

WINEGRAPE BERRY SKIN THICKNESS DETERMINATION: COMPARISON BETWEEN HISTOLOGICAL OBSERVATION AND TEXTURE ANALYSIS DETERMINATION

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ABSTRACT

We analyzed the relation between the assessment of grape berry skin thickness by means of histology sections and instrumental mechanical properties measurements. Berry skin of *Vitis vinifera* L. cultivar Corvina vineyards from Valpolicella Valpantena zone (Verona, Italy) were tested, evidencing a strong correlation between the two thickness determination methods. The middle or equatorial berry skin portion was found to be the less variable in instrumental skin thickness determination. In addition, unlike other studies, no correlation between the skin thickness and cell layers number was found.

- Keywords: grape skin, cell layers, thickness, histology, mechanical properties -

INTRODUCTION

The skin or exocarp forms the grape's dermal system: depending on thickness and berry size, it accounts for between 5 and 18 % of the fresh weight of ripe berries (OJEDA *et al.*, 2002). The skin is composed of epidermis, covered with a waxy cuticle, and a underlying outer hypodermis (CONSIDINE and KNOX, 1979).

During grape berry development from fecundation to ripening the skin thickness varies consistently (COOMBE and McCARTHY, 2000). Immediately after the fruit set until the véraison, when berry weight increases, epidermis and hypodermis cells expands in the tangential direction, increasing their area by 15% and 33%, respectively. After the véraison, the mesocarp cells continue their expansion, and the hypodermal and epidermal cells lose size, acting inversely in comparison with mesocarp cells (SCHLOSSER *et al.*, 2008). Thus, the skin become thinner in the berry during ripening: the relative thickness of the skin decreases from one-eighth to one-hundredth of the total berry diameter between fruit set and maturity stages (KELLER, 2010).

The skin thickness is one of the most important grape skin morphological characteristics affecting the gas exchange regulation, the berry susceptibility to fungal diseases and the resistance to mechanical injuries (ROSENQUIST and MORRISON, 1989; KÖK and ÇELİK, 2004). The skin thickness varies depending on variety (MUGANU *et al.*, 2011; GIACOSA *et al.*, 2012) and clone (ROLLE *et al.*, 2012a), confirming that this parameter is genetically influenced: this could be useful to further understand some different varietal characteristics, such as the susceptibility to fungal diseases or the aptitude to the post-harvest dehydration process. Furthermore, the skin thickness seems to be related with the environmental conditions: in the alpine area cv. Nebbiolo berries with similar sugar content showed a generally thickest skin than in the hill side (ROLLE *et al.*, 2012b). This highlights that the skin thickness is very sensitive to the climate and the bunch microclimate conditions (PORRO *et al.*, 2008; MUGANU *et al.*, 2011), although a direct relation with water regimes in cv. Muscat blanc in open field conditions was not found (GIORDANO *et al.*, 2013).

Since epidermis and hypodermis cells contain chloroplasts and phenolic-rich vacuoles (KELLER, 2010), skin berry properties (break force and thickness) can aid in the assessment of the phenolic content during the ripening. In particular, the skin thickness represents a useful indicator to predict anthocyanin extractability, and thinner skins seems to be characterized by higher anthocyanin extractability (RÍO SEGADÉ *et al.*, 2011a). So, thickness can be useful to support the choice of the harvest data and to rationalize maceration and winemaking processes, thus allowing winemakers to best ex-

plot the grape potential reached in the vineyard. Berry skin thickness assessment can be obtained with histological observation or instrumental methods, i.e. texture analysis (LETAIEF *et al.*, 2008a; ROLLE *et al.*, 2012c). The instrumental skin thickness measurement permits to minimize the sample treatment without using reagents or special procedures, speeding up the analysis process.

The aim of this study was to compare the two cited skin thickness measurement methods (histology and texture analysis) among several vineyards, in order to assess differences between the two techniques and also to investigate the relationship between the thickness and the cell layer number of the analyzed samples. A preliminary test on the influence of the sampling berry skin portion on the instrumental skin thickness determination was also carried out.

