1^{-05}).

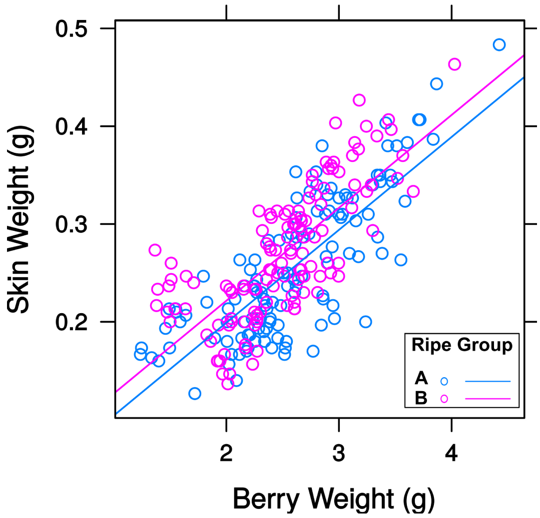

Figure 1. Results of the ANCOVA with equation SW = BW + Mat. The analysis shows differences in SW between the two ripening stage, while controlling for the nuisance effect of BW.

3.2. Classifying berry ripening classes using TA parameters

Because the two ripening classes were significantly different in the berry texture characteristics, the analysis was extended to evaluate if TA parameters were able to discriminate ripe A and B berries. The reason of this analysis was to evaluate the performances of the classification across cultivars, and therefore to know if, at this late stage of ripening, different cultivars show similar changes in TA parameters, or not. If cultivars show similar changes, the performance of the classification should be equal for all cultivars; conversely, if changes in TA parameters are cultivar-specific, the performance should vary across cultivars, with cultivars having greater changes being easier to classify than cultivars with little changes. This was evaluated, in a straightforward and single step approach, using a multiple logistic regression, where the response was the ripening class (which was binary) and the predictors were the physical-mechanical parameters. Because the number of TA parameters was large, feature selection was performed to identify the most informative independent variables. Two models were selected, the first minimised the BIC and just included SW and S as independent variables. Both SW and S parameters were significant in the model (p-value < 0.01). This regression model correctly classified 58% of the observations. The second model minimised the AIC, and contained as variables the SW, G, Ch, and Sp_{sk} (p-value < 0.01 for G, Ch and SW, p-value < 0.1 for Sp_{sk}); the model correctly classified 62% of the observations. It is worth noting that S is correlated to G and Ch (r = 0.61 and 0.38, respectively, p-value < 0.05). This can be a reason why S is excluded in this second model when G and Ch are included. The two models were then compared using cross-validation, which suggested the selection of the second model (AIC minimised). However, the significant effect of S in the model can also be taken into account for a future discussion. The final logistic regression predicting the probability that an observed sample X belongs to the B group has equation 1:

$$\hat{p}(X) = \frac{e^{-2.448-0.008Sp_{sk}+13.205SW-4.243G-1.606Ch}}{1+e^{-2.448-0.008Sp_{sk}+13.205SW-4.243G-1.606Ch}} \quad (1)$$

Where p(X) is the probability of X, *e* is the natural logarithm base, Sp_{sk} is skin thickness, SW is skin weight, G is gumminess, Ch is chewiness.
Table 1 shows coefficient estimate, standard error and *p*-value for the regressions minimising either the AIC or the BIC. Errors do not appear equally spread across all cultivars in the experiment.

Table 1. Coefficients of the logistic regression classifying berries in two ripening classes according to their physical-mechanical characteristics.

	Logistic Regression Minimising AIC (equation 1)		
	Estimate	**Std. Error**	**p-value**
Intercept	-2.448	0.948	9.8e-03
Sp_{sk}	-0.008	0.004	5.5e-02
SW	13.205	3.293	6.1e-05
G	-4.243	1.031	3.9e-05
Ch	-1.606	0.375	1.9e-05
	Logistic Regression Minimising BIC		
Intercept	3.113	1.277	1.5e-02
S	7.820	2.405	1.1e-03
SW	-2.135	0.652	1.1e-03

Sp_{sk} Skin Thickness; SW: Skin Weight; G: Gumminess; Ch: Chewiness; S: Springiness; SW: Skin Weight.

