Use of instrumental acoustic parameters of winegrape seeds as possible predictors of extractable phenolic compounds.

UNIVERSITÀ DEGLI STUDI DI TORINO

USE OF INSTRUMENTAL ACOUSTIC PARAMETERS OF WINEGRAPE SEEDS AS POSSIBLE PREDICTORS OF EXTRACTABLE PHENOLIC COMPOUNDS

Luca Rolle,[†,⊥] Simone Giacosa, [†,⊥] Fabrizio Torchio, [†] Daniele Perenzoni, [§] Susana Río Segade[*†], Vincenzo Gerbi,[†] and Fulvio Mattivi [§]

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

[§]Fondazione Edmund Mach, Research and Innovation Centre, Department of Food Quality and Nutrition, Via E. Mach 1, 38010 San Michele all'Adige, Italy.

[⊥] These authors contributed equally to this study.

*Corresponding author. Telephone: +39 0116708558; fax: +39 0116708549; email: susana.riosegade@unito.it

ABSTRACT

The use of instrumental acoustic parameters produced during a compression test as reliable predictors of the extractable phenolic composition in intact winegrape seeds, determined by reference chemical methods, was evaluated by means of the analytical performance of calibration models. These models were developed only for those phenolic compounds most significantly and strongly correlated with the acoustic parameters. The analytical performance of the models was expressed in terms of standard error of cross-validation (SECV) and residual predictive interquartile amplitude (RPIQ), among other statistics. Several acoustic parameters showed satisfactory predictive accuracy for the percentage of galloylation in the terminal units, the content of (-)-epicatechin and the mean degree of polymerization. Most of the reliable models developed are fairly recommended not for quantitative purposes but for fast screening (SECV%<19, 1.6<RPIQ<2.1).

Keywords: instrumental texture analysis, acoustic parameters, seeds crunchiness, extractable phenols, LC-MS

INTRODUCTION

The phenolic composition of a wine plays an important role in the assessment of the perceived quality that is governed by the criteria of expert tasters along with consumer acceptance.[1] Flavanols are one of the main families of phenolic compounds that contribute to the wine quality. A significant amount of these compounds is located in the grape seeds,[2,3] which are a rich source of monomeric catechins ((+)-catechin, (-)-epicatechin and (-)-epicatechin-3-*O*-gallate), as well as their oligomers and polymeric procyanidins.[4-7] These compounds are of sensory relevance because they are directly involved in bitterness and astringency as a function of their chemical structure,[8-10] and indirectly in promoting the colour stability through the formation of flavanol-anthocyanin complexes.[11,12]

The grape cultivar is an important factor in the qualitative and quantitative phenolic composition of the seeds, although it also depends on environmental conditions, cultural practices and degree of grape ripeness.[5,6,13-16] The compositional changes occurring in the seeds affect the concentration and extraction of phenolic compounds. The extractable amount of seed flavanols gradually declines during grape ripening.[7,14,16-20] This decrease may be consistent with the oxidation of flavanols,[21] which favors their increased association with cell wall components[17,18] reducing their extractability. The changes in the phenolic composition of the seeds influence their sensory properties.[7] Some authors have reported that astringency varies with the changes in the flavanol structure.[9] During ripening, galloylated flavanolic compounds undergo a strong decrease in their content,[7,22] which may be accompanied by a reduction in astringency.[7] Although it is widely accepted that astringency increases with the chain length for low molecular weight flavanols,[8] the decreased content of monomeric flavanols and the increased polymeric fraction are sensory perceived as low astringency.[10] Instead, bitterness is restricted to low molecular weight flavanols.[8,10,11] During winemaking, more phenolic compounds being extracted from the seeds, larger impediment to any

enhancement in the colour wine promoted by the anthocyanin extraction from the skin because of anti-copigmentation effects.[23] Therefore, the phenolic composition of the seeds plays a key role in the production of quality red wines.

