Berry Heterogeneity as a Possible Factor Affecting the Potential of Seed Mechanical Properties to Classify Wine Grape Varieties and Estimate Flavanol Release in Wine-like Solution

F. Torchio†, S. Giacosa†, S. Río Segade*, V. Gerbi, L. Rolle

Università di Torino, DISAFA – Dipartimento di Scienze Agrarie, Forestali e Alimentari, Via Leonardo da Vinci 44, 10095 Grugliasco, Italy

† These authors contributed equally to this study

Submitted for publication: August 2013
Accepted for publication: October 2013

Key words: Instrumental texture analysis, mechanical parameters, wine grape seeds, berry heterogeneity, varietal classification, extractable phenols prediction

Seed mechanical properties were instrumentally measured by compression testing in thirty white and red wine grape varieties at harvest. The effect of berry heterogeneity in a vineyard on these seed texture parameters was also evaluated to improve the understanding of intra-sample variability. Furthermore, the mechanical properties of the seeds were assessed as possible predictors of their phenolic extractability. The results show that the texture parameters of the seeds are independent of the location of the berry in the vineyard and the soluble solid content at harvest. Densimetric flotation of the berries permits the reduction of the intra-sample variability that could hinder the differentiation and/or classification of wine grape varieties according to seed mechanical attributes. Cluster analysis classified the wine grape varieties studied into three groups according to seed hardness (low: 32.51 to 40.80 N, intermediate: 42.84 to 44.99 N, high: 46.71 to 57.78 N). The relationships between the seed mechanical properties and the extractable content of phenolic compounds, determined by spectrophotometric and chromatographic reference chemical methods, were evaluated by means of correlation analysis. Linear regression calibration models were developed for each cluster. The statistical parameters highlighted that total flavonoids, proanthocyanidins and flavanols reactive to vanillin can be predicted successfully from the seed mechanical properties for the varieties having low and intermediate seed hardness (SEC% ca. 20, RPIQ > 1.6). For varieties with harder seeds, a satisfactory predictive accuracy seems to require the construction of separate calibration models for each cultivar (Nebbiolo, SEC% ca. 20, RPIQ > 2.2).

INTRODUCTION

The phenolic composition of the grape strongly contributes to the sensorial quality of the wine. A study performed to determine the relationship between the phenolic composition of the grapes and the projected market price of the resulting wines showed that wines in a lower price category presented lower flavanol contents (Cáceres *et al.*, 2012). Flavanols are important phenolic compounds in the grape and wine because these compounds govern perceived quality parameters like astringency, bitterness and colour (Peleg *et al.*, 1999; Cheynier *et al.*, 2006; McRae & Kennedy, 2011; Obreque-Slier *et al.*, 2011). Grape seeds are a rich source of gallic acid and flavanols, particularly monomeric catechins (catechin, epicatechin and epicatechin-3-O-gallate), as well as their oligomeric and polymeric procyanidins (Monagas *et al.*, 2003; Rodríguez Montealegre *et al.*, 2006; Mattivi *et al.*, 2009; Ferrer-Gallego *et al.*, 2010). Low molecular weight flavanols are important determinants of bitterness, whereas astringency increases with an increase in the chain length and galloylation percentage (Peleg *et al.*, 1999; Cheynier *et al.*, 2006; Ferrer-Gallego *et al.*, 2010; Obreque-Slier *et al.*, 2011). Otherwise, flavanols undergo complex interactions with anthocyanins during winemaking and wine ageing, and therefore play a key role in the colour of red wines (Cheynier *et al.*, 2006; Pérez-Magariño & González-San José, 2006; Puškaš & Miljić, 2012).

Wine grape varieties differ widely in the qualitative and quantitative flavanolic composition of the seeds (Rodríguez Montealegre *et al.*, 2006; Mattivi *et al.*, 2009; Kotseridis *et al.*, 2012; Obreque-Slier *et al.*, 2012). This compositional diversity is of great technological relevance for the adaptation

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**Corresponding author: E-mail address: susana.riosegade@unito.it [Tel.: +39 0116708558; Fax: +39 0116708549]*

Aknowledgements: Part of this study was funded by the Fondazione Fojanini di Studi Superiori (Sondrio, Italy), and part by the project Ricerca & Innovazione per il Miglioramento della Sostenibilità della Filiera Agro-alimentare ECOFOOD – Finanziamento PSR-FEASR – cofinanziamento dall'UE, dal Ministero dell'economia e delle Finanze e dalla Regione Piemonte; Pubblicazione n° 05. The authors thank Anna Schneider, for the samples collected in Piedmont, and Graziano Murada, for kindly providing the grape samples from Valtellina

of the winemaking techniques to the oenological potential of a particular cultivar (Sacchi *et al.*, 2005). Furthermore, the changes occurring during grape ripening affect the content and extraction of phenolic compounds in the seeds. The extractable amount of seed flavanols gradually declines with the advance of ripening because of oxidation reactions that favour the increased association of these compounds with cell-wall components (Kennedy *et al.*, 2000a, 2000b; Downey *et al.*, 2003; Cadot *et al.*, 2006; Ferrer-Gallego *et al.*, 2010; Lorrain *et al.*, 2011; Obreque-Slier *et al.*, 2012). In this sense, the knowledge at harvest of the flavanolic composition of the seeds that can be extracted into the wine facilitates winemaking management.

The medium integument undergoes an intensive lignification during ripening, which results in hardening of the grape seed (Cadot *et al.*, 2006) and, therefore, the hardness has been proposed as a sensory descriptor of the seeds (Le Moigne *et al.*, 2008). Although wine grape tasting is a well-recognised tool that is used by many wine professionals to support harvesting decisions, the subjectivity associated with the sensory perception of this texture property and the berry heterogeneity make it difficult to discriminate between ripening stages. High data variability can be reduced by means of objective measurements based on instrumental texture analysis. The compression parameters are closely related to the extractable content of phenolic compounds during ripening, particularly in the seeds. The resistance of the seed to axial deformation is negatively correlated with the phenol extractability (Rolle *et al.*, 2012a), and the seed break force is also intrinsically linked to the extractable content of oligomeric flavanols (Torchio *et al.*, 2012). Nevertheless, the robustness of these correlations was low, probably due to the fact that the studies were conducted either for total phenols or on heterogeneous berries in terms of ripeness at each harvest date.

The berry heterogeneity inside the vineyard is a limiting factor for characterising grape varieties and discriminating between ripening stages because of the variability in the physicochemical characteristics. Some authors have even proposed exploiting the separation of berries from different cluster positions for the elaboration of different quality wines (Noguerol-Pato *et al.*, 2012). Others have chosen the densimetric sorting of the berries as the best option in the winery to separate grapes with different quality parameters, this process being automatised through the use of densimetric berry-sorting machines (Rolle *et al.*, 2012b).

In this work, thirty wine grape varieties were characterised according to the mechanical properties of the intact seeds measured during compression testing. The effect of the berry heterogeneity in a vineyard was evaluated to improve the understanding of the intra-sample variability that could influence the discriminating ability of the seed texture parameters. The densimetric sorting allowed the berries to be separated at harvest into more homogeneous groups in terms of ripeness, thereby also facilitating the study of the ripening effect on the texture attributes of the seeds. Furthermore, the performance of the instrumental mechanical parameters of grape seeds as possible predictors of the extractable phenolic composition determined by reference chemical methods was comprehensively investigated by means of the establishment of robust relationships using the varieties studied. The modelling of those strongest and most significant correlations may provide insight into the rapid estimation of the amount of phenolic compounds in grape seeds that can be extracted during winemaking.

MATERIALS AND METHODS

Grape samples

Whole bunches of seven white (Arneis, Chardonnay, Erbaluce, Malvasia Bianca, Moscato Bianco, Riesling Italico, Sauvignon Blanc) and 23 red (Albarossa, Avanà, Barbera, Brachetto d'Acqui, Cabernet Sauvignon, Calabrese, Cinsault, Croatina, Dolcetto, Freisa, Grenache, Grignolino, Malvasia di Schierano, Merlot, Mourvèdre, Nebbiolo, Petit Rouge, Petit Verdot, Pignolo Spano, Pinot Noir, Ruchè, Sangiovese, Syrah) grape cultivars (*Vitis vinifera* L.) were harvested at technological maturity from the same vineyard located in Grinzane Cavour (Piedmont, Cuneo Province, north-west Italy) in 2010. Furthermore, grape samples of the Nebbiolo red cultivar were collected at two different ripening stages in ten commercial vineyards located in Valtellina (Sondrio Province, Lombardy, northern Italy) in 2010 and 2011. The study was performed separately for each wine grape cultivar, harvest date and vineyard. Bunches (n = 10) were randomly collected from ten vines (one bunch per vine) selected to ensure a representative sampling. Once in the laboratory, a subsample of approximately 1.5 kg of grapes (ca. 1 000 to 1 200 berries) was randomly selected by picking berries from different positions in the cluster (shoulders, middle and bottom). For each subsample, one set of 100 berries was randomly selected and used for determining the technological ripeness parameters in the grape must obtained by manual crushing and centrifugation at 3 000 × g for 10 min at 20ºC (Universal 32 R, Hettich, Tuttlingen, Germany).

For the estimation of the variability in the instrumental texture parameters of the seeds within and among clusters, the seeds (n = 5) of Pinot Noir berries located in a given position of the cluster were analysed separately from those of the berries located in the other two positions, with a total of 15 seeds per cluster (Torchio *et al.*, 2012). The study of the effect of berry heterogeneity on the mechanical properties of the seeds also required an analysis of all the seeds belonging to the same berry for one set of 10 Pinot Noir berries randomly sampled. Afterwards, in order to better define the different ripening states present at the same harvest date and to improve intra-sample homogeneity, the remaining Pinot Noir berries were sorted according to their density by flotation using different saline solutions (from 100 to 170 g/L sodium chloride) and following the protocol described by Rolle *et al.* (2011). The berry density classes selected were: A = 1 075 kg/m^3, B = 1 081 kg/m^3, C = 1 088 kg/m^3 and D = 1 094 kg/m^3. For each density class, a subsample of 50 sorted berries was used to evaluate the effect of the density class on the mechanical properties of the seeds (n = 50). An average subsample of 50 unsorted berries (UnS) was also used with the same last aim, with the total number of seeds analysed being 250.

The remaining berries of the other 29 wine grape varieties were also sorted densimetrically as mentioned above, with the exception of the Nebbiolo cultivar from the Valtellina growing zone. In this case, the selected berry density classes were: D = 1 094 kg/m^3, E = 1 100 kg/m^3 and F = 1 107 kg/m^3, with the exception of the Cinsault and Sangiovese cultivars, in which density class C (1 088 kg/m^3) predominated over the F. The criterion was to select berries with three ripeness grades that corresponded to the most representative values of probable alcohol (12.5, 13.5 and 14.5% v/v, respectively). The berries in each density class were weighed and the distribution percentage was established. For both variety and density class (or harvest date and vineyard for the Nebbiolo from Valtellina), one set of 30 sorted berries was randomly selected and subdivided into three replicates of 10 grape berries that were weighed with a technical balance (Gibertini E1700, Modena, Italy). Subsequently, the three replicates of 10 berry seeds obtained were also weighed. The intact seeds of each replicate were individually compressed and quickly immersed in an extracting wine-like solution. In all studies, unless stated otherwise, only one randomly selected seed per berry was used for analysis. These were previously separated from the pulp and carefully cleaned with adsorbent paper. This permitted a wider variation range with the same number of seeds to be covered, facilitating the characterisation of wine grape varieties according to the mechanical properties of the seeds, the evaluation of the ripening effect on these texture parameters, and the prediction of extractable phenolic compounds of the seeds, determined by reference chemical methods, from the mechanical attributes.

Instrumental mechanical properties

The mechanical properties were determined directly on the intact seeds by a compression test. Each seed was individually compressed at 1 mm/s speed under 50% deformation using a TA.XT2i Plus Texture Analyzer (Stable Micro Systems, Surrey, UK) equipped with an SMS HDP/90 platform, an SMS P/35 probe and a 50 kg load cell (Torchio *et al.*, 2012). The following instrumental mechanical parameters were determined: the seed break force (N, as F_s), the seed break energy (mJ, as W_s), the resistance of the seed to the axial deformation (N/mm, as E_s), and the seed deformation index (%, as DI_s) (Rolle *et al.*, 2012c). This last index was calculated as the distance of the seed break point/seed height × 100. Before each test session, the instrument was calibrated for force and distance.

Chemical analysis

Solvents of HPLC gradient grade and all other chemicals of analytical reagent grade were purchased from Sigma (Milan, Italy). The solutions were prepared in deionised water produced by a Purelab Classic system (Elga Labwater, Marlow, United Kingdom). Of the phenol standards, gallic acid (GA), (+)-catechin (CA), (-)-epicatechin (EC) and (-)-epicatechin gallate (ECG) were obtained from Sigma, and cyanidin chloride and procyanidins B_1 and B_2 were purchased from Extrasynthèse (Genay, France).

Technological ripeness parameters. The concentration of total soluble solids (°Brix, as SSC) was measured with an Atago 0 to 32°Brix temperature-compensating refractometer (Atago Corporation, Tokyo, Japan), and the pH was determined by potentiometry using a Crison electrode (Carpi, Italy). Titratable acidity (TA), expressed as g/L of tartaric acid, was estimated using the method of the International Organisation of Vine and Wine (OIV, 2008). Malic acid and tartaric acid were quantified (g/L) using a P100-AS3000 HPLC system (Thermo Electron Corporation, Waltham, MA, USA) equipped with a UV detector (UV3000) set to 210 nm. The analyses were performed according to the method proposed by Giordano *et al.* (2009). The data analysis was carried out using the ChromQuest chromatography data system (ThermoQuest, Inc., San Jose, CA, USA).

Extraction and determination of seed phenols. In the reference method, each replicate of 10 berry seeds previously compressed was immediately immersed in 10 mL of a wine-like solution and maintained at 25°C for seven days (Torchio *et al.*, 2012). This wine-like hydroalcoholic solution consisted of ethanol/water (12/88 v/v) containing 2 g/L $Na_2S_2O_5$ (to avoid the possible oxidation of phenolic compounds) and 5 g/L tartaric acid, which was buffered at pH 3.2 using NaOH 0.1N. The extracts were filtered through a 0.20 μm filter, bottled and stored at 4°C until they were analysed. Spectrophotometric methods were used to determine absorbance at 280 nm (as A_{280}) and the extractable content of total flavonoids [mg (+)-catechin/kg grape or mg/g seed, as TF], proanthocyanidins (viz. polymeric flavanols, expressed as mg cyanidin chloride/kg grape or mg/g seed, as PRO) and flavanols reactive to vanillin [viz. oligomeric flavanols, expressed as mg (+)-catechin/kg grape or mg/g seed, as FRV] (Di Stefano & Cravero, 1991; Torchio *et al.*, 2012). Proanthocyanidins were determined after acid hydrolysis with warming (Bate-Smith reaction), using a ferrous salt ($FeSO_4$) as catalyst. A UV-1800 spectrophotometer (Shimazdu Corporation, Kyoto, Japan) was used. The relative standard deviations of phenolic compound determinations, based on repeated analyses (n = 20) of 10 sample extracts, were 0.93, 1.74 and 2.80% for TF, PRO and FRV, respectively (Torchio *et al.*, 2010).