Cultivar-specific errors in the classification are presented in Table 2 (12 samples of three berries for cultivar). In eight of the 21 cultivars (Cannonau-Grenache, Croatina, Franconia, Malbech, Malvasia Nera Di Lecce, Marzemino, Merlot, and Raboso), the classification is not higher than that attended by chance.

Table 2. Percentage of correctly classified samples for the logistic regression in equation 1 across cultivars.

Cultivars	Correctly classified samples (%)	Cultivars	Correctly classified samples (%)
Merlot	50	Cabernet-Franc	67
Raboso	50	Corvina	67
Cannonau	50	Gamay	67
Malvasia Nera di Lecce	50	Montepulciano	67
Croatina	50	Pinot Noir	67
Franconia	50	Ancellotta	75
Marzemino	50	Refosco	75
Malbech	50	Negramaro	75
Bonarda	58	Barbera	83
Schiava Gentile	58	Primitivo	92
Teroldego	58		

Therefore, in these cultivars at this stage of ripening, the changes in physical-mechanical parameters do not consistently vary and do not allow to differentiate between the two classes. In all other cultivars, the results of the classification are sensibly better than chance. Very good results were reached for Primitivo, Barbera, Ancellotta, Negramaro, and Refosco (correct classification equal to 92%, 83%, and 75% for the last three cultivars, respectively). In this last group of cultivars, changes in physical-mechanical properties continue even in a late period of ripening.
Differences in the variation percentage between the two ripening stages are summarised in Fig. 2. The well classified cultivars show higher variation between the two stages than the bad classified cultivars; for bad classified cultivars, variation is close to zero excepting for SW. This suggests that the changes of physical-mechanical properties in the last ripening stages are cultivar dependent.

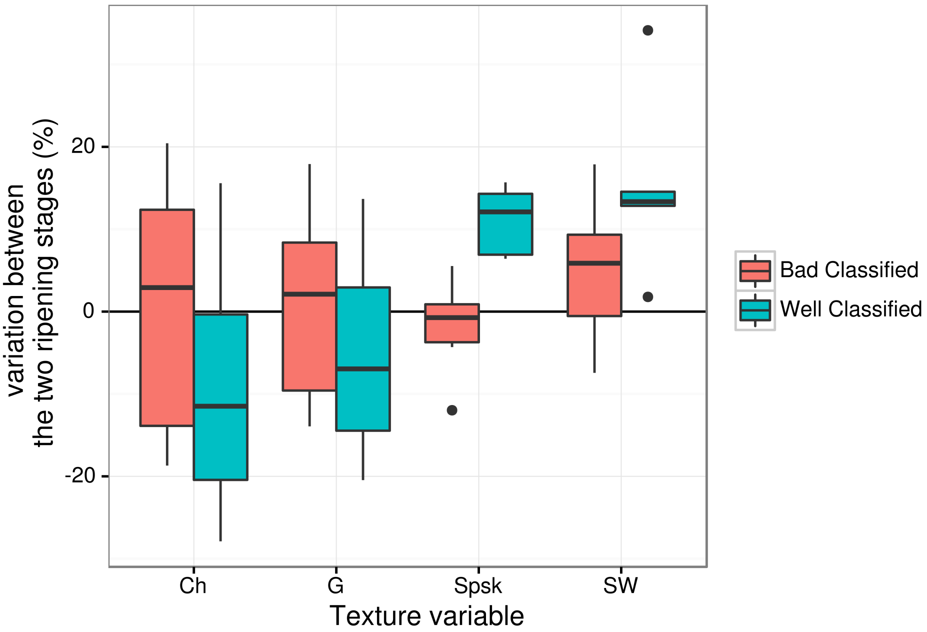

Figure 2. Variation percentage between the two ripening stages for TA parameters and SW.