MATERIALS AND METHODS

Grape Samples

Grapes were collected from four vineyards located in the "Valpolicella Valpantena" denomination of origin, just to the north of Verona, Italy (45°29'22"N, 11°0'49"E). The vineyards were fifteen years old, planted with Corvina (clone ISV-13) grafted on Kober 5BB. The vines were trained with simple Guyot and the rows were oriented North to South. The number of the vineyards analyzed was considered to be sufficient for this kind of study, following previous studies involving berry skin thickness variation analysis which considered from 3 to 7 vineyards (RÍO SEGADÉ *et al.* 2011b; ROLLE *et al.*, 2012b). Each vineyard was analyzed in duplicate (two subsamples) using this random sampling schema: each subsample was obtained by sampling fifteen bunches (one per chosen grapevine). From each bunch, twenty intact berries were selected, then the 300 resulting berries were used for the following analysis.

Histology

For the histological characterization, ten berries from each subsample were randomly chosen, and the protocol by BOZZOLA and RUSSELL (1998) for histological observation followed. A berry section was cut from each berry and immediately fixed in 2.5% glutaraldehyde (Ted Pella Inc., Redding, CA, USA) diluted with 0.1 mol/L sodium cacodylate buffer solution at pH 7.4 overnight at 4 °C. Then, they were incubated for 1 hour at 4 °C in osmium tetroxide 1 % in 0.1 mol/L sodium cacodylate buffer, and then water washed three times. After that, the samples were dehydrated in a graded ethanol series and embedded in an epoxy resin (Sigma-Aldrich, St. Louis, MO, USA). Semithin sections (1 µm) were obtained with an Ultratome V (LKB)

ultramicrotome, counterstained with toluidine blue 1 % and observed with a Leica DMR optical microscope equipped with a camera (Leica DFC 480). Measurements of skin thickness and cell skin number were made using an imaging software (ImageJ 1.38; Wayne Rasband; National Institutes of Health, USA) considering the Epidermis and Hypodermis.

Instrumental skin thickness

A TA.XTplus Universal Testing Machine (Stable Micro Systems, Godalming, UK) was used, operating in the following conditions (LETAIEF *et al.*, 2008a): 5 kg load cell, P/2 2-mm cylindrical flat probe, HDP/90 platform, test speed 0.2 mm/s, data rate 500 points per second, data acquisition and integration using the Exponent software from the same manufacturer. All the analysis were done at 20 ± 2 °C.

The probe was calibrated by force and distance before each session, the latter to define the starting point 1 mm above the platform. A pulp-free clean portion of the peeled skin sample was then placed on the HDP/90 platform base, letting it adhere on the platform surface, and a 0.2 mm/s compression movement was applied by the probe. The berry skin thickness (Sp_{sk}) instrumental parameter was defined as the distance (in μm) between the point when the probe touched the skin sample and the platform base. In correctly defining the touch point, it was necessary to include a 0.05 N instrumental trigger to avoid the "tail" effect (LETAIEF *et al.*, 2008a), as described in Fig. 1.

In order to assess the variability of the instrumental measurements influenced by the analysis position and intra-berry, a preliminary test was carried out. Ten berries were randomly taken from all the previously-formed subsamples: these berries were analyzed in fifteen different spots each, equally distributed in the top (close to the peduncle), equatorial (middle), and bottom side of the berry. Instrumental berry skin thickness (Sp_{sk}) values were then calculated, the results normalized based on the equatorial position, and the relative standard deviation (%RSD) calculated for the three spots and for the intra-berry measurements variation in the same spot.

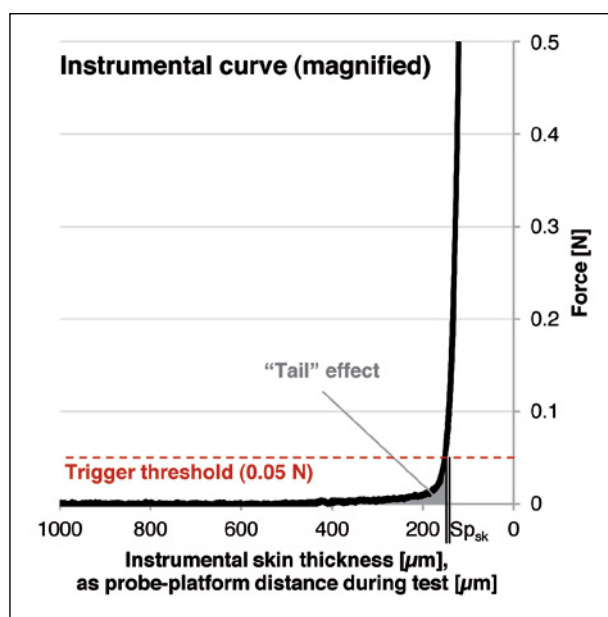


Fig. 1 - Typical force-distance curve of berry skin thickness mechanical test, magnified in the y-axis in order to highlight the "tail" effect influence and the need of a trigger threshold.