The determination of gallic acid and individual monomeric and dimeric flavanols was performed according to a method adapted from Downey *et al.* (2003). An Agilent 1260 Infinity HPLC system (Milford, MA, USA) equipped with a diode array detector (DAD) was used. The chromatographic separation was carried out at 25°C on a LiChroCART 250-2 Purospher STAR RP-18 analytical column (5 μm, 250 mm × 2 mm i.d.) purchased from Merck (Darmstadt, Germany). The injection volume was 20 μL. The mobile phases consisted of 0.2% aqueous phosphoric acid (mobile phase A) and a mixture of acetonitrile/0.2% phosphoric acid (4:1) (mobile phase B) at a flow rate of 0.2 mL/min. The two mobile phases were filtered through a 0.20 μm filter. A linear gradient was used for the separation of flavanols, starting at 0% B to 10% B in 5 min, maintaining at 10% B for 35 min, increasing to 17% B in 15 min, to 19% B in 10 min, and maintaining at 19% B for 10 min. The column was then washed with 100% B for 10 min and equilibrated for 10 min prior to each analysis. The UV-VIS spectra were acquired from 230 to 400 nm, and the detection wavelength was set at 280 nm. The identification was achieved by comparing the absorption spectra and retention times with those of pure standards. The quantification (mg/kg grape or mg/g seed) was carried out by the external standard method. All analyses were performed in triplicate.

Statistical analysis

Statistical analyses were carried out using the SPSS software package version 19.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to establish significant differences. A cluster analysis was performed to classify wine grape varieties according to the mechanical properties of the seeds, using the average linkage between groups and squared Euclidean distance. Pearson's correlation coefficients were calculated to determine significant relationships between the instrumental texture parameters and the phenolic composition of the seeds. For prediction purposes, the sample set was randomly subdivided into the calibration set (about 2/3) and the validation set (about 1/3), the two sets being associated with comparable ranges of phenolic compounds. The performance of calibration models developed by regression analysis was assessed from the correlation coefficient of calibration (R_c) and the standard error of calibration (SEC). The standard error of calibration was also standardised (SEC%) by rating its value to the mean of the calibration population, and it is related to the mean error of the model. On the other hand, the predictive accuracy of the calibration models was evaluated from the correlation coefficient of validation (R_v) and the standard error of prediction (SEP). Furthermore, the coefficient of variation (SEP%) was calculated as the ratio of the SEP value to the mean of the validation population. Other indices evaluated with this objective were the residual predictive deviation (RPD) and the residual predictive interquartile amplitude (RPIQ). The first statistical index is the most commonly used to account for model reliability and was defined as the ratio between the standard deviation (SD) of the validation set and the SEP value. The other is based on quartiles and was calculated as the ratio of the interquartile amplitude of the validation population to the SEP value (Bellon-Maurel *et al.*, 2010).

RESULTS AND DISCUSSION

Effect of berry heterogeneity on seed mechanical properties

A preliminary study was performed on the Pinot Noir cultivar to assess if the berry heterogeneity typically present in a vineyard influences the variability in the mechanical properties of the seeds at harvest. The differences in the texture parameters of the seed tissues were not significant ($p > 0.05$) within the same berry or among grape berries randomly sampled. On the other hand, the mechanical attributes also agreed for the seeds belonging to berries from different positions within the cluster ($p > 0.05$). This confirms the results reported in another work, where no influence of the position of the grape berry within the cluster was observed on the mechanical properties of the seeds (Torchio *et al.*, 2012). For most of the clusters analysed, the variability (as relative standard deviation) was higher in the middle position than in the shoulder and bottom positions. The mechanical attributes of the seeds agreed statistically for berries from different clusters ($p > 0.05$). After verifying that the location of the berry in the vineyard was not an influencing factor on the texture parameters of the seeds, the study was completed with the evaluation of the berry density effect on these parameters at the harvest date. There were no significant differences ($p > 0.05$) in the mechanical parameters of the seeds from berries belonging to the four density classes studied. Figure 1 shows that the highest variability corresponded to the average subsample (unsorted berries), whereas the ripest berries (density classes C and D) had the lowest variability in all of the mechanical properties of the seeds, although this behaviour was not as evident for the seed deformation index. The variation within the average subsample was even higher than that found among clusters.

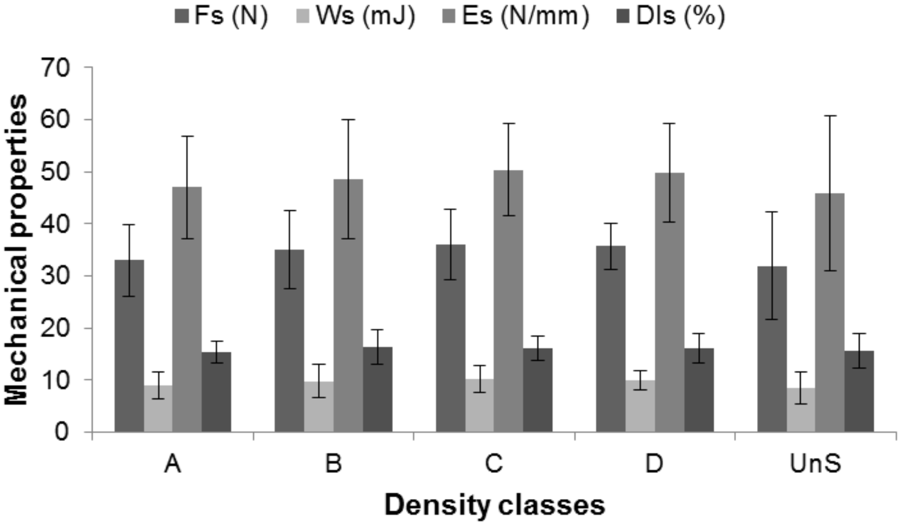

FIGURE 1

Instrumental mechanical properties of Pinot Noir seeds from densimetrically sorted grape berries at harvest. A = 1 075 kg/m^3, B = 1 081 kg/m^3, C = 1 088 kg/m^3, D = 1 094 kg/m^3, UnS = unsorted. F_s = seed break force, W_s = seed break energy, E_s = resistance of the seed to the axial deformation, DI_s = seed deformation index.

Therefore, the densimetric sorting of the berries permits the reduction of the intrasample variability that could hinder the differentiation and/or classification of wine grape varieties according to the mechanical attributes of the seeds.

Effect of ripening on seed mechanical properties

Table 1 shows the distribution percentage of the berries in four density classes and average technological ripeness parameters at harvest for a total of thirty wine grape varieties. For each variety, berries with different total soluble solids contents (SSC) are present in a vineyard because of a heterogeneous ripening process. Furthermore, the distribution percentage depended mainly on the variety. In fact, varieties with the same value of SSC had different distribution percentages, and no other technological ripeness parameter seemed to be a determining factor in this distribution. The average SSC at harvest ranged from 19.5 to 25.4 °Brix, corresponding to the Syrah and Avaná cultivars, respectively. Within each density class there were differences in the SSC values among varieties with variation ranges of 17.9 to 21.4, 18.7 to 23.5, 21.8 to 24.9 and 21.4 to 25.4 ºBrix for the density classes C, D, E and F, respectively. This could be due to the size effect on berry density. Berries with the same SSC can have different sizes and therefore would belong to different density classes. The sugar concentration is directly linked to berry size and berry fresh weight by means of a negative correlation (Dai *et al.*, 2009). These last two parameters also showed an inverse trend with berry density (Rolle *et al.*, 2012b). Therefore, it was necessary to study the effect of berry maturity in terms of density on the texture parameters of

TABLE 1
Distribution percentage of berries in four density classes and average technological ripeness parameters at harvest.

Grape variety	Colour	Berries distribution in density classes				Technological ripeness parameters				
		C (%)	D (%)	E (%)	F (%)	SSC (ºBrix)	TA (g/L tartaric acid)	pH	Malic acid (g/L)	Tartaric acid (g/L)
Albarossa	R	5.1	30.7	26.3	28.7	23.6	10.72	2.87	2.6	10.0
Arneis	W	11.5	17.0	10.1	37.2	23.1	6.56	3.10	1.7	7.1
Avaná	R	10.9	8.7	9.0	32.2	25.4	5.55	3.24	1.4	7.3
Barbera	R	17.9	8.3	8.7	25.4	24.3	10.59	2.96	2.8	8.9
Brachetto d'Acqui	R	3.1	22.9	28.0	34.6	22.6	8.84	3.11	3.8	7.4
Cabernet Sauvignon	R	13.0	27.8	42.8	16.4	22.6	6.43	3.19	2.1	6.8
Calabrese	R	49.1	38.5	7.6	4.9	21.0	7.99	3.02	2.5	7.8
Chardonnay	W	16.2	36.2	36.7	6.3	22.6	7.40	3.24	2.6	6.9
Cinsault	R	21.6	51.8	21.6	1.7	22.7	4.98	3.24	2.4	5.7
Croatina	R	22.3	11.1	33.0	28.0	22.2	6.45	3.19	2.4	6.6
Dolcetto	R	43.0	24.9	17.6	11.8	20.5	6.24	3.30	2.4	6.8
Erbaluce	W	32.9	35.7	17.7	10.6	21.9	7.14	3.08	2.1	7.6
Freisa	R	0.8	9.7	43.1	38.2	22.8	8.62	3.15	3.4	6.9
Grenache	R	4.3	15.1	28.8	21.4	23.6	5.49	3.25	2.0	5.9
Grignolino	R	11.5	11.0	37.5	26.7	22.5	6.84	3.20	2.7	6.3
Malvasia Bianca	W	46.1	12.7	24.0	16.3	20.8	6.54	3.19	2.8	6.2
Malvasia di Schierano	R	22.4	35.3	30.6	9.1	21.7	6.00	3.17	2.3	6.3
Merlot	R	3.7	34.2	39.4	20.2	23.2	5.55	3.47	2.4	6.6
Moscato Bianco	W	19.2	14.0	24.8	25.4	22.4	7.21	3.18	2.7	7.0
Mourvèdre	R	43.2	35.6	15.1	3.9	21.1	7.15	3.28	4.1	5.5
Nebbiolo	R	1.6	16.0	65.5	16.3	23.3	8.50	2.96	2.2	8.2
Petit Rouge	R	11.4	18.6	24.8	31.8	24.1	6.08	3.23	2.2	6.7
Petit Verdot	R	0.0	14.3	38.6	45.2	22.4	8.85	3.19	4.5	6.4
Pignolo Spano	R	5.6	18.9	35.7	19.0	22.6	5.72	3.24	2.4	6.1
Pinot Noir	R	9.6	23.9	34.4	22.0	23.1	7.32	3.22	2.8	6.7
Riesling Italico	W	33.1	29.7	15.7	11.7	22.6	6.12	3.18	1.6	7.6
Ruchè	R	13.8	21.7	29.4	24.3	21.8	7.04	3.21	2.7	6.9
Sangiovese	R	27.2	47.4	15.1	0.0	21.6	7.55	3.08	2.7	7.1
Sauvignon Blanc	W	4.1	27.8	33.2	30.9	23.6	8.79	3.04	2.4	8.5
Syrah	R	71.6	19.2	6.3	2.8	19.5	6.98	3.19	3.4	6.1

SSC = total soluble solids content, TA = titratable acidity. R = red, W = white. C = 1 088 kg/m^3, D = 1 094 kg/m^3, E = 1 100 kg/m^3, F = 1 107 kg/m^3.

the seeds for wine grape varieties with different distribution percentages of the berries in density classes. As can be seen in Table 2, the mechanical properties of the seeds were not significantly influenced by the berry density at harvest for most of the varieties. Furthermore, the few significant differences found did not show a clear trend for the different varieties studied. Despite the intensive lignification of the tissues during ripening that involved seed hardening (Cadot *et al.*, 2006), some works have also reported no significant change in the mechanical attributes of berry seeds (F_s, W_s, E_s and DI_s) for densimetrically sorted Barbera grapes harvested at the same date (Torchio *et al.*, 2010). In contrast, significant differences were found in the compression parameters of Cabernet Sauvignon seeds (W_s, E_s and DI_s) when the ripening effect was studied for several weeks (Rolle *et al.*, 2012d). Nevertheless, the changes occurred only in seed stiffness (E_s) for sorted Cabernet Sauvignon grapes sampled at different harvest dates, even if one density class per sampling date was selected to emphasise the physiological differences among grape ripening stages (Rolle *et al.*, 2012a). Some researchers also noted that most of the texture parameters of the seeds became steady three weeks after the end of *véraison* (Letaief *et al.*, 2013). During the on-vine dehydration process of Mondeuse grapes, stability in the values of seed mechanical properties was reached after the 75th withering day (Rolle *et al.*, 2009). Therefore, the sampling date is a key factor in the visualisation of the ripening effect on the instrumental texture parameters of the seeds. On the other hand, the discriminating ability of the mechanical properties of the seeds may depend on the operative conditions used during the compression test (Torchio *et al.*, 2012).

Characterisation and differentiation of wine grape varieties according to seed hardness

After verifying that the mechanical properties of the seeds were not influenced by the berry heterogeneity present in the vineyard at the harvest date, the potential of the instrumental texture parameters of seed tissues as varietal markers was evaluated. The statistical analysis of average data for the three density classes in each wine grape cultivar revealed that the seed break force (F_s) was the best varietal marker, and that it was correlated significantly with the seed break energy (W_s), the resistance of the seed to the axial deformation (E_s) and the seed deformation index (DI_s), with Pearson's correlation

TABLE 2
Instrumental mechanical properties of seeds from densimetrically sorted berries at harvest.