4. DISCUSSION

The production of high-quality red wines requires the assessment of grape phenolic ripening indexes through the determination of the content of phenolic compounds and of their extractability during winemaking (RÍO SEGADE *et al.*, 2008). Texture analysis has been already used to develop rapid methods for the evaluation of total phenolic content and phenol extractability in grape seeds (ROLLE *et al.*, 2012), and of anthocyanin extractability in grape skins with a good accuracy (ROLLE *et al.*, 2008; RÍO SEGADE *et al.*, 2011a; RÍO SEGADE *et al.*, 2011b). The scientific literature is scarce on the description of changes in physical-mechanical parameters, instrumentally measured by TA, in the late stages of ripening, when variation in sugar content is not huge. In fact, several studies suggested that a steady value is achieved close to ripeness for some mechanical parameters, which could limit their choice as ripeness indicators in grape berries (MAURY *et al.*, 2009). In the present work, this observation is confirmed for a group of the cultivars studied: Cannonau-Grenache, Croatina, Franconia, Malbech, Malvasia Nera Di Lecce,

Marzemino, Merlot, and Raboso. Nevertheless, the observation is not confirmed for other cultivars in this study (Primitivo, Barbera, Ancellotta, Negramaro, and Refosco), which can be easily classified because physical-mechanical properties still change in the late ripening. The reason of this variation can have a genetic origin, and it deserves to be further investigated in future studies. A variety effect was already found in the relationship between flavonoid content and TA parameters (BRILLANTE *et al.*, 2015a; BRILLANTE *et al.*, 2015b). Variability in physical-mechanical parameters across cultivars can also be related to the climatic conditions of the ripening period, as shown in ROLLE et al., 2011b. That study showed that while the differences between cultivars for some texture parameters are, at least qualitatively, stable across vintages (an example is F_{sk}), others can also be affected by the climate (an example is W_{sk}).
This work carefully treats the BW effect on physical-mechanical parameters. When the BW effect is excluded from the analysis, results for other physical-mechanical characteristics are more reliable. In details, the study evidences an increase in SW with ripening. Although some authors showed that the percentage of skin cell wall material decreases during ripening (HERNÁNDEZ-HIERRO *et al.*, 2014) probably due to the cell walls become thinner (ORTEGA-REGULES *et al.*, 2006), others reported that cell wall material slightly but continuously increases as ripening progresses before decreasing (VICENS *et al.*, 2009). This increase in SW, even if modest in absolute terms, can become significant when considered as a ratio of the SW with BW, and is equal to 10%. This is a huge variation, especially if we consider that the accumulation of sugars in berries between the two ripening classes (approx. 34 g) accounts for just the 3% in average of BW.
Among the mechanical parameters measured by TA, and therefore excluding SW, whole berry characteristics, such as S, G, and Ch, were better related to berry ripeness than skin properties. Among all tested skin-related properties, Sp_{sk} was the only one showing a little effect. Since the texture properties of the whole berry depend on different characteristics, such as cell wall composition, cell structure and pulp turgescence (GOULAO and OLIVEIRA, 2008), and fruit softening occurring during ripening (Nunan 1998), it is not surprising to find larger evidence in the modification of the mechanical properties of the whole berry, as also reported in ZOUID *et al.* (2013).
In future, it could be interesting to couple TA analysis to the determination of pectins in grape berries. This will probably allow to better understand the direct relations between the physiological activity in grape berries during ripening, molecular structure and the macroscopic modifications of texture.

5. CONCLUSIONS

This work highlights that changes in physical-mechanical properties of grape berries are cultivar-specific in the final ripening stages. However, among all tested physical-mechanical parameters, a general behaviour was shown by skin weight. This parameter showed larger variation with ripening than the others considered. The observed increase in SW is particularly evident once considered its ratio over berry weight, which is equal to 10%. Proportionally, between early harvested and full ripe berries, berry weight changed more because of increased skin weight than because of sugar accumulation. Differences in sugar content between the two ripening classes accounted for just 3% in average of BW.

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