Sensory analysis is widely used to evaluate the perceived characteristics of foods, and many descriptors have been also proposed for the sensory evaluation of grape seeds.[24-26] Astringency that is considered one of the most important sensory attributes of red wines is not always easy to assess sensorially in the seeds because its perception in the latter varies with residence time in the mouth and the number of repeated exposures.[24] Furthermore, there are residual and carryover effects between samples. The intensive lignification of the medium integument and the dehydration of the outer integument have been suggested as causes of hardening of the grape seed during ripening,[19] and some authors have reported that the decrease in the perceived astringency is accompanied by an increase in the hardness and cracking of the seeds.[25,26] This fact suggests that the instrumental techniques used to determine the hardness and crispness/crunchiness of foods could be useful in the prediction of the phenolic composition of the grape seeds and therefore of their astringency.

The use of fast, simple, reproducible and economically profitable instrumental techniques is increasingly demanded in the wine industry to accurately predict texture attributes, and presents the additional advantage of reducing the variability associated with the subjectivity of sensory analysis. In recent years, instrumental acoustic methods have attracted interest for the investigation on structural properties of foods. In fact, the recording of the acoustic emission produced during the fracturing process of food tissues permits the instrumental assessment of crispness/crunchiness.[27-30] Most of these acoustic methods are based on the use of acoustic sensors placed close or attached to the sample via a solid body during mechanical testing. In grape seeds, some significant correlations among instrumental mechanical parameters and the phenol content or extractability have also been found.[31]

Nevertheless, their low robustness has led to the evaluation of the instrumental measurement of the acoustic response produced during the compression test to predict the ripeness of winegrape seeds.[32,33] In a first attempt, the effect of the developmental changes occurring in the last stages of grape ripening on the acoustic energy and average acoustic pressure of the seeds has been reported.[32] A novel application in this field is based on the use of instrumental acoustic parameters as predictors of the extractable content of phenolic compounds in the seeds. In particular, correlation studies have highlighted that the average acoustic pressure level of Merlot seeds could be proposed as predictor of the spectrophotometric indices closely related to the extractable content of total flavonoids and total phenols, and that the maximum acoustic pressure level is intrinsically linked to extractable flavanols reactive to vanillin.[33]

The aim of the present work was to evaluate more comprehensively the performance of the instrumental acoustic parameters of intact grape seeds as predictors of the phenolic composition. This required the chromatographic determination of individual flavanolic compounds, which are strongly involved in astringency and bitterness, in the seeds of six red winegrape cultivars, in addition to the spectrophotometric indices. The relationships between the acoustic parameters measured during compression testing and the extractable content of different phenolic compounds determined by reference chemical methods were then established, and calibration models were developed. The seeds from the six red winegrape cultivars were used for the construction and validation of the predictive models. A high variability calibration data set for each individual cultivar was assured by harvesting the grapes at different ripening stages in two growing zones. Furthermore, the predictive ability was individually studied for one cultivar attempting to improve the prediction robustness.

MATERIALS AND METHODS

Grape Samples. Grape berries of six red cultivars (*Vitis vinifera* L. cv. Cabernet Sauvignon, Freisa, Merlot, Nebbiolo, Sangiovese and Teroldego) were harvested at three different ripening stages (S1-S2-S3) in vineyards located in two growing zones (Piedmont, Cuneo province, North-West Italy and Trentino, Trento province, North-East Italy), in 2011. Grape samples of the Nebbiolo cultivar were collected at two different ripening stages in ten commercial vineyards located in Valtellina (Sondrio province, Lombardy, Northern Italy) in 2011. For each winegrape cultivar, harvest date and growing zone or vineyard, ten bunches were collected from ten vines randomly selected to be representative of the vineyard (one bunch per vine), and each sample was separately processed. Once in the laboratory, a subsample of approximately 0.5 kg of grapes (ca. 350-400 berries) was randomly selected by picking at least twelve berries from each different position in the cluster (shoulders, middle and bottom), with a total of at least 36 berries by cluster. For each subsample, two sets of 30 berries were randomly selected. The first set was subdivided into three replicates of 10 grape berries that were weighed. The seeds were carefully separated from the pulp and cleaned with adsorbent paper before determining extractable phenolic compounds by the reference methods. In the second set, the seeds were also separated and cleaned before instrumental texture analysis. In this case, one seed per berry was used to cover a wider variation range with the same number of seeds. Each seed was individually analyzed up to acquire the acoustic parameters for a total of 30 intact grape seeds. A sample size of least 30 seeds is required to minimize the intrasample variability.[33]