Grape variety	Density class	F_s (N)	W_s (mJ)	E_s (N/mm)	DI_s (%)
Albarossa	D	36.88 ± 2.58	9.81 ± 0.63	62.16 ± 7.95	14.78 ± 0.72
	E	35.54 ± 2.92	8.97 ± 1.07	62.81 ± 2.08	14.15 ± 0.34
	F	32.15 ± 2.82	7.86 ± 1.37	58.98 ± 1.79	13.62 ± 0.88
Sign[a]		ns	ns	ns	ns
Arneis	D	54.28 ± 0.78b	13.91 ± 0.25b	90.95 ± 3.58	14.37 ± 0.52
	E	54.88 ± 2.58b	13.87 ± 0.08b	97.72 ± 10.17	13.98 ± 1.00
	F	48.45 ± 1.91a	11.76 ± 0.60a	93.11 ± 9.29	12.92 ± 1.05
Sign		*	***	ns	ns
Avaná	D	49.50 ± 3.98b	14.32 ± 1.21	74.25 ± 7.12b	15.14 ± 0.43
	E	36.52 ± 7.58a	11.67 ± 1.88	51.58 ± 12.39a	16.20 ± 1.61
	F	47.00 ± 4.11ab	13.77 ± 1.34	70.48 ± 6.78ab	15.56 ± 0.34
Sign		*	ns	*	ns
Barbera	D	45.28 ± 3.84	11.59 ± 0.94	80.03 ± 9.03	14.37 ± 0.94
	E	44.35 ± 2.40	10.64 ± 0.44	81.20 ± 4.83	14.53 ± 0.68
	F	44.24 ± 2.97	10.48 ± 1.44	86.20 ± 0.83	14.00 ± 1.45
Sign		ns	ns	ns	ns
Brachetto d'Acqui	D	40.43 ± 0.79ab	11.42 ± 0.29	62.67 ± 0.36	15.53 ± 0.61
	E	42.46 ± 1.68b	12.49 ± 1.67	69.50 ± 7.36	15.15 ± 1.82
	F	38.63 ± 1.88a	10.70 ± 1.58	67.03 ± 3.41	13.84 ± 1.73
Sign		*	ns	ns	ns
Cabernet Sauvignon	D	42.38 ± 1.85	10.88 ± 1.28	72.66 ± 2.95	15.46 ± 1.21
	E	44.91 ± 1.31	11.59 ± 0.39	77.33 ± 1.72	15.39 ± 0.29
	F	41.25 ± 3.19	10.24 ± 1.14	74.32 ± 2.06	14.28 ± 0.81
Sign		ns	ns	ns	ns
Calabrese	D	50.86 ± 9.37	12.61 ± 2.50	82.98 ± 8.52	14.83 ± 0.74
	E	49.86 ± 0.50	13.07 ± 0.05	79.17 ± 2.00	15.39 ± 0.26
	F	49.07 ± 2.45	12.12 ± 1.01	84.39 ± 4.37	14.72 ± 1.54
Sign		ns	ns	ns	ns

TABLE 2 (CONTINUED)

Grape variety	Density class	F_s (N)	W_s (mJ)	E_s (N/mm)	DI_s (%)
Chardonnay	D	37.13 ± 1.63	10.07 ± 0.49	61.46 ± 2.51	14.80 ± 0.25b
	E	36.41 ± 1.62	9.63 ± 0.65	63.64 ± 1.86	14.15 ± 0.65ab
	F	36.73 ± 1.78	9.23 ± 0.35	66.52 ± 4.55	13.12 ± 0.41a
Sign		ns	ns	ns	*
Cinsault	C	53.11 ± 2.73	14.81 ± 0.71	74.07 ± 8.01	18.68 ± 2.11
	D	59.00 ± 8.07	16.98 ± 1.38	81.53 ± 12.35	18.41 ± 1.98
	E	61.22 ± 2.56	16.47 ± 1.04	89.56 ± 7.10	16.14 ± 0.51
Sign		ns	ns	ns	ns
Croatina	D	41.23 ± 1.47	10.21 ± 0.31	69.05 ± 4.09	14.47 ± 0.11
	E	43.99 ± 2.10	11.12 ± 0.89	73.70 ± 1.46	14.88 ± 0.75
	F	45.04 ± 4.53	11.45 ± 1.53	75.17 ± 4.87	15.09 ± 0.75
Sign		ns	ns	ns	ns
Dolcetto	D	38.74 ± 3.48	9.65 ± 1.48	65.12 ± 1.99	14.64 ± 1.17
	E	37.57 ± 0.19	9.08 ± 0.55	66.66 ± 4.72	14.35 ± 0.86
	F	37.76 ± 1.26	8.93 ± 0.44	69.09 ± 3.44	13.28 ± 0.29
Sign		ns	ns	ns	ns
Erbaluce	D	35.49 ± 0.37a	9.25 ± 0.64ab	64.50 ± 2.87	13.24 ± 1.07
	E	39.29 ± 0.58b	10.03 ± 0.14b	72.08 ± 2.48	14.20 ± 1.97
	F	35.38 ± 2.18a	8.72 ± 0.30a	70.03 ± 5.62	12.04 ± 0.19
Sign		*	*	ns	ns
Freisa	D	50.60 ± 4.93	14.88 ± 1.56	75.44 ± 9.21	16.44 ± 0.73
	E	56.20 ± 4.25	18.13 ± 2.56	74.47 ± 3.21	18.32 ± 1.43
	F	56.33 ± 1.26	17.20 ± 0.31	79.67 ± 3.82	17.90 ± 1.04
Sign		ns	ns	ns	ns
Grenache	D	32.89 ± 0.18	8.42 ± 0.03	53.89 ± 1.16	14.28 ± 0.08
	E	31.94 ± 2.12	8.18 ± 1.06	51.81 ± 1.75	16.29 ± 1.81
	F	32.69 ± 0.71	8.46 ± 0.34	54.77 ± 1.47	14.84 ± 0.67
Sign		ns	ns	ns	ns
Grignolino	D	39.25 ± 3.53	10.50 ± 0.77	67.81 ± 6.69	13.57 ± 0.43a
	E	39.28 ± 2.76	10.66 ± 1.07	67.36 ± 1.30	14.52 ± 0.27b
	F	37.95 ± 1.06	9.62 ± 0.61	68.87 ± 2.51	13.66 ± 0.26a
Sign		ns	ns	ns	*
Malvasia Bianca	D	47.88 ± 4.91	12.38 ± 2.18	77.84 ± 1.08	15.11 ± 1.30
	E	45.76 ± 1.88	11.53 ± 0.49	74.86 ± 6.28	14.86 ± 0.97
	F	46.48 ± 3.04	11.91 ± 1.74	78.34 ± 0.19	14.69 ± 1.07
Sign		ns	ns	ns	ns
Malvasia di Schierano	D	34.19 ± 2.33	8.90 ± 0.60	56.32 ± 5.73	14.80 ± 0.87
	E	36.25 ± 2.66	9.53 ± 1.00	60.96 ± 3.54	14.55 ± 0.26
	F	34.26 ± 1.86	8.96 ± 0.87	59.93 ± 1.92	13.99 ± 0.60
Sign		ns	ns	ns	ns
Merlot	D	47.31 ± 2.65	13.03 ± 1.29	77.46 ± 1.97a	14.55 ± 0.90b
	E	46.94 ± 3.71	11.43 ± 1.22	86.82 ± 1.64b	12.99 ± 0.57a
	F	47.04 ± 1.50	11.43 ± 0.85	90.43 ± 2.51b	12.71 ± 0.31a
Sign		ns	ns	***	*
Moscato Bianco	D	31.91 ± 1.76a	8.01 ± 0.49a	57.19 ± 2.67a	13.49 ± 0.11a
	E	36.76 ± 0.21b	9.60 ± 0.47b	64.82 ± 1.15b	14.21 ± 0.14b
	F	35.21 ± 1.50b	8.84 ± 0.46ab	61.77 ± 4.02ab	14.04 ± 0.21b
Sign		*	*	*	**

TABLE 2 (CONTINUED)

Grape variety	Density class	F_s (N)	W_s (mJ)	E_s (N/mm)	DI_s (%)
Mourvèdre	D	52.06 ± 3.31	14.73 ± 1.83	79.89 ± 2.44	14.74 ± 0.46
	E	49.76 ± 1.11	14.25 ± 1.38	75.65 ± 3.84	14.73 ± 0.97
	F	48.71 ± 1.60	13.60 ± 1.10	77.81 ± 2.22	13.56 ± 0.24
Sign		ns	ns	ns	ns
Nebbiolo	D	53.29 ± 4.06	14.58 ± 1.94	81.45 ± 1.37	16.80 ± 1.36
	E	47.30 ± 0.82	12.39 ± 0.82	78.86 ± 1.57	15.24 ± 0.26
	F	45.46 ± 3.73	11.09 ± 1.40	80.16 ± 2.62	14.94 ± 0.99
Sign		ns	ns	ns	ns
Petit Rouge	D	55.32 ± 0.62b	15.84 ± 0.15b	83.74 ± 2.84	14.93 ± 0.39
	E	49.76 ± 1.45a	12.70 ± 0.88a	86.87 ± 2.03	13.50 ± 0.71
	F	48.78 ± 2.64a	12.67 ± 0.68a	81.53 ± 6.67	14.26 ± 0.65
Sign		**	**	ns	ns
Petit Verdot	D	52.74 ± 0.64a	14.96 ± 0.77	80.82 ± 3.76	16.41 ± 0.98
	E	52.60 ± 0.47a	14.24 ± 0.38	83.52 ± 3.31	16.19 ± 0.44
	F	55.45 ± 0.19b	15.01 ± 0.54	89.44 ± 4.17	16.42 ± 0.57
Sign		***	ns	ns	ns
Pignolo Spano	D	47.23 ± 1.93	12.78 ± 0.26	77.39 ± 3.70	15.53 ± 0.48
	E	48.35 ± 1.66	13.24 ± 0.21	81.92 ± 6.13	16.52 ± 1.87
	F	50.81 ± 2.17	13.39 ± 1.15	84.98 ± 1.16	15.68 ± 0.50
Sign		ns	ns	ns	ns
Pinot Noir	D	38.46 ± 2.36	12.37 ± 1.39	51.73 ± 2.24a	18.88 ± 1.06b
	E	41.98 ± 1.75	12.27 ± 1.27	62.73 ± 1.60b	15.49 ± 0.89a
	F	41.96 ± 3.61	11.96 ± 1.99	66.72 ± 1.20c	15.15 ± 1.38a
Sign		ns	ns	***	*
Riesling Italico	D	34.87 ± 1.21	9.35 ± 0.87	59.88 ± 1.71a	14.81 ± 0.54
	E	37.96 ± 2.79	9.99 ± 1.14	65.82 ± 2.13b	16.23 ± 2.44
	F	37.85 ± 0.96	9.93 ± 0.37	65.54 ± 1.52b	16.25 ± 2.50
Sign		ns	ns	*	ns
Ruchè	D	38.80 ± 1.87	10.52 ± 0.39	65.91 ± 5.06	14.38 ± 0.47
	E	41.36 ± 0.34	11.29 ± 0.48	71.20 ± 1.71	14.63 ± 0.43
	F	40.05 ± 2.21	10.25 ± 0.90	72.88 ± 1.72	13.67 ± 0.08
Sign		ns	ns	ns	ns
Sangiovese	C	51.85 ± 2.02	15.40 ± 1.00	72.90 ± 2.56	16.39 ± 0.56
	D	53.02 ± 2.30	16.31 ± 0.80	69.11 ± 3.53	17.35 ± 0.30
	E	50.86 ± 0.75	15.16 ± 0.21	69.68 ± 5.22	16.75 ± 0.56
Sign		ns	ns	ns	ns
Sauvignon Blanc	D	38.12 ± 7.82a	10.18 ± 2.20	64.66 ± 12.13a	13.88 ± 1.33
	E	48.59 ± 1.87b	12.40 ± 0.57	83.51 ± 2.03b	13.78 ± 0.39
	F	48.27 ± 0.40b	12.23 ± 0.74	86.75 ± 4.74b	13.56 ± 0.77
Sign		*	ns	*	ns
Syrah	D	35.63 ± 1.48	9.45 ± 0.64	58.86 ± 1.86	14.90 ± 0.37
	E	35.87 ± 1.37	9.53 ± 0.87	59.72 ± 0.98	14.85 ± 0.41
	F	34.20 ± 4.17	9.54 ± 2.04	54.97 ± 5.25	15.75 ± 2.16
Sign		ns	ns	ns	ns

Data are expressed as mean value ± standard deviation (n = 3). Different Latin letters within the same column indicate significant differences (^{a}) among density classes according to the Tukey-b test ($p < 0.05$). Sign^a: *, **, *** indicate significance at $p < 0.05$, 0.01 and 0.001, respectively. ns = not significant. F_s = seed break force, W_s = seed break energy, E_s = resistance of the seed to axial deformation, DI_s = seed deformation index. C = 1 088 kg/m^3, D = 1 094 kg/m^3, E = 1 100 kg/m^3, F = 1 107 kg/m^3.

factors ranging from 0.486 to 0.943 ($p < 0.01$). A work recently published also highlighted that the seed texture properties measured by compression testing are positively correlated with each other (Letaief *et al.*, 2013).

The differentiating power of F_s is shown in Fig. 2, where the wine grape varieties studied were classified according to this compression parameter by cluster analysis. The varieties characterised by the softest seeds (F_s values ranging from 32.51 to 40.80 N) were included in the first cluster (upper side), whereas those having the hardest seeds (F_s values between 42.84 and 57.78 N) were grouped in the second cluster (bottom side). Nevertheless, the Cinsault cultivar was well differentiated inside this last cluster, and the other sub-cluster was composed of two other groups, well separated, including those varieties with F_s values ranging from 42.84 to 44.99 N and from 46.71 to 54.38 N. The differences found in seed hardness among the wine grape varieties studied showed that this mechanical parameter can be considered an ampelographic characteristic of each variety independently of the berry ripening grade, and therefore an efficient varietal marker.