For the instrumental skin thickness determination, ten berries from each subsample were randomly chosen. The berries were singularly treated, they were peeled and a skin portion from the equatorial berry position analyzed using the aforementioned method, with the thickness calculated as Sp_{sk} (μm).

Statistical analysis

Statistical analysis was performed using the statistical software package IBM SPSS Statistics (IBM Corporation, Armonk, NY, US). The Tukey-b test at $p < 0.05$ was used in order to establish statistical differences by one-way analysis of variance (ANOVA).

RESULTS AND CONCLUSIONS

The instrumental skin thickness preliminary test results were shown in Table 1. Given 100 the equatorial position skin thickness average,

Table 1 - Comparison between instrumental mechanical skin thickness evaluation on top, equatorial and bottom positions of the berry, and mean variation of intra-berry measurements.

Berry analysis position	Normalized Sp_{sk}^a	%RSD	Average intra-berry %RSD
Top	78.3 ^a	20.1	14.84
Equatorial	100.0 ^b	9.9	9.09
Bottom	102.3 ^b	17.2	14.07

Instrumental berry skin thickness (Sp_{sk}) data is expressed as normalized result ($n = 50$) with respect to the equatorial side (given as 100). For each measurement the relative standard deviation (%RSD) is reported.

Intra-berry %RSD calculated as average of the ten %RSD values found analyzing each berry in 5 different spots.

^a Sp_{sk} normalized result values are significantly different at $p < 0.001$. Different letters in Sp_{sk} normalized results mean significance at $p < 0.05$ (Tukey-b test) among berry analysis position.

the other measures were normalized accordingly, and the relative standard deviation (%RSD) calculated. The bottom section gave similar results compared to the equatorial one, however the relative standard deviation measured is about 75 % higher than that of the latter position. The berry top skin section (close to the peduncle) showed lower Sp_{sk} values in relation to the other two sections considered, but the higher relative standard deviation of the group.

Regarding the intra-berry variation, the equatorial position was found the less variable, that means several measurements of the skin thickness on the same berry gave the more similar results when done on the equatorial position, with respect on measurements done only on the top position, or only on the bottom position.

The difference in the berry skin mechanical behavior induced by the analysis position was also found by LETAIEF *et al.* (2008b), which found significantly different berry skin break force and energy values depending on the puncture position when testing berries of Cabernet sauvignon, Pinot noir and Nebbiolo cultivars. The berry skin break force and energy values in the top position (labeled A3) were found the lower ones in most cases, as found for the instrumental thickness parameter in the present study. This can lead to the hypothesis of a link between these skins mechanical parameters, however no evidence of a meaningful correlation between skin break

force and thickness analyzed on the same position (berry lateral side) was found in a previous study conducted on grapes from several cv. Mencía vineyards (RÍO SEGADE *et al.*, 2011a).

Fig. 2 shows a picture of the berry skin section taken with the optical microscope: in the picture there were reported the different tissues that form the skin, Epidermis and Hypodermis. The outermost two-three cell layers were considered to be the Epidermis, while the seven to nine cell layers immediately below the Epidermis were considered to be the Hypodermis (HARDIE *et al.*, 2008). Immediately below to these layers there were polygonal shape cells that were considered to be the Mesocarp (SCHLOSSER *et al.*, 2008).

The data collected with the histological method is reported in Table 2. The measured skin cell layers number ranged between 8 to 11, with the average vineyard values resulted from 8.87 to 9.65, in agreement with the values reported for other varieties (CONSIDINE and KNOX, 1979; MUGANU *et al.*, 2011). The cell layers number showed significant differences between vineyards: A and C showed smaller values (8.87 and 8.95 respectively) compared with B (9.65), while D showed an intermediate average cell number (9.23). Also the skin thickness measured from the histological samples showed significant differences between vineyards. The vineyard A showed the thickest skin (173 μm) while the vineyard D showed the thinnest one (152 μm), with B