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 deionized water produced by a Purelab Classic system (Elga Labwater, Marlow, United Kingdom). Among pure HPLC-grade phenol standards, 98% (+)-catechin hydrate (C), 98% (-)-epicatechin (EC), 98% (-)-gallocatechin (GC), 95% (-)-epigallocatechin (EGC) and 98% (-)-

epicatechin gallate (ECG) were obtained from Sigma, and 96% cyanidin chloride, 80% procyanidin B_1 and 90% procyanidin B_2 were purchased from Extrasynthèse (Genay, France). Phloroglucinol was supplied by Aldrich (Steinheim, Germany).

Seed phenols extraction, phloroglucinolysis and determination. In the reference method, the whole seeds were immediately immersed in a variable volume (80-100 mL) of a wine-like solution and soaked for 5 days at 30 °C. The hydroalcoholic solution consisted of ethanol/water (12/88 v/v) containing 100 mg/L sulphur dioxide and 5 g/L tartaric acid, which was buffered at pH 3.2. The volume of the hydroalcoholic solution was adjusted to a ratio of 1.25 between volume (mL) of the solution and weight (g) of the berries from which the seeds were obtained.[34] The extracts were filtered through a 0.20 μm PTFE filter (Puradisc 25, Whatman International Ltd, Maidstone, Kent, UK), bottled and stored at 4 °C until their analysis.

Spectrophotometric methods were used to measure absorbance at 280 nm (as A_{280}), and to determine the extractable content of total polyphenols (mg (+)-catechin/kg grape, as TP), total flavonoids (mg (+)-catechin/kg grape, as TF), proanthocyanidins (mg cyanidin chloride/kg grape, as PRO) and flavanols reactive to vanillin (mg (+)-catechin/kg grape, as FRV) in the hydroalcoholic extracts.[35,36] A UV-1800 spectrophotometer (Shimazdu Corporation, Kyoto, Japan) was used.

Phloroglucinolysis of the seed extracts was carried out according to a method adapted from the previously described wine analysis protocol by Fortes Gris et al. (2011).[37] Ten milliliters of the extract were dealcoholized reducing the volume to 5 mL under reduced pressure at 30 °C, brought back to the initial volume with water, and loaded on the C_{18}-SPE cartridge (1 g, Waters, Milford, MA) previously activated sequentially with 4 mL of methanol and 10 mL of water. The hydrophilic fraction was washed with 50 mL of water, and flavanols were then recovered with 40 mL of methanol. The eluate was evaporated to dryness at 30 °C,

redissolved in 2 mL of methanol and filtered at 0.22 μm PTFE filters (Millipore, Bedford, MA). Thereafter, an 100 μL aliquot of the concentrated extract was allowed to react for 20 min at 50 °C with 100 μL of phloroglucinol reagent consisting of 100 g/L phloroglucinol and 20 g/L ascorbic acid in methanol containing 0.2 M hydrochloric acid. The reaction was terminated by adding 1 mL of 40 mM aqueous sodium acetate. The final extracts (taken before and after phloroglucinolysis) were filtered through 0.22 μm PTFE filters into LC vials and immediately injected into a HPLC-DAD-MS system. This assay provides information on the mean degree of polymerization (mDP) and the percentage of galloylation (%G).