Prediction of the extractable content of phenolic compounds from seed mechanical properties

Table 3 shows the reference values of some spectrophotometric indices often used in wineries to evaluate the phenolic composition extractable from the seeds into the wine-like solution for all wine grape varieties studied at the three ripening stages defined by berry density. Thus, the high natural variability in the quantitative phenolic composition of the seeds was considered. A total of 90 seed samples were analysed. Table 4 summarises the reference values for the extractable content of gallic acid and monomeric and dimeric flavanols in the same samples of seeds. All results were expressed per grape weight. For each wine grape variety, the spectrophotometric indices of the seeds were similar for berries belonging to different density classes, with very few exceptions. The lowest values of absorbance at 280 nm (A_{280}), as well as of the extractable content of total flavonoids (TF) and flavanols reactive to vanillin (FRV), corresponded to the Cinsault cultivar, followed by Grenache, whereas those of the extractable content of proanthocyanidins (PRO) were associated with the Cinsault cultivar, followed by Barbera and Sauvignon Blanc. In contrast, Petit Verdot and Pinot

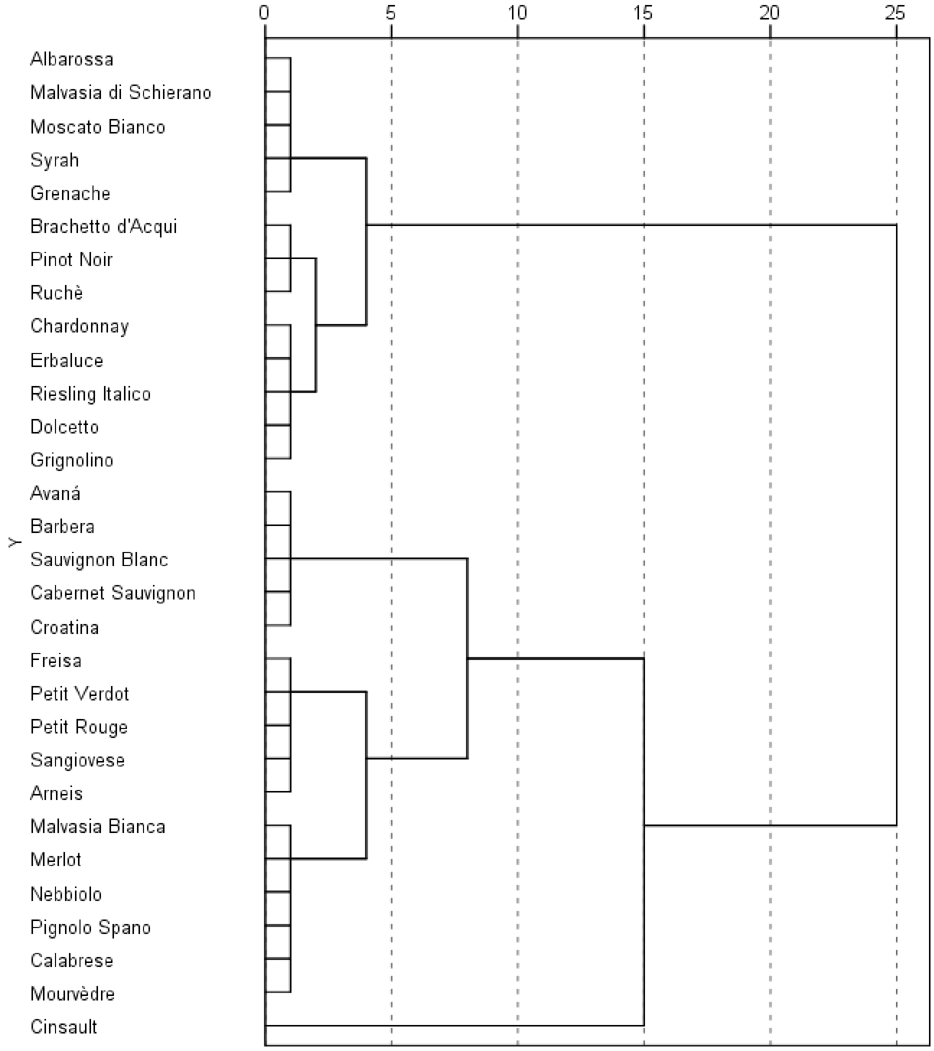

FIGURE 2

Dendogram of wine grape varieties by applying the average linkage between groups and squared Euclidean distance for hierarchical cluster analysis according to the seed break force.

TABLE 3
Spectrophotometric indices related to the phenolic composition of seeds per grape weight from densimetrically sorted berries at harvest.

Grape variety	Density class	A_{280} (1/kg)	TF (mg/kg)	PRO (mg/kg)	FRV (mg/kg)	FRV/PRO
Albarossa	D	43.5 ± 4.0	3 606 ± 261	2 391 ± 226b	1 677 ± 185	0.70 ± 0.05
	E	36.5 ± 2.9	3 136 ± 238	1 851 ± 119a	1 441 ± 144	0.78 ± 0.06
	F	43.0 ± 5.8	3 581 ± 411	2 309 ± 237b	1 779 ± 384	0.76 ± 0.08
Sign[a]		ns	ns	*	ns	ns
Arneis	D	38.4 ± 1.1	2 945 ± 33	1 945 ± 40	1 619 ± 144	0.83 ± 0.06
	E	37.6 ± 5.4	2 918 ± 298	2 026 ± 230	1 674 ± 160	0.83 ± 0.03
	F	35.3 ± 6.5	2 684 ± 469	1 787 ± 307	1 484 ± 214	0.83 ± 0.03
Sign		ns	ns	ns	ns	ns
Avaná	D	28.3 ± 2.0	2 026 ± 171	1 271 ± 208	1 065 ± 92	0.85 ± 0.08
	E	26.2 ± 2.5	1 986 ± 199	1 238 ± 143	972 ± 107	0.79 ± 0.09
	F	23.9 ± 3.5	1 752 ± 247	1 088 ± 137	951 ± 187	0.87 ± 0.07
Sign		ns	ns	ns	ns	ns
Barbera	D	30.0 ± 5.5	2 152 ± 372	1 090 ± 215	1 063 ± 87	0.99 ± 0.13
	E	26.3 ± 2.1	1 975 ± 110	1 040 ± 105	1 025 ± 79	0.99 ± 0.03
	F	24.7 ± 2.6	2 009 ± 194	1 114 ± 242	984 ± 106	0.90 ± 0.18
Sign		ns	ns	ns	ns	ns
Brachetto d'Acqui	D	60.9 ± 11.6	4 328 ± 576	2 573 ± 321	2 124 ± 254	0.83 ± 0.02
	E	48.1 ± 2.4	3 421 ± 244	2 038 ± 226	1 696 ± 159	0.83 ± 0.02
	F	53.2 ± 8.3	3 620 ± 389	2 236 ± 381	1 896 ± 125	0.86 ± 0.11
Sign		ns	ns	ns	ns	ns
Cabernet Sauvignon	D	45.7 ± 8.5	3 426 ± 463	2 603 ± 142	1 904 ± 45	0.73 ± 0.03
	E	48.4 ± 11.0	3 790 ± 667	2 887 ± 397	2 006 ± 158	0.70 ± 0.04
	F	46.9 ± 4.1	3 684 ± 291	2 620 ± 591	1 894 ± 187	0.74 ± 0.11
Sign		ns	ns	ns	ns	ns
Calabrese	D	36.5 ± 1.2	2 798 ± 229	1 477 ± 22	1 585 ± 70	1.07 ± 0.06ab
	E	33.3 ± 3.9	2 394 ± 324	1 401 ± 246	1 387 ± 140	1.00 ± 0.08a
	F	31.9 ± 0.9	2 273 ± 115	1 183 ± 55	1 413 ± 62	1.19 ± 0.02b
Sign		ns	ns	ns	ns	*
Chardonnay	D	40.4 ± 7.0	2 951 ± 347	1 879 ± 364	1 777 ± 263	0.95 ± 0.05
	E	37.2 ± 2.7	2 948 ± 285	1 764 ± 214	1 725 ± 152	0.98 ± 0.03
	F	32.0 ± 1.6	2 516 ± 33	1 583 ± 50	1 489 ± 32	0.94 ± 0.04
Sign		ns	ns	ns	ns	ns
Cinsault	C	19.1 ± 2.7	1 521 ± 222	984 ± 124	802 ± 118	0.81 ± 0.05
	D	16.6 ± 2.8	1 301 ± 197	832 ± 161	625 ± 140	0.75 ± 0.08
	E	16.2 ± 1.3	1 266 ± 51	829 ± 95	654 ± 77	0.79 ± 0.11
Sign		ns	ns	ns	ns	ns
Croatina	D	45.2 ± 6.0	3 370 ± 330	1 950 ± 266	1 792 ± 251	0.92 ± 0.04
	E	42.7 ± 2.3	3 169 ± 134	1 914 ± 25	1 606 ± 67	0.84 ± 0.04
	F	42.6 ± 3.5	3 175 ± 228	1 786 ± 203	1 693 ± 134	0.95 ± 0.10
Sign		ns	ns	ns	ns	ns
Dolcetto	D	36.8 ± 6.8	2 803 ± 273	1 879 ± 359	1 351 ± 112	0.73 ± 0.09
	E	34.3 ± 5.7	2 620 ± 504	1 683 ± 383	1 242 ± 218	0.75 ± 0.06
	F	38.4 ± 5.7	2 895 ± 428	1 881 ± 181	1 467 ± 275	0.78 ± 0.07
Sign		ns	ns	ns	ns	ns

TABLE 3 (CONTINUED)

Grape variety	Density class	A_{280} (1/kg)	TF (mg/kg)	PRO (mg/kg)	FRV (mg/kg)	FRV/PRO
Erbaluce	D	25.4 ± 2.6	2 057 ± 80	1 360 ± 109	1 026 ± 37	0.76 ± 0.06
	E	28.4 ± 1.1	2 101 ± 147	1 394 ± 34	1 041 ± 46	0.75 ± 0.02
	F	25.5 ± 3.1	1 891 ± 73	1 250 ± 57	906 ± 88	0.73 ± 0.09
Sign		ns	ns	ns	ns	ns
Freisa	D	55.7 ± 4.3	4 031 ± 373	2 325 ± 262	1 570 ± 108	0.68 ± 0.03
	E	55.9 ± 4.6	3 803 ± 511	2 295 ± 175	1 446 ± 227	0.63 ± 0.05
	F	48.9 ± 5.2	3 467 ± 116	2 041 ± 50	1 323 ± 93	0.65 ± 0.03
Sign		ns	ns	ns	ns	ns
Grenache	D	23.7 ± 1.7	1 862 ± 133	1 303 ± 109	842 ± 59	0.65 ± 0.02
	E	22.5 ± 6.0	1 776 ± 497	1 297 ± 307	814 ± 221	0.62 ± 0.03
	F	23.9 ± 0.8	1 868 ± 68	1 351 ± 54	888 ± 85	0.66 ± 0.06
Sign		ns	ns	ns	ns	ns
Grignolino	D	37.3 ± 8.1	3 149 ± 404	2 346 ± 470	1 817 ± 219	0.78 ± 0.08
	E	42.6 ± 3.2	3 315 ± 93	2 510 ± 215	1 859 ± 136	0.74 ± 0.02
	F	33.6 ± 2.2	3 008 ± 333	2 407 ± 443	1 691 ± 157	0.71 ± 0.08
Sign		ns	ns	ns	ns	ns
Malvasia Bianca	D	30.6 ± 0.8	2 181 ± 328	1 419 ± 200	1 063 ± 174	0.75 ± 0.04
	E	36.2 ± 6.0	2 624 ± 358	1 664 ± 328	1 261 ± 201	0.76 ± 0.07
	F	29.2 ± 4.9	2 178 ± 280	1 480 ± 285	1 209 ± 223	0.82 ± 0.07
Sign		ns	ns	ns	ns	ns
Malvasia di Schierano	D	33.6 ± 3.1	2 465 ± 122	1 454 ± 48	1 204 ± 87	0.83 ± 0.04
	E	37.1 ± 6.1	2 746 ± 315	1 686 ± 166	1 341 ± 167	0.79 ± 0.05
	F	37.6 ± 5.2	2 810 ± 350	1 745 ± 270	1 437 ± 109	0.83 ± 0.09
Sign		ns	ns	ns	ns	ns
Merlot	D	40.0 ± 4.6	2 971 ± 119	2 095 ± 162	1 799 ± 92	0.86 ± 0.07
	E	40.7 ± 3.1	2 979 ± 36	2 094 ± 113	1 741 ± 15	0.83 ± 0.04
	F	39.0 ± 2.0	2 830 ± 471	2 250 ± 64	1 771 ± 70	0.79 ± 0.03
Sign		ns	ns	ns	ns	ns
Moscato Bianco	D	36.2 ± 4.8	2 652 ± 306	1 653 ± 179	1 260 ± 43	0.77 ± 0.06
	E	37.3 ± 2.1	2 590 ± 114	1 487 ± 84	1 297 ± 73	0.87 ± 0.08
	F	34.8 ± 2.4	2 541 ± 87	1 541 ± 135	1 255 ± 74	0.82 ± 0.07
Sign		ns	ns	ns	ns	ns
Mourvèdre	D	53.7 ± 3.9	4 174 ± 365	2 965 ± 319	1 949 ± 196	0.66 ± 0.04
	E	54.2 ± 3.9	4 205 ± 294	3 029 ± 216	1 973 ± 138	0.65 ± 0.06
	F	51.4 ± 2.9	3 916 ± 254	2 993 ± 225	2 070 ± 100	0.70 ± 0.08
Sign		ns	ns	ns	ns	ns
Nebbiolo	D	35.1 ± 4.2	2 613 ± 515	1 702 ± 186	1 314 ± 132	0.77 ± 0.03
	E	30.0 ± 4.9	2 453 ± 313	1 551 ± 161	1 193 ± 53	0.77 ± 0.05
	F	30.5 ± 1.8	2 377 ± 183	1 661 ± 114	1 198 ± 59	0.72 ± 0.05
Sign		ns	ns	ns	ns	ns
Petit Rouge	D	40.2 ± 8.5	2 908 ± 469	2 083 ± 256	1 825 ± 318	0.87 ± 0.06
	E	39.7 ± 2.1	2 959 ± 137	1 999 ± 64	1 846 ± 135	0.92 ± 0.05
	F	36.6 ± 3.5	2 772 ± 288	1 974 ± 182	1 817 ± 198	0.92 ± 0.02
Sign		ns	ns	ns	ns	ns
Petit Verdot	D	65.9 ± 1.5	4 676 ± 144	3 043 ± 61	2 546 ± 127	0.84 ± 0.03b
	E	66.3 ± 7.7	4 728 ± 424	3 281 ± 387	2 422 ± 320	0.74 ± 0.01a
	F	65.9 ± 7.6	4 872 ± 609	3 201 ± 388	2 640 ± 401	0.82 ± 0.06b
Sign		ns	ns	ns	ns	*

TABLE 3 (CONTINUED)