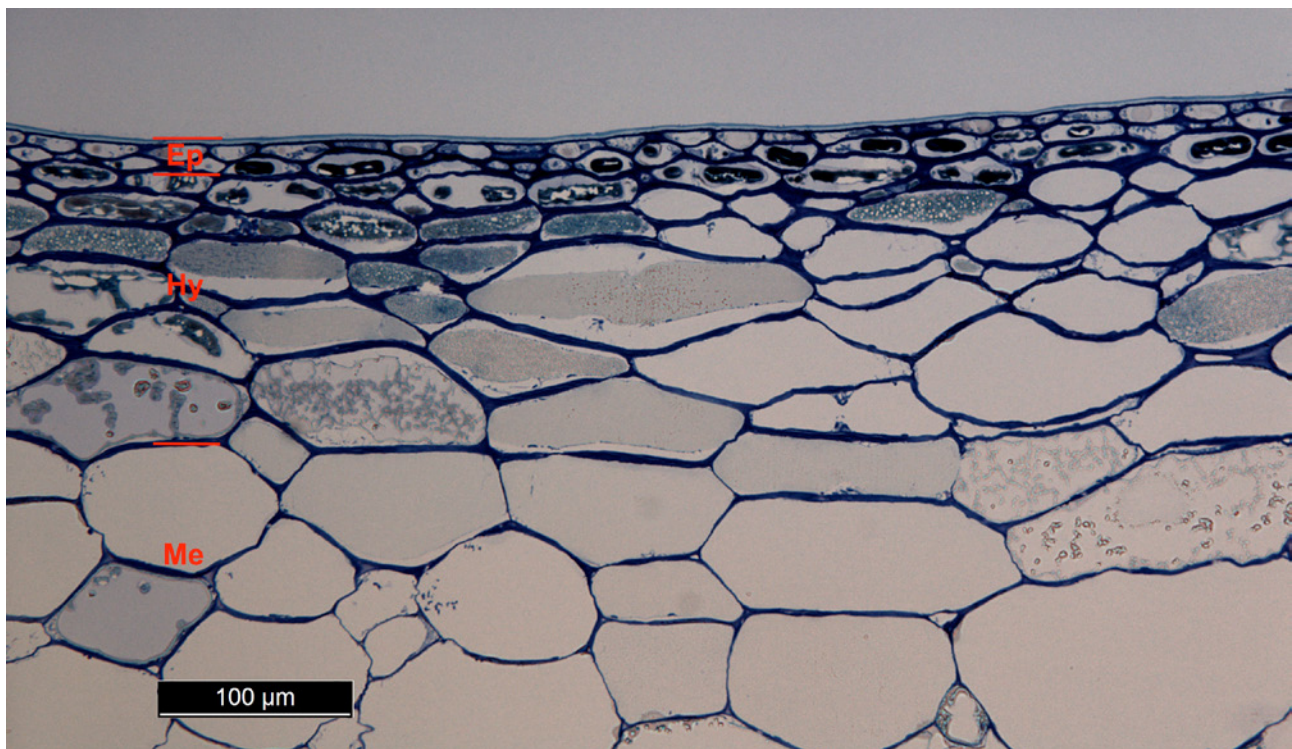


Fig. 2 - Picture of traverse section of Corvina berry skin at maturity taken with Leica DMR optical microscope. In the picture there are indicated the Epidermis (Ep), Hypodermis (Hy), and Mesocarp (Me).

Table 2 - Skin cell layers number and thickness evaluated by histology, and skin thickness by instrumental mechanical properties technique, of the analyzed vineyards (two groups per vineyard).

Vineyard	Cell layers number [by histology]	Thickness [by histology, μm]	Thickness [mechanical as Sp_{sk} , μm]	Sign ^b
A	8.87±0.69 ^a	173±5 ^c	176±8 ^c	ns
B	9.65±0.65 ^b	163±5 ^b	164±8 ^b	ns
C	8.95±0.72 ^a	161±5 ^b	160±7 ^b	ns
D	9.23±0.92 ^{ab}	152±5 ^a	153±5 ^a	ns
Sign ^a	**	***	***	

Data is expressed as average±standard deviation ($n = 20$). Different letters means significance at $p < 0.05$ (Tukey-b test) among vineyards.
^a: ** and *** means significance among vineyards at $p < 0.01$ and 0.001 , respectively.
^b: ns means not significant differences between thickness determinations (in a same vineyard sample).