The determination of individual flavanols was performed by HPLC-DAD-MS using a Waters 2690 HPLC system (Milford, MA, USA) equipped with a Waters 996 diode array detector (DAD) and a Waters Micromass ZQ electrospray ionization-mass spectrometer (ESI-MS). The chromatographic separation was carried out at 40 °C on an Atlantis C_{18} column (5.0 μm, 250 mm × 4.6 mm i.d.) purchased from Waters (Manchester, UK). The injection volume was 20 μL. The mobile phases consisted of 2.5% v/v acetic acid in water (A) and methanol (B) at a flow rate of 0.90 mL/min. The two mobile phases were filtered through a 0.20 μm PTFE membrane filter (Whatman International Ltd, Maidstone, Kent, UK). A linear gradient was used for the flavanols separation in 48 min starting at 5% B and increasing to 6% B in 5 min, to 18% B in 25 min, to 30% B in 1 min, to 100% B in 16 min and back to 5% B in 1 min. The column was then equilibrated for 7 min prior to each analysis. The UV-VIS spectra were recorded from 210 to 400 nm, and the detection wavelength was set at 280 nm with a bandwidth of 4 nm. The MS detector operated at a capillary voltage of 3000 V, extractor voltage of 6 V, cone voltage of 30 V, source temperature of 150 °C, desolvation temperature of 500 °C, cone gas flow rate (nitrogen) of 50 L/h and desolvation gas flow rate (nitrogen) of 1200 L/h. The ESI-MS spectra ranging from 100 to 1500 *m/z* were acquired in negative ion mode with a dwell time of 0.1 s. The identification of monomeric and dimeric

flavanols was achieved by comparing their absorption spectra and retention times (RT) with those of pure standards. All of them were confirmed and then quantified by ESI-MS. The quantification of monomeric and dimeric flavanols was carried out by the external standard method using the molecular ion $(M-H)^-$ of 289.3 *m/z* for C (RT 18.0 min) and EC (RT 28.0 min), 305.3 *m/z* for GC (RT 9.5 min) and EGC (RT 18.0 min), 441.4 *m/z* for ECG (RT 36.5 min), and 577.5 *m/z* for B_1 and B_2 dimers. The phloroglucinol adducts were identified on the basis of their retention times and the molecular ion of 413.3 *m/z* for C- (RT 9.3 min) and EC-phloroglucinol (RT 10.0 min), 429.4 *m/z* for EGC-phloroglucinol (RT 6.3 min) and 565.5 *m/z* for ECG-phloroglucinol (RT 20.5 min). The presence of other main fragments by MS was also used. The phloroglucinol adducts were quantified from the absorbance measurement at 280 nm (external standard method) and their respective molar absorptivity.[38] The mDP value was calculated as the molar ratio of the sum of all of the flavanols units produced by phloroglucinolysis (phloroglucinol adducts plus terminal units, as total polymers or TPP) to the sum of the terminal units.[6] The %G value was calculated as the ratio of the sum of galloylated flavanols to the sum of all flavanols. All of the analyses were performed in duplicate and then averaged.

Instrumental Acoustic Properties. The acoustic emission produced during the compression test of the intact seeds was measured using an acoustic envelope detector (AED) (SMS, Stable Micro Systems, Surrey, UK) equipped with a 12.7 mm diameter Brüel & Kjær 4188-A-021 microphone (Nærum, DK). The microphone was positioned at a 20 mm distance from the sample at an angle of 45 º, and connected to a TA-XT Plus texture analyzer (SMS) equipped with a SMS HDP/90 platform, a SMS P/35 probe and a 50 kg load cell.[33] Each one of the intact grape seeds was individually compressed (1 mm/s speed and 50% deformation), and the recording of the acoustic emission produced was carried out at two different instrumental gain

SPL values (0 and 24 dB). The calibration was performed before each measurement session using an acoustic calibrator type 4231 (94 and 114 dB-1000 Hz).

For each gain, the following instrumental acoustic parameters were measured: the acoustic pressure level at the breakage (dB), the maximum acoustic pressure level (dB), the acoustic energy (dB × mm, as AE), the linear distance (as LD), the number of acoustic peaks higher than 15 dB (as $N_{pk>15\ dB}$), the number of acoustic peaks higher than 5 dB (as $N_{pk>5\ dB}$), the average acoustic pressure level for peaks with threshold higher than 15 dB (dB, as $AV_{pk>15\ dB}$) and the average acoustic pressure level for peaks with threshold higher than 5 dB (dB, as $AV_{pk>5\ dB}$).[27,33] With the exception of the two first parameters, all remaining ones were separately determined before and after breaking, and their total value during the compression test was also assessed. All data acquisitions were made at 500 points per second (PPS) involving the Texture Exponent software.

Statistical Analysis. Statistical analyses were carried out using the SPSS software package version 17.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to establish significant differences. Pearson's correlation coefficients were calculated to determine significant relationships between the instrumental acoustic parameters and the phenolic composition of the