Grape variety	Density class	A_{280} (1/kg)	TF (mg/kg)	PRO (mg/kg)	FRV (mg/kg)	FRV/PRO
Pignolo Spano	D	42.5 ± 1.1	3 403 ± 78	2 176 ± 36	1 791 ± 6	0.82 ± 0.01
	E	42.8 ± 0.8	3 435 ± 49	2 245 ± 74	1 843 ± 61	0.82 ± 0.01
	F	43.5 ± 1.4	3 463 ± 124	2 297 ± 90	2 068 ± 334	0.90 ± 0.17
Sign		ns	ns	ns	ns	ns
Pinot Noir	D	66.2 ± 4.1	4 646 ± 285	2 913 ± 217	3 015 ± 208	1.04 ± 0.01
	E	61.9 ± 6.2	4 661 ± 472	2 926 ± 91	2 850 ± 288	0.98 ± 0.11
	F	65.3 ± 10.2	4 684 ± 147	3 039 ± 343	3 036 ± 223	1.00 ± 0.04
Sign		ns	ns	ns	ns	ns
Riesling Italico	D	48.6 ± 4.0	3 906 ± 482	2 927 ± 310	2 167 ± 298	0.74 ± 0.04
	E	44.3 ± 4.9	3 569 ± 318	2 647 ± 166	1 974 ± 187	0.74 ± 0.03
	F	45.3 ± 6.2	3 632 ± 474	2 661 ± 520	2 158 ± 233	0.82 ± 0.09
Sign		ns	ns	ns	ns	ns
Ruchè	D	40.9 ± 1.5b	3 305 ± 71	2 100 ± 79	1 683 ± 100	0.80 ± 0.02
	E	38.1 ± 0.8ab	2 948 ± 221	1 889 ± 226	1 611 ± 96	0.86 ± 0.08
	F	36.1 ± 2.2a	2 917 ± 242	1 917 ± 148	1 793 ± 269	0.93 ± 0.10
Sign		*	ns	ns	ns	ns
Sangiovese	C	40.7 ± 0.9b	3 018 ± 80	2 119 ± 85	1 771 ± 136b	0.84 ± 0.09
	D	39.0 ± 2.1ab	2 821 ± 108	2 083 ± 120	1 513 ± 81ab	0.73 ± 0.01
	E	35.8 ± 2.0a	2 607 ± 307	1 824 ± 324	1 404 ± 174a	0.78 ± 0.07
Sign		*	ns	ns	*	ns
Sauvignon Blanc	D	28.9 ± 2.5	2 133 ± 164	1 133 ± 71	1 023 ± 49	0.90 ± 0.03
	E	26.0 ± 2.3	2 091 ± 136	1 112 ± 123	1 008 ± 64	0.91 ± 0.07
	F	27.2 ± 4.6	2 071 ± 270	988 ± 19	1 019 ± 121	1.03 ± 0.14
Sign		ns	ns	ns	ns	ns
Syrah	D	36.2 ± 4.5	3 010 ± 382	2 002 ± 200b	1 562 ± 167b	0.78 ± 0.05
	E	34.8 ± 4.9	2 839 ± 380	1 941 ± 82b	1 592 ± 143b	0.82 ± 0.04
	F	26.8 ± 3.8	2 225 ± 259	1 583 ± 159a	1 158 ± 110a	0.73 ± 0.02
Sign		ns	ns	*	*	ns

Data are expressed as mean value ± standard deviation (n = 3). Different Latin letters within the same column indicate significant differences (a) among density classes according to the Tukey-b test ($p < 0.05$). Sign[a]: * and ns indicate significance at $p < 0.05$ and not significant, respectively. A_{280} = absorbance measured at 280 nm, TF = total flavonoids, PRO = proanthocyanidins, FRV = flavanols reactive to vanillin. C = 1 088 kg/m^3, D = 1 094 kg/m^3, E = 1 100 kg/m^3, F = 1 107 kg/m^3.

Noir seeds were characterised by the highest values of these spectrophotometric indices, although the Mourvèdre cultivar had also a high extractable content of PRO in the seeds.

Regarding extractable monomeric and dimeric flavanols (Table 4), the Cinsault cultivar was characterised by the lowest content in the seeds of (+)-catechin (CA) and procyanidin B_1, although low amounts of other compounds like (-)-epicatechin gallate (ECG) were also found. Grenache seeds accounted for the lowest amount of (-)-epicatechin (EC) and ECG, although low contents of CA and procyanidins B_1 and B_2 were also observed. Malvasia Bianca seeds were characterised by the lowest amount of procyanidin B_2 and by a comparatively low content of EC and ECG. Erbaluce and Avaná seeds had also low contents of EC. Barbera, Sauvignon Blanc and Moscato Bianco seeds contained low concentrations of ECG, procyanidin B_1 and B_2, respectively. In contrast, the Pinot Noir cultivar showed the highest content of CA in the seeds, whereas Petit Verdot seeds were the richest in ECG and accounted for a high amount of procyanidin B_1. Furthermore, the Pinot Noir and Petit Rouge cultivars had the highest content of EC and procyanidins B_1 and B_2 in the seeds, but also showed quite high amounts of ECG. Pignolo Spano seeds were rich in EC and procyanidin B_2, whereas Merlot, Freisa and Mourvèdre seeds contained high amounts of ECG. The presence of gallic acid was higher in Brachetto d'Acqui, Merlot and Albarossa seeds.

The effect of berry density on the extractable content of gallic acid and monomeric and dimeric flavanols in the seeds of the wine grape varieties studied was quite small at harvest, and few significant differences were found in these contents among density classes when each variety was evaluated individually. However, a decreasing trend was mostly observed, as also occurred for the spectrophotometric indices. This agreed with the increased association of seed flavanols with cell-wall components resulting in the gradual decline of the extractable amount as ripening advanced (Kennedy *et al.*, 2000a, 2000b; Downey *et al.*, 2003; Cadot *et al.*, 2006; Ferrer-Gallego *et al.*, 2010; Lorrain *et al.*, 2011; Obreque-Slier *et al.*, 2012).

TABLE 4
Gallic acid and monomeric and dimeric flavanols of seeds per grape weight from densimetrically sorted berries at harvest.

Grape variety	Density class	GA (mg/kg)	CA (mg/kg)	EC (mg/kg)	ECG (mg/kg)	B_1 (mg/kg)	B_2 (mg/kg)	TM (mg/kg)	TD (mg/kg)
Albarossa	D	4.87 ± 0.24	58.22 ± 2.45a	57.66 ± 1.79a	1.46 ± 0.09	23.05 ± 1.22	36.70 ± 2.77	117.34 ± 2.89a	59.75 ± 3.62
	E	4.32 ± 0.07	65.13 ± 7.96ab	64.25 ± 4.64ab	1.58 ± 0.09	20.34 ± 1.82	36.35 ± 3.43	130.96 ± 12.56ab	56.69 ± 5.20
	F	4.59 ± 0.54	77.16 ± 10.01b	72.49 ± 4.13b	1.84 ± 0.28	24.99 ± 3.89	41.97 ± 4.71	151.49 ± 13.86b	66.95 ± 8.39
Sign[a]		ns	*	**	ns	ns	ns	*	ns
Arneis	D	3.96 ± 0.21	114.71 ± 19.42	82.46 ± 5.70	1.47 ± 0.17	20.21 ± 1.94	41.16 ± 2.75	198.64 ± 24.55	61.36 ± 4.68
	E	3.13 ± 1.09	109.28 ± 12.49	78.76 ± 8.87	1.40 ± 0.18	20.79 ± 5.25	41.40 ± 5.43	189.44 ± 19.15	62.19 ± 10.46
	F	2.54 ± 0.28	133.69 ± 37.11	74.96 ± 14.61	1.28 ± 0.25	18.45 ± 4.38	36.77 ± 7.48	209.93 ± 51.94	55.22 ± 11.81
Sign[a]		ns	ns	ns	ns	ns	ns	ns	ns
Avaná	D	3.02 ± 0.86	69.83 ± 8.40	51.52 ± 8.74	2.05 ± 0.17	18.47 ± 1.05	20.89 ± 1.93b	123.40 ± 16.83	39.36 ± 1.97
	E	3.17 ± 0.72	50.23 ± 19.36	35.07 ± 12.19	1.69 ± 0.22	14.81 ± 3.45	12.94 ± 2.96a	86.99 ± 30.90	27.76 ± 5.51
	F	2.04 ± 0.58	51.45 ± 13.31	37.98 ± 10.74	1.83 ± 0.34	14.29 ± 3.66	16.61 ± 3.56ab	91.27 ± 24.38	30.89 ± 7.13
Sign[a]		ns	ns	ns	ns	ns	*	ns	ns
Barbera	D	1.81 ± 0.51	124.43 ± 20.13b	115.10 ± 26.44	1.03 ± 0.16	19.45 ± 4.72	44.84 ± 8.67	240.56 ± 44.85	64.30 ± 13.38
	E	1.36 ± 0.19	107.78 ± 23.58ab	95.68 ± 11.97	0.93 ± 0.02	16.96 ± 4.29	37.38 ± 2.30	204.40 ± 35.56	54.33 ± 5.76
	F	2.01 ± 0.53	75.10 ± 5.48a	85.77 ± 7.00	0.83 ± 0.04	14.48 ± 0.12	36.29 ± 2.28	161.71 ± 4.09	50.77 ± 2.32
Sign[a]		ns	*	ns	ns	ns	ns	ns	ns
Brachetto d'Acqui	D	6.07 ± 1.07	122.53 ± 12.77b	82.11 ± 9.68b	1.97 ± 0.19b	29.01 ± 4.06	34.86 ± 3.48b	206.61 ± 22.51b	63.88 ± 7.51
	E	4.19 ± 0.74	88.46 ± 14.59a	53.02 ± 6.63a	1.57 ± 0.10a	22.17 ± 2.38	26.67 ± 4.09a	143.06 ± 20.30a	48.85 ± 6.33
	F	4.85 ± 1.78	88.40 ± 17.43a	48.42 ± 2.65a	1.44 ± 0.20a	26.22 ± 4.11	25.60 ± 1.30a	138.27 ± 18.32a	51.83 ± 5.41
Sign[a]		ns	*	**	*	ns	*	*	ns
Cabernet Sauvignon	D	4.65 ± 0.85	135.13 ± 14.17	78.15 ± 9.27	1.84 ± 0.11	26.96 ± 1.49	40.38 ± 1.04	215.12 ± 23.01	67.34 ± 1.42
	E	2.64 ± 2.07	143.42 ± 7.73	85.51 ± 5.11	2.00 ± 0.07	27.54 ± 5.62	43.25 ± 4.54	230.93 ± 12.44	70.79 ± 10.02
	F	1.74 ± 1.30	135.81 ± 19.12	83.54 ± 19.26	2.05 ± 0.34	30.59 ± 8.99	39.92 ± 4.79	221.40 ± 38.28	70.51 ± 12.53
Sign[a]		ns	ns	ns	ns	ns	ns	ns	ns
Calabrese	D	2.60 ± 0.84	254.02 ± 13.83b	58.63 ± 0.85b	1.77 ± 0.20	29.61 ± 3.52	38.42 ± 3.39	314.42 ± 12.93b	68.03 ± 6.87
	E	2.53 ± 1.05	180.42 ± 2.26a	50.00 ± 3.41a	1.57 ± 0.23	29.51 ± 4.41	32.93 ± 4.80	231.99 ± 4.68a	62.44 ± 8.81
	F	2.48 ± 0.96	200.44 ± 24.10a	49.73 ± 1.81a	1.49 ± 0.10	29.55 ± 1.28	32.75 ± 0.60	251.66 ± 22.72a	62.30 ± 1.16
Sign[a]		ns	**	**	ns	ns	ns	**	ns
Chardonnay	D	2.70 ± 2.57	166.82 ± 24.00	152.51 ± 5.99	1.68 ± 0.22	21.12 ± 6.63	39.83 ± 3.86	321.02 ± 30.01	60.95 ± 9.81
	E	3.13 ± 0.24	145.21 ± 25.21	144.28 ± 33.01	1.77 ± 0.21	26.77 ± 1.69	39.57 ± 5.76	291.26 ± 58.04	66.34 ± 7.39
	F	3.23 ± 0.15	135.49 ± 11.95	127.32 ± 9.87	1.47 ± 0.04	21.49 ± 0.96	33.92 ± 0.61	264.28 ± 10.88	55.41 ± 1.37
Sign[a]		ns	ns	ns	ns	ns	ns	ns	ns

TABLE 4 (CONTINUED)