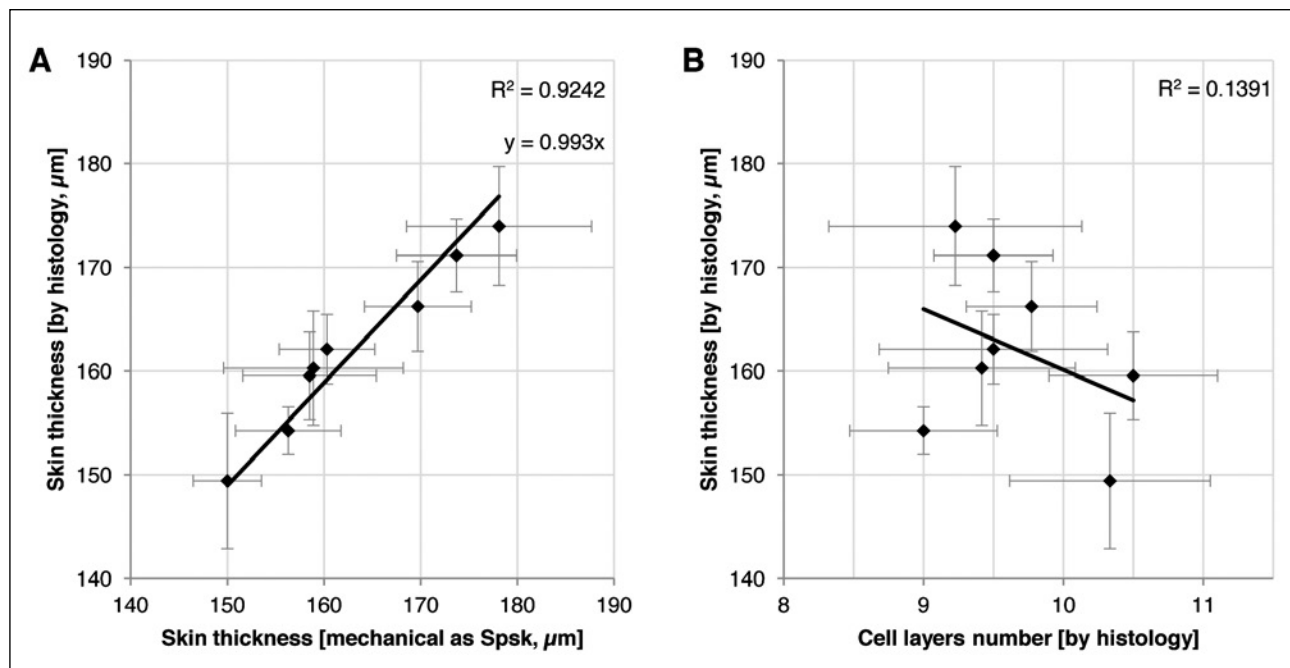


Fig. 3 - Correlation between skin thickness measured by means of instrumental mechanical properties technique and histology, and between histological cell layers number and skin thickness. Each point represents a berry group ($n = 10$, two groups per vineyard).

and C showing intermediate values (163 and 161 μm , respectively). The skin thickness estimated with the texture analysis equipment (as Sp_{sk}) showed similar values with respect to those measured on the histological sections. Indeed, there was not statistical difference between the values measured using the two different techniques. This was confirmed by the high correlation ($R^2 = 0.9242$) found between the values recorded with the two methods, as shown in the correlation graph in Fig. 3A.

The obtained values highlighted that there was no correlation ($R^2 = 0.1391$) between the skin thickness and the cell layers number, both analyzed by histology (Fig. 3B). This means that the different skin thickness recorded between cv. Corvina vineyards was due essentially to

the different Epidermis and Hypodermis cells size. Some authors reported that the skin thickness is strictly dependent on the different number of cell layers (CONSIDINE and KNOX, 1979; ROUDOT, 2006; HARDIE *et al.*, 2008; MUGANU *et al.*, 2011) but they based their observation on different grape varieties with respect to the present study.

We can conclude that the comparison between histological observation and texture analysis determination confirmed that the instrumental skin thickness technique by texture analysis give similar values of skin thickness in relation to those obtained by histology. The use of the texture analysis method can speed up the analysis process, minimizing the sample treatment.

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