Grape variety	Density class	GA (mg/kg)	CA (mg/kg)	EC (mg/kg)	ECG (mg/kg)	B_1 (mg/kg)	B_2 (mg/kg)	TM (mg/kg)	TD (mg/kg)
Cinsault	C	3.32 ± 0.57	35.08 ± 5.84	68.97 ± 12.72	1.09 ± 0.22	9.55 ± 0.63	29.19 ± 4.23	105.13 ± 18.70	38.75 ± 4.86
	D	2.11 ± 0.55	28.97 ± 6.43	57.35 ± 7.58	0.83 ± 0.14	8.42 ± 1.77	25.16 ± 3.66	87.15 ± 14.10	33.58 ± 5.33
	E	2.49 ± 0.51	28.40 ± 1.53	50.49 ± 6.61	0.76 ± 0.07	9.79 ± 0.46	23.62 ± 1.76	79.66 ± 7.41	33.41 ± 2.18
Sign[a]		ns	ns	ns	ns	ns	ns	ns	ns
Croatina	D	3.26 ± 0.63	132.65 ± 23.25	150.93 ± 15.70b	1.50 ± 0.43	21.09 ± 6.71	39.27 ± 2.80	285.08 ± 38.84	60.36 ± 8.76
	E	3.48 ± 1.27	139.72 ± 9.29	127.75 ± 13.98ab	1.22 ± 0.08	19.72 ± 0.70	36.95 ± 2.43	268.69 ± 13.04	56.67 ± 2.93
	F	1.44 ± 2.10	157.62 ± 19.28	108.05 ± 10.97a	1.15 ± 0.05	25.09 ± 5.12	32.10 ± 4.39	266.81 ± 22.91	57.19 ± 7.40
Sign[a]		ns	ns	*	ns	ns	ns	ns	ns
Dolcetto	D	2.89 ± 0.61	50.89 ± 4.74	66.58 ± 0.62	1.37 ± 0.10	20.40 ± 1.44	37.28 ± 1.68	118.84 ± 4.65	57.68 ± 2.34
	E	3.20 ± 1.43	45.51 ± 6.63	75.98 ± 29.59	1.45 ± 0.34	18.89 ± 2.88	36.00 ± 7.75	122.93 ± 36.48	54.89 ± 10.40
	F	2.21 ± 0.77	58.80 ± 13.33	71.10 ± 9.59	1.44 ± 0.17	40.05 ± 18.37	34.99 ± 5.37	131.34 ± 22.73	75.05 ± 21.03
Sign[a]		ns	ns	ns	ns	ns	ns	ns	ns
Erbaluce	D	1.53 ± 1.16	53.17 ± 7.17	41.41 ± 1.75	1.42 ± 0.06b	21.94 ± 1.57	18.48 ± 2.72	95.99 ± 8.53	40.41 ± 3.87
	E	2.08 ± 1.61	56.71 ± 14.18	47.41 ± 9.33	1.50 ± 0.14b	22.66 ± 5.21	19.24 ± 1.94	105.62 ± 23.53	41.90 ± 5.89
	F	1.33 ± 1.93	45.36 ± 5.06	33.60 ± 3.26	1.19 ± 0.05a	20.36 ± 3.06	18.48 ± 4.30	80.15 ± 8.09	38.84 ± 3.70
Sign[a]		ns	ns	ns	*	ns	ns	ns	ns
Freisa	D	2.78 ± 0.43ab	128.06 ± 10.97	115.49 ± 4.99b	3.03 ± 0.39b	29.24 ± 1.28b	44.69 ± 3.95b	246.59 ± 9.71	73.94 ± 4.83b
	E	5.19 ± 1.59b	140.59 ± 12.17	118.92 ± 5.55b	3.02 ± 0.28b	29.93 ± 1.18b	41.37 ± 3.05ab	262.53 ± 17.52	71.30 ± 1.99b
	F	0.93 ± 1.07a	142.14 ± 13.58	102.83 ± 4.99a	2.42 ± 0.10a	23.35 ± 2.25a	35.53 ± 0.05a	247.38 ± 17.22	58.88 ± 2.28a
Sign[a]		*	ns	*	*	**	*	ns	**
Grenache	D	1.60 ± 0.34ab	36.65 ± 4.71	23.95 ± 0.98	0.67 ± 0.14	10.88 ± 0.21	15.06 ± 2.30	61.27 ± 5.23	25.94 ± 2.29
	E	0.59 ± 0.70a	35.49 ± 10.08	23.47 ± 7.16	0.64 ± 0.22	12.62 ± 3.33	14.85 ± 4.33	59.59 ± 17.45	27.47 ± 7.38
	F	1.84 ± 0.11b	42.10 ± 6.21	23.27 ± 3.53	0.70 ± 0.05	14.31 ± 0.73	14.57 ± 2.30	66.07 ± 9.68	28.89 ± 2.75
Sign[a]		*	ns	ns	ns	ns	ns	ns	ns
Grignolino	D	3.45 ± 0.42	98.85 ± 11.57	77.03 ± 9.39	1.93 ± 0.28	32.85 ± 4.79	34.00 ± 7.37	177.81 ± 20.87	66.85 ± 9.77
	E	4.02 ± 1.85	85.84 ± 6.13	80.73 ± 6.92	1.92 ± 0.36	33.93 ± 4.30	36.01 ± 1.24	168.49 ± 12.05	69.95 ± 3.81
	F	4.43 ± 1.07	76.51 ± 10.55	68.91 ± 8.09	1.86 ± 0.27	34.83 ± 3.09	35.33 ± 5.19	147.29 ± 18.85	70.17 ± 7.90
Sign[a]		ns	ns	ns	ns	ns	ns	ns	ns
Malvasia Bianca	D	1.81 ± 0.58	104.89 ± 9.85	40.23 ± 3.09	0.72 ± 0.04	18.74 ± 2.56	10.80 ± 2.53	145.83 ± 12.82	29.54 ± 5.08
	E	1.56 ± 0.46	118.85 ± 27.81	43.15 ± 6.93	0.82 ± 0.09	23.22 ± 4.77	12.90 ± 2.56	162.83 ± 34.67	36.13 ± 7.18
	F	1.43 ± 0.01	104.87 ± 10.36	36.87 ± 6.15	0.61 ± 0.22	17.63 ± 5.00	9.97 ± 3.72	142.36 ± 16.67	27.60 ± 8.71
Sign[a]		ns	ns	ns	ns	ns	ns	ns	ns

TABLE 4 (CONTINUED)

Grape variety	Density class	GA (mg/kg)	CA (mg/kg)	EC (mg/kg)	ECG (mg/kg)	B_1 (mg/kg)	B_2 (mg/kg)	TM (mg/kg)	TD (mg/kg)
Malvasia di Schierano	D	0.72 ± 0.91a	80.17 ± 12.81	94.32 ± 25.01	1.87 ± 0.26	14.65 ± 1.05	22.93 ± 2.13	176.36 ± 32.67	37.58 ± 3.15
	E	2.41 ± 0.04b	97.84 ± 13.90	101.06 ± 14.27	2.11 ± 0.24	18.12 ± 1.99	27.17 ± 0.58	201.01 ± 28.39	45.29 ± 2.10
	F	2.46 ± 0.39b	89.28 ± 24.66	93.15 ± 11.33	1.93 ± 0.42	19.02 ± 6.79	22.74 ± 4.33	184.36 ± 34.43	41.76 ± 10.67
Sign[a]		*	ns	ns	ns	ns	ns	ns	ns
Merlot	D	5.25 ± 0.32b	105.36 ± 11.64	112.62 ± 11.15	3.08 ± 0.10	28.75 ± 1.02	41.98 ± 2.51	221.07 ± 20.82	70.74 ± 1.49
	E	4.94 ± 0.07b	117.33 ± 2.30	112.86 ± 4.61	3.03 ± 0.09	36.42 ± 10.79	39.72 ± 1.96	233.23 ± 3.20	76.14 ± 12.27
	F	4.11 ± 0.44a	118.80 ± 1.23	114.86 ± 9.19	2.90 ± 0.12	36.55 ± 10.34	40.62 ± 2.59	236.56 ± 8.34	77.17 ± 8.31
Sign[a]		*	ns	ns	ns	ns	ns	ns	ns
Moscato Bianco	D	3.20 ± 0.21	127.58 ± 39.88	65.22 ± 14.03	1.33 ± 0.19	15.98 ± 2.00	14.10 ± 0.52	194.13 ± 54.10	30.08 ± 2.50
	E	4.91 ± 1.92	147.12 ± 25.39	66.44 ± 6.28	1.57 ± 0.10	17.41 ± 2.00	13.51 ± 1.42	215.13 ± 30.92	30.92 ± 2.20
	F	3.88 ± 0.50	128.55 ± 15.75	60.93 ± 14.50	1.41 ± 0.14	16.47 ± 0.51	13.36 ± 1.14	190.89 ± 28.14	29.83 ± 0.96
Sign[a]		ns	ns	ns	ns	ns	ns	ns	ns
Mourvèdre	D	1.18 ± 1.61a	67.80 ± 13.95	58.57 ± 9.75	2.81 ± 0.32	25.11 ± 10.11	22.21 ± 4.58	129.17 ± 22.70	47.33 ± 13.85
	E	4.35 ± 0.40b	63.20 ± 8.75	51.35 ± 3.14	2.89 ± 0.02	30.89 ± 4.14	18.19 ± 0.21	117.44 ± 11.74	49.09 ± 3.95
	F	2.94 ± 0.68ab	60.37 ± 16.31	47.24 ± 7.98	2.57 ± 0.24	18.73 ± 0.53	16.68 ± 0.47	110.18 ± 18.92	35.42 ± 0.77
Sign[a]		*	ns	ns	ns	ns	ns	ns	ns
Nebbiolo	D	2.07 ± 1.58	111.33 ± 9.85	64.51 ± 2.38b	1.32 ± 0.12	17.43 ± 3.18	24.04 ± 2.13	177.15 ± 10.69	41.47 ± 4.85
	E	1.91 ± 1.45	112.40 ± 9.42	59.12 ± 4.16ab	1.22 ± 0.07	18.24 ± 4.01	22.24 ± 2.62	172.74 ± 7.39	40.48 ± 1.73
	F	2.03 ± 0.09	127.68 ± 18.71	54.15 ± 3.22a	1.09 ± 0.10	19.11 ± 3.00	20.08 ± 1.88	182.92 ± 21.20	39.19 ± 3.92
Sign[a]		ns	ns	*	ns	ns	ns	ns	ns
Petit Rouge	D	2.72 ± 1.46	106.34 ± 20.94	142.83 ± 20.52	2.93 ± 0.43	41.25 ± 6.42	65.41 ± 7.00	252.11 ± 41.70	106.65 ± 13.41
	E	4.09 ± 1.02	130.97 ± 25.56	149.20 ± 7.84	2.81 ± 0.31	52.22 ± 14.16	67.78 ± 3.25	282.98 ± 33.09	120.00 ± 17.39
	F	4.54 ± 0.21	138.87 ± 9.89	147.41 ± 17.95	2.52 ± 0.08	40.52 ± 2.08	61.43 ± 3.65	288.80 ± 27.89	101.95 ± 5.50
Sign[a]		ns	ns	ns	ns	ns	ns	ns	ns
Petit Verdot	D	4.33 ± 1.06	228.61 ± 33.45	109.02 ± 12.04	3.32 ± 0.14	38.56 ± 3.83	32.15 ± 2.46	340.95 ± 44.78	70.71 ± 4.82
	E	3.55 ± 2.84	175.89 ± 32.36	88.54 ± 11.53	3.14 ± 0.47	39.61 ± 10.21	30.04 ± 1.80	267.57 ± 44.00	69.64 ± 10.03
	F	5.65 ± 0.80	197.35 ± 30.35	94.11 ± 16.88	3.32 ± 0.56	40.10 ± 3.97	32.82 ± 6.25	294.78 ± 47.78	72.92 ± 9.79
Sign[a]		ns	ns	ns	ns	ns	ns	ns	ns
Pignolo Spano	D	3.70 ± 0.61	143.39 ± 7.33	153.37 ± 20.15	2.02 ± 0.19	27.39 ± 1.75	45.08 ± 2.98	298.78 ± 17.54	72.47 ± 3.54
	E	4.48 ± 0.53	131.28 ± 9.64	139.58 ± 24.20	1.99 ± 0.05	29.65 ± 6.31	44.35 ± 1.49	272.85 ± 29.28	74.00 ± 5.00
	F	2.43 ± 1.91	149.10 ± 6.05	144.97 ± 7.70	2.02 ± 0.10	30.70 ± 2.36	51.21 ± 5.90	296.08 ± 13.63	81.91 ± 8.07
Sign[a]		ns	ns	ns	ns	ns	ns	ns	ns

TABLE 4 (CONTINUED)

Grape variety	Density class	GA (mg/kg)	CA (mg/kg)	EC (mg/kg)	ECG (mg/kg)	B_1 (mg/kg)	B_2 (mg/kg)	TM (mg/kg)	TD (mg/kg)
Pinot Noir	D	5.81 ± 0.78b	616.76 ± 10.76	208.75 ± 14.83	2.97 ± 0.13	59.36 ± 4.12	56.97 ± 2.93	828.48 ± 14.16	116.33 ± 4.69
	E	2.55 ± 2.25a	681.85 ± 101.97	217.72 ± 38.51	2.99 ± 0.37	61.89 ± 5.75	58.10 ± 12.54	902.57 ± 131.34	119.99 ± 16.97
	F	0.23 ± 0.01a	675.89 ± 41.84	200.99 ± 29.53	2.75 ± 0.29	57.31 ± 4.89	53.21 ± 7.50	879.63 ± 69.98	110.52 ± 12.33
Sign[a]		**	ns	ns	ns	ns	ns	ns	ns
Riesling Italico	D	4.15 ± 1.37	197.73 ± 42.23	106.80 ± 18.41	1.71 ± 0.28	31.47 ± 7.94	36.32 ± 8.25	306.23 ± 60.43	67.79 ± 15.01
	E	3.78 ± 0.62	173.53 ± 36.69	92.90 ± 16.71	1.58 ± 0.20	25.88 ± 2.79	35.21 ± 5.62	268.02 ± 53.59	61.09 ± 8.23
	F	3.84 ± 0.26	189.35 ± 47.92	102.26 ± 17.89	1.64 ± 0.32	27.95 ± 4.55	33.97 ± 5.97	293.25 ± 62.03	61.92 ± 9.61
Sign[a]		ns	ns	ns	ns	ns	ns	ns	ns
Ruchè	D	5.37 ± 0.85	101.19 ± 13.97	114.84 ± 20.84	2.37 ± 0.13b	23.43 ± 1.50	27.76 ± 4.17	218.40 ± 31.08	51.19 ± 5.05
	E	3.48 ± 1.23	104.04 ± 13.35	128.51 ± 24.41	2.15 ± 0.05b	20.73 ± 1.27	25.97 ± 0.55	234.71 ± 37.05	46.70 ± 1.80
	F	2.71 ± 2.38	66.11 ± 50.90	100.88 ± 11.97	1.86 ± 0.15a	21.54 ± 4.66	42.77 ± 34.24	168.85 ± 62.20	64.31 ± 37.93
Sign[a]		ns	ns	ns	**	ns	ns	ns	ns
Sangiovese	C	2.70 ± 0.37b	62.37 ± 3.24	88.98 ± 1.51	2.46 ± 0.14b	16.02 ± 2.15a	39.77 ± 0.96b	153.81 ± 3.28	55.79 ± 1.27ab
	D	0.24 ± 0.00a	59.76 ± 7.48	92.23 ± 26.20	2.28 ± 0.27ab	40.53 ± 14.06b	33.80 ± 4.11ab	154.27 ± 31.57	74.33 ± 17.74b
	E	0.23 ± 0.02a	53.56 ± 11.89	60.85 ± 10.57	1.76 ± 0.27a	15.98 ± 3.22a	28.36 ± 2.23a	116.17 ± 22.59	44.34 ± 5.23a
Sign[a]		***	ns	ns	*	*	**	ns	*
Sauvignon Blanc	D	2.76 ± 0.50	106.33 ± 15.61	71.87 ± 20.14	1.64 ± 0.21	14.70 ± 2.00	17.07 ± 3.44	179.84 ± 34.70	31.77 ± 4.93
	E	2.06 ± 0.18	123.46 ± 6.01	82.37 ± 5.58	1.64 ± 0.07	14.53 ± 0.64	18.61 ± 0.96	207.46 ± 11.45	33.14 ± 1.58
	F	1.81 ± 0.70	117.10 ± 24.21	73.93 ± 12.06	1.56 ± 0.24	14.07 ± 1.78	16.56 ± 1.79	192.59 ± 35.21	30.63 ± 3.27
Sign[a]		ns	ns	ns	ns	ns	ns	ns	ns
Syrah	D	2.71 ± 2.11	93.82 ± 14.00	87.57 ± 11.87	2.31 ± 0.28b	31.91 ± 6.99	26.23 ± 3.20	183.70 ± 25.96	58.14 ± 10.11
	E	1.23 ± 1.71	89.43 ± 19.26	86.69 ± 16.77	2.27 ± 0.40b	26.48 ± 6.69	25.56 ± 3.76	178.39 ± 36.42	52.04 ± 10.37
	F	2.21 ± 1.76	65.61 ± 6.41	73.52 ± 2.16	1.66 ± 0.06a	21.09 ± 1.87	21.44 ± 0.61	140.79 ± 8.51	42.53 ± 1.98
Sign[a]		ns	ns	ns	*	ns	ns	ns	ns

Data are expressed as mean value ± standard deviation (n = 3). Different Latin letters within the same column indicate significant differences ([a]) among density classes according to the Tukey-b test ($p < 0.05$). Sign[a]: *, **, *** indicate significance at $p < 0.05$, 0.01 and 0.001, respectively. ns = not significant. GA = gallic acid, CA = catechin, EC = epicatechin, ECG = epicatechin gallate, B_1 = procyanidin B_1, B_2 = procyanidin B_2, TM = total monomers, TD = total dimers. C = 1 088 kg/m^3, D = 1 094 kg/m^3, E = 1 100 kg/m^3, F = 1 107 kg/m^3.

The next aim was the establishment of robust relationships between the instrumental mechanical properties of grape seeds (Table 2) and the phenolic composition determined by the reference chemical methods. A correlation study was performed using the reference values of the extractable content of phenolic compounds in the seeds, expressed per grape weight (Tables 3 and 4) and seed weight. When all of the wine grape varieties and density classes studied were used, the performance of the texture parameters of the seeds as predictors of the extractable content of phenolic compounds was quite poor, with significant correlation coefficients ranging from 0.121 ($p < 0.05$) to 0.363 ($p < 0.001$). The weakness of these relationships led to a separate correlation study for each cluster established according to seed hardness (Fig. 2). Table 5 shows the variation range of the spectrophotometric indices and the extractable content of gallic acid and flavanolic monomers and dimers in the seeds, expressed per grape and seed weight, using all of the varieties included in each cluster and density class studied. Likewise, Table 6 reports the significant correlation coefficients (R) between the mechanical and chemical parameters of the seeds summarised in Table 5. The most significant and strongest correlations were found for the varieties belonging to cluster 1 (softest seeds), the coefficients lying between 0.504 and 0.640 ($p < 0.001$) for the correlations of A_{280}, TF, FRV and TD expressed per grape weight with F_s and W_s, and those of PRO, CA, EC, ECG, procyanidin B_1 and TM expressed per grape weight or FRV and TD expressed per seed weight with W_s. It is important to take into account that, although most of the chemical parameters showed significant correlations in cluster 1 with the break force, break energy and deformation index of the seeds, no significant relationship was found with the resistance of the seed to axial deformation. Instead, different chemical parameters were significantly correlated with E_s ($p < 0.05$) in clusters 2 and 3, particularly if they were expressed per seed weight. Significant correlations were also observed in clusters 2 and 3 between the chemical and mechanical parameters of the seeds, but the coefficients were too small (R < 0.500), except for the relationship ($p < 0.001$) between PRO expressed per seed weight and E_s (R = -0.525), FRV/PRO and DI_s (R = -0.508), GA expressed per seed weight and E_s (R = -0.538), and ECG, also expressed per seed weight, and W_s (R = 0.504) in cluster 2.

This work confirmed the relationship reported in a preliminary study performed on Cabernet Sauvignon seeds, where the strongest and most significant correlation for the A_{280} values per seed weight measured in the extracts obtained after seed treatment with a wine-like solution was found with E_s (Rolle *et al.*, 2012a). In the present study, wine grape varieties that belong to cluster 2, including Cabernet Sauvignon, showed a significant correlation between these two parameters, but the correlation coefficient improved (R = 0.406 instead of 0.190, $p < 0.01$). In Merlot seeds, the only significant correlation reported between mechanical and chemical parameters corresponded to the relationship of the extractable content of FRV expressed per grape weight with F_s (R = 0.452, $p < 0.05$) (Torchio *et al.*, 2012). In the present work, this later chemical parameter was the only spectrophotometric index that showed significant correlations for the Merlot cultivar (as it belongs to cluster 3) when the results were expressed per grape weight, although not with F_s but DI_s. However, the extractable content of FRV and F_s were correlated significantly when the results were expressed per seed weight (R = 0.205, $p < 0.05$).

Univariate linear regression calibration models were only constructed for the most significant and strongest correlations between the phenolic composition of the seeds, determined by the reference chemical methods,

TABLE 5
Spectrophotometric indices, gallic acid and monomeric and dimeric flavanols of seeds per grape and seed weight for all varieties belonging to the clusters defined by the seed hardness and density classes.

Chemical parameter	Cluster 1				Cluster 2				Cluster 3			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
					mg/kg grape							
A_{280}	16.8	76.5	39.2	11.6	20.2	59.8	34.2	10.2	13.6	75.1	40.4	12.6
TF	1319	5146	3015	798	1525	4483	2587	763	1106	5368	3016	907
PRO	1004	3435	1984	549	933	3339	1589	674	650	3707	2010	646
FRV	589	3293	1614	550	768	2179	1334	422	508	2946	1600	478
GA	0.14	7.19	3.15	1.75	0.21	5.56	2.48	1.23	0.22	6.93	2.95	1.59
CA	7.56	793.41	137.41	158.34	28.45	173.93	111.34	36.07	22.89	269.93	119.06	54.21
EC	16.62	244.13	88.16	48.17	21.45	160.53	85.55	32.82	30.01	175.38	86.53	36.89
ECG	0.47	3.41	1.73	0.55	0.79	2.26	1.53	0.43	0.37	3.71	2.05	0.88
B_1	9.66	68.50	25.89	12.26	11.13	40.61	19.52	6.45	6.82	68.09	26.72	11.27
B_2	9.90	82.29	30.44	12.85	10.30	54.53	30.20	11.68	5.80	71.51	34.30	14.14
TM	43.03	1032.33	227.30	200.45	51.34	315.31	198.42	64.05	71.12	390.18	207.64	73.49
TD	19.55	135.20	56.32	23.31	21.43	82.69	49.72	16.59	17.74	139.60	61.01	22.62

TABLE 5 (CONTINUED)

Chemical parameter	Cluster 1				Cluster 2				Cluster 3			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
mg/g seed												
A$_{280}$	0.77	1.58	1.09	0.21	0.76	1.36	1.04	0.15	0.67	1.43	1.03	0.16
TF	59.7	116.2	84.2	13.4	64.6	98.0	78.3	9.9	55.5	100.9	76.9	11.5
PRO	39.1	82.2	55.3	8.9	31.0	61.0	46.8	8.8	36.2	79.5	51.1	9.4
FRV	27.8	74.9	44.7	10.2	30.4	54.6	40.2	5.8	20.7	54.9	41.1	7.8
FRV/PRO	0.59	1.07	0.81	0.11	0.65	1.19	0.87	0.12	0.57	1.21	0.81	0.13
GA	0.00	0.24	0.09	0.05	0.01	0.15	0.08	0.04	0.01	0.18	0.08	0.04
CA	0.24	17.91	3.68	3.62	1.27	4.89	3.40	0.95	1.06	8.64	3.09	1.52
EC	0.79	5.86	2.44	1.17	0.96	4.48	2.64	0.97	1.02	4.61	2.25	0.86
ECG	0.02	0.09	0.05	0.01	0.02	0.09	0.05	0.02	0.01	0.09	0.05	0.02
B$_1$	0.38	1.58	0.71	0.25	0.42	0.86	0.59	0.10	0.31	1.61	0.69	0.25
B$_2$	0.43	2.66	0.83	0.27	0.46	1.59	0.91	0.29	0.21	1.69	0.91	0.38
TM	2.04	23.31	6.17	4.55	2.29	9.03	6.09	1.79	2.16	10.51	5.39	1.71
TD	0.92	3.48	1.54	0.45	0.96	2.32	1.50	0.33	0.64	3.30	1.59	0.54

n = 117 for cluster 1, n = 45 for cluster 2, n = 108 for cluster 3. SD = standard deviation. A$_{280}$ = absorbance measured at 280 nm, TF = total flavonoids, PRO = proanthocyanidins, FRV = flavanols reactive to vanillin, GA = gallic acid, CA = catechin, EC = epicatechin, ECG = epicatechin gallate, B$_1$ = procyanidin B$_1$, B$_2$ = procyanidin B$_2$, TM = total monomers, TD = total dimers.

TABLE 6

Significant Pearson's correlation coefficients between instrumental mechanical properties and chemical parameters related to the phenolic composition of seeds for varieties belonging to the clusters defined by seed hardness.

Parameter	Cluster 1			Cluster 2				Cluster 3			
	F$_s$ (N)	W$_s$ (mJ)	DI$_s$ (%)	F$_s$ (N)	W$_s$ (mJ)	E$_s$ (N/mm)	DI$_s$ (%)	F$_s$ (N)	W$_s$ (mJ)	E$_s$ (N/mm)	DI$_s$ (%)
mg/kg grape											
A$_{280}$	0.521***	0.639***	0.408***								
TF	0.505***	0.602***	0.382***								
PRO	0.450***	0.529***	0.392***								
FRV	0.527***	0.640***	0.403***								-0.229*
GA		0.190*		-0.297*		-0.368*				0.274**	
CA	0.362***	0.521***	0.419***						-0.238*	0.224*	
EC	0.416***	0.525***	0.359***		-0.342*						
ECG	0.470***	0.574***	0.308***		0.327*	-0.311*			0.196*		
B$_1$	0.477***	0.563***	0.331***								-0.215*
B$_2$	0.463***	0.496***	0.283**		-0.337*					0.216*	
TM	0.387***	0.539***	0.418***		-0.330*					0.219*	
TD	0.507***	0.570***	0.331***		-0.297*						
mg/g seed											
A$_{280}$	0.293**	0.460***	0.343***			-0.406**					
TF	0.261**	0.423***	0.336***			-0.431**					
PRO	0.184*	0.334***	0.394***	-0.342*		-0.525***	0.446**				-0.212*
FRV	0.390***	0.543***	0.367***			-0.372*		-0.205*	-0.318***		-0.374***

TABLE 6 (CONTINUED)

Parameter	Cluster 1			Cluster 2				Cluster 3			
	F_s (N)	W_s (mJ)	DI_s (%)	F_s (N)	W_s (mJ)	E_s (N/mm)	DI_s (%)	F_s (N)	W_s (mJ)	E_s (N/mm)	DI_s (%)
FRV/PRO	0.406^{***}	0.477^{***}				0.375^{*}	-0.508^{***}		-0.325^{***}	0.252^{**}	-0.274^{**}
GA				-0.361^{*}		-0.538^{***}				0.283^{**}	
CA	0.308^{***}	0.472^{***}	0.391^{***}			0.315^{*}	-0.329^{*}	-0.204^{*}	-0.379^{***}	0.204^{*}	-0.208^{*}
EC	0.296^{**}	0.400^{***}	0.278^{**}								
ECG	0.200^{*}	0.300^{**}			0.504^{***}	-0.413^{**}					
B_1	0.374^{***}	0.473^{***}	0.284^{**}				0.305^{*}		-0.265^{**}		-0.306^{**}
B_2	0.371^{***}	0.400^{***}	0.226^{*}			0.326^{*}		0.220^{*}		0.243^{*}	
TM	0.322^{***}	0.479^{***}	0.383^{***}			0.316^{*}	-0.327^{*}		-0.293^{**}	0.261^{**}	
TD	0.431^{***}	0.504^{***}	0.294^{**}							0.235^{*}	

n = 117 for cluster 1, n = 45 for cluster 2, n = 108 for cluster 3. Sign: *, **, *** indicate significance at $p < 0.05$, 0.01 and 0.001, respectively. F_s = seed break force, W_s = seed break energy, E_s = resistance of the seed to axial deformation, DI_s = seed deformation index. A_{280} = absorbance measured at 280 nm, TF = total flavonoids, PRO = proanthocyanidins, FRV = flavanols reactive to vanillin, GA = gallic acid, CA = catechin, EC = epicatechin, ECG = epicatechin gallate, B_1 = procyanidin B_1, B_2 = procyanidin B_2, TM = total monomers, TD = total dimers.

and the mechanical parameters instrumentally determined (R > 0.500, $p < 0.001$). Table 7 shows that better statistical parameters of calibration corresponded to the relationships between the chemical parameters and the seed break energy than to the break force for wine grape varieties belonging to cluster 1. Regarding these varieties, the relationship of FRV expressed per seed weight with W_s was statistically the most satisfactory in terms of error ($R_c = 0.500$, SEC% < 20), although the correlation coefficient of calibration was not too good. Other chemical parameters like A_{280}, TF, FRV and ECG expressed per grape weight showed better correlation coefficients of calibration with W_s ($R_c > 0.600$), but the standard error of calibration (SEC) was slightly higher than 20%. On the other hand, the extractable content of TM in the seeds, expressed per grape weight, accounted for extremely high SEC% values, which may be due to a discontinuous distribution of the CA content (92.3% of seed samples contained amounts of CA lower than 245 mg/kg grape, whereas the amounts in the remaining samples ranged from 593 to 793 mg/kg grape). When the univariate calibration models were developed for wine grape varieties belonging to cluster 2, the most satisfactory statistical parameters corresponded to the relationships of the spectrophotometric index PRO expressed per seed weight with E_s ($R_c = 0.521$, SEC% < 16).

An external validation was performed to assess the robustness of the linear regression calibration models using a sample set that did not belong to the calibration set. The calibration equations obtained were applied to the validation set, and the chemical parameters determined in the seeds by the reference method were compared with those predicted by the calibration models obtained (Table 7). The lower the differences between the reference values and those predicted by the calibration models, the smaller the value of the standard error of prediction (SEP). Because of the wide range of samples analysed to provide adequate variability in the parameters evaluated, the variation range effect (measurement range or mean of this range) on the SEP value was removed by standardising the predictive accuracy of each calibration model using three statistical parameters (SEP%, RPD and RPIQ). SEP% values lower than 20 are considered acceptable for most analytical purposes (Cozzolino *et al.*, 2008), and therefore also to determine FRV and PRO in intact berry seeds for wine grape varieties belonging to clusters 1 and 2, respectively.

The SEP value was better standardised by the residual predictive deviation (RPD) and residual predictive interquartile amplitude (RPIQ) indices (Table 7). When the SEP value is small when compared to the population spread of a certain chemical parameter, a relatively high index is obtained. Therefore, the higher the RPD value, the greater the predictive accuracy. Some authors have established standards indicating that RPD values higher than 2.0 correspond to very satisfactory calibration models for prediction purposes, whereas values ranging between 1.4 and 2.0 are indicative of fair models (Chang *et al.*, 2001). More recently, other researchers have proposed the use of the RPIQ index to better assess the predictive accuracy of the calibration models (Cozzolino *et al.*, 2011). According to this criterion, the extractable content of PRO in the seeds, expressed by seed weight, may be satisfactorily predicted from E_s for wine grape varieties belonging to cluster 2 (RPIQ > 2). The predictive ability of W_s was unreliable for quantitative purposes, but acceptable for screening of the extractable content of TF expressed by grape weight, and of FRV expressed by seed weight (RPIQ = 1.59-1.64) for wine grape varieties included in cluster 1. The remaining chemical parameters could not be reliably predicted from the mechanical parameters evaluated using univariate calibration models.

In an attempt to improve the statistical parameters, multivariate calibration models were developed (Table 7).

The results obtained in cluster 2 showed that the predictive accuracy was good for the determination of the extractable content of ECG expressed by seed weight (R_c = 0.836, SEC% = 19.3, RPIQ = 3.5), satisfactory for TF and TD expressed by grape weight and for PRO expressed by seed weight (R_c = 0.771-0.859, SEC% < 20, RPIQ = 2.1-2.6), and unreliable for quantitative purposes but acceptable for screening of the FRV/PRO ratio, A_{280} expressed by grape and seed weight, as well as of FRV and TM expressed by grape weight (R_c = 0.621-0.765, SEC% < 25, RPIQ = 1.5-2.0). Taking into account the smaller number of samples in cluster 2, the calibration models were also constructed using full cross-validation (leave-one-out splitting). In this case, the accuracy was satisfactory for the prediction of PRO and ECG expressed by seed weight (R_c = 0.794-0.840, SEC% < 20, RPIQ ca. 2.5), but acceptable only for screening of FRV/PRO and TF expressed by seed weight (R_c = 0.578-0.736, SEC% < 12, RPIQ ca. 1.5). Other improvements were also achieved for the prediction of the chemical parameters in the three clusters by means of multivariate calibration models if compared to univariate calibrations. However, some of the statistical parameters studied could hinder the predictive ability.

The relevance for the wine industry of a fast estimation of the extractable content of ECG, PRO, FRV and even the FRV/PRO ratio in grape seeds is supported by the impact of these determinations on the sensory characteristics of the seeds, like astringency and bitterness, which have great repercussions in the wine quality. In this work, galloylation was restricted to ECG because this compound was the only galloylated monomeric flavanol found in the seeds. It is well known that galloylation increases the astringency perceived (Ferrer-Gallego *et al.*, 2010). The spectrophotometric index PRO is mainly associated with the concentration of high molecular weight proanthocyanidins, whereas the FRV index is strongly sensitive to the presence of monomeric flavanols and is partially related to low molecular weight flavanols (Peleg *et al.*, 1999; Cheynier *et al.*, 2006; Obreque-Slier *et al.*, 2011). Furthermore, oligomeric flavanols represent the main phenolic fraction released from the intact seeds during winemaking. Therefore, the extractable contents of PRO and FRV are important factors determining astringency and bitterness, respectively. Astringency was the most appropriate sensory attribute for the assessment of grape seed quality, and the compression parameters of the seeds were likely correlated with perceived astringency, bitterness,

TABLE 7
Analytical performance of calibration models developed for the chemical parameters related to phenolic composition from the instrumental mechanical properties of seeds for varieties belonging to the clusters defined by seed hardness.

Chemical parameter	Mechanical parameter	R_c	SEC	SEC%	R_v	SEP	SEP%	RPD	RPIQ
Cluster 1									
FRV (mg/kg grape)	F_s	0.507	461.05	28.5	0.577	488.23	30.5	1.20	1.25
TD (mg/kg grape)		0.502	20.20	35.5	0.519	20.13	36.4	1.17	1.08
A_{280} (1/kg grape)	W_s	0.625	8.82	22.6	0.670	9.05	23.0	1.35	0.99
TF (mg/kg grape)		0.618	629.71	20.9	0.578	659.76	21.9	1.22	1.59
FRV (mg/kg grape)		0.643	409.65	25.3	0.636	452.48	28.3	1.30	1.35
EC (mg/kg grape)		0.500	41.13	46.5	0.579	41.25	47.1	1.22	1.18
ECG (mg/kg grape)		0.603	0.43	24.9	0.522	0.49	28.9	1.16	1.14
B_1 (mg/kg grape)		0.552	10.23	39.1	0.582	10.09	39.8	1.23	0.99
TM (mg/kg grape)		0.500	172.60	76.0	0.623	163.02	71.6	1.27	0.80
TD (mg/kg grape)		0.569	19.22	33.8	0.572	19.29	34.9	1.22	1.12
FRV (mg/g seed)		0.500	8.77	19.6	0.628	8.29	18.7	1.27	1.64
A_{280} (1/kg grape)	F_s, W_s, E_s, DI_s	0.642	8.66	22.2	0.666	9.09	23.1	1.34	0.99
TF (mg/kg grape)		0.630	622.35	20.6	0.576	664.25	22.1	1.21	1.58
PRO (mg/kg grape)		0.579	424.29	21.4	0.501	527.58	26.6	1.15	1.70
FRV (mg/kg grape)		0.653	405.08	25.0	0.633	454.55	28.4	1.29	1.34
CA (mg/kg grape)		0.541	132.95	97.1	0.655	121.93	88.1	1.32	0.61
EC (mg/kg grape)		0.529	40.17	45.4	0.584	40.95	46.8	1.23	1.19
ECG (mg/kg grape)		0.621	0.43	24.5	0.517	0.50	29.4	1.14	1.12
B_1 (mg/kg grape)		0.555	10.20	39.0	0.583	10.09	39.8	1.23	0.99
B_2 (mg/kg grape)		0.511	11.32	36.9	0.502	10.71	35.8	1.15	1.52
TM (mg/kg grape)		0.557	164.97	72.6	0.653	156.97	69.0	1.32	0.83
TD (mg/kg grape)		0.575	19.12	33.6	0.581	19.10	34.6	1.23	1.14
FRV (mg/g seed)		0.534	8.53	19.0	0.631	8.19	18.4	1.29	1.66
TM (mg/g seed)		0.500	3.99	64.6	0.639	3.49	56.8	1.29	1.03

TABLE 7 (CONTINUED)

Chemical parameter	Mechanical parameter	R_c	SEC	SEC%	R_v	SEP	SEP%	RPD	RPIQ
Cluster 2									
PRO (mg/g seed)	E_s	0.521	7.11	15.2	0.533	8.38	17.9	1.18	2.23
A_{280} (1/kg grape)	F_s, W_s, E_s, DI_s	0.651	8.07	23.3	0.668	7.36	22.1	1.30	1.94
TF (mg/kg grape)		0.771	506.46	19.2	0.689	550.34	22.2	1.31	2.08
PRO (mg/kg grape)		0.629	516.02	32.0	0.574	594.61	38.5	1.21	1.57
FRV (mg/kg grape)		0.679	314.59	23.2	0.630	315.61	24.4	1.33	1.92
CA (mg/kg grape)		0.697	27.69	24.2	0.540	28.78	27.3	1.07	2.05
EC (mg/kg grape)		0.744	23.47	26.6	0.713	20.38	25.5	1.37	2.07
B_1 (mg/kg grape)		0.528	5.88	29.3	0.585	4.47	24.3	1.22	1.43
B_2 (mg/kg grape)		0.741	7.99	25.8	0.630	8.93	31.0	1.29	2.53
TM (mg/kg grape)		0.765	44.29	21.7	0.689	42.63	22.8	1.26	1.97
TD (mg/kg grape)		0.793	10.11	19.8	0.694	11.30	24.0	1.37	2.61
A_{280} (1/g seed)		0.701	0.12	11.4	0.603	0.11	10.5	1.19	1.74
PRO (mg/g seed)		0.859	4.27	9.1	0.660	7.44	15.9	1.33	2.51
FRV/PRO		0.621	0.10	11.4	0.525	0.11	13.0	1.05	1.57
GA (mg/g seed)		0.615	0.03	34.3	0.580	0.03	39.7	1.22	2.07
EC (mg/g seed)		0.593	0.82	30.7	0.566	0.79	30.7	1.14	1.78
ECG (mg/g seed)		0.836	0.01	19.3	0.854	0.01	24.2	1.67	3.45
TM (mg/g seed)		0.633	1.48	24.0	0.566	1.56	26.3	1.01	2.13
Cluster 3									
ECG (mg/kg grape)	F_s, W_s, E_s, DI_s	0.612	0.71	34.1	0.523	0.73	36.6	1.16	1.92

n = 117 for cluster 1, n = 45 for cluster 2, n = 108 for cluster 3. R_c = correlation coefficient of calibration, SEC = standard error of calibration, SEC% = (SEC/Mean) x 100, R_v = correlation coefficient of validation, SEP = standard error of prediction, SEP% = (SEP/Mean) x 100, RPD = residual predictive deviation (SD/SEP), SD = standard deviation, RPIQ = residual predictive interquartile amplitude (IQ/SEP), IQ = interquartile amplitude. F_s = seed break force, W_s = seed break energy, E_s = resistance of the seed to the axial deformation, DI_s = seed deformation index. A_{280} = absorbance measured at 280 nm, TF = total flavonoids, PRO = proanthocyanidins, FRV = flavanols reactive to vanillin, GA = gallic acid, CA = catechin, EC = epicatechin, ECG = epicatechin gallate, B_1 = procyanidin B_1, B_2 = procyanidin B_2, TM = total monomers, TD = total dimers.

vegetal aroma and roasted aroma (Letaief *et al.*, 2013).

Regarding wine grape varieties belonging to cluster 3, no instrumental mechanical property may reliably predict any chemical parameter related to the phenolic composition of the seeds (Tables 6 and 7). At this point it was necessary to determine whether linear regression calibration models could be developed to predict the spectrophotometric indices (A_{280}, TF, PRO and FRV) with adequate reliability from direct instrumental measurement of the mechanical parameters of the seeds (F_s, W_s, E_s and DI_s) for some of the varieties belonging to cluster 3, but working with one cultivar individually, particularly Nebbiolo. A suitable variability in the reference values was assured by means of the analysis of seeds from grape berries sampled in different vineyards, at different ripening stages, during two consecutive years. In this case, only spectrophotometric indices were evaluated because they are more usually used in wineries to assess the phenolic maturity of the seeds, and were better correlated with the mechanical properties of the varieties in clusters 1 and 2. A correlation study carried out on Nebbiolo seeds showed that the highest significant correlation coefficients between the spectrophotometric indices and the instrumental mechanical parameters corresponded to TF with F_s and W_s, but the relationships found were weak (R = 0.418-0.459, $p < 0.001$). This shortcoming was overcome using multivariate calibration models. Table 8 shows the mean and standard deviation values for each chemical and mechanical parameter, showing significant correlations between them ($p < 0.01$), as well as the statistical parameters of calibration and validation. The best analytical performance of the multivariate calibration models corresponded to the prediction of the chemical parameters expressed per grape weight. The ability of three mechanical attributes (F_s, W_s and E_s) to predict the extractable content of TF and PRO in the seeds, expressed per grape weight, was satisfactory, indicating an acceptable robustness of the multivariate calibration models for quantitative purposes ($R_c > 0.68$, SEC% ca. 20, RPIQ > 2.2). Nevertheless, the high SEC% values found for the FRV/PRO ratio hindered the recommendation of the corresponding calibration model, even for screening purposes.

CONCLUSIONS

This study provides an interesting approach for understanding the associations between the instrumental mechanical properties and the phenolic composition of the

TABLE 8
Analytical performance of multivariate calibration models developed for the spectrophotometric indices related to the phenolic composition of Nebbiolo seeds from the instrumental mechanical properties.

Chemical parameter	Mean ± SD[a]	Mechanical parameters	Mean ± SD[a]	R_c	SEC	SEC%	R_v	SEP	SEP%	RPD	RPIQ
TF (mg/kg grape)	2249 ± 928			0.858	483.48	21.6	0.886	424.41	18.6	2.14	3.95
PRO (mg/kg grape)	903 ± 273	F_s	48.19 ± 4.22	0.682	203.00	22.3	0.665	199.61	22.5	1.33	2.24
TF (mg/g seed)	58.7 ± 22.7	W_s	11.50 ± 1.53	0.782	14.43	24.6	0.798	13.43	22.9	1.65	2.79
PRO (mg/g seed)	23.7 ± 6.9	E_s	92.96 ± 7.43	0.534	5.93	24.7	0.501	5.93	25.9	1.15	1.85
FRV/PRO	0.79 ± 0.39			0.713	0.24	31.4	0.674	0.34	41.8	1.34	1.56

[a]n = 136. R_c = correlation coefficient of calibration, SEC = standard error of calibration, SEC% = (SEC/Mean) x 100, R_v = correlation coefficient of validation, SEP = standard error of prediction, SEP% = (SEP/Mean) x 100, RPD = residual predictive deviation (SD/SEP), SD = standard deviation, RPIQ = residual predictive interquartile amplitude (IQ/SEP), IQ = interquartile amplitude. F_s = seed break force, W_s = seed break energy, E_s = resistance of the seed to the axial deformation. TF = total flavonoids, PRO = proanthocyanidins, FRV = flavanols reactive to vanillin.

seeds. At harvest, the berry heterogeneity in the vineyard had a small effect on the compression parameters of the seeds. Nevertheless, the seed hardness represents an important factor in the characterisation and differentiation of wine grape varieties. This varietal classification was useful in the assessment of the mechanical properties as possible predictors of the phenolic composition of the seeds**.** In fact, the robustness of the prediction for some spectrophotometric indices, as well as for monomeric and dimeric flavanols, was highly related to seed hardness. Among the calibration models developed, a few could be recommended for quantitative purposes (total flavonoids, proanthocyanidins, epicatechin gallate and total dimer flavanol content), while others were only acceptable for screening. The predictive accuracy could be improved for varieties with higher seed hardness, where the significant correlations between mechanical and chemical parameters were low, by constructing separate calibration models for each cultivar. Harvesting wine grapes at optimal seed maturity is one of the first steps in producing high quality wines and, in this sense, the knowledge of the mechanical properties of the seeds would enable rapid prediction of the extractable content of phenolic compounds affecting sensory characteristics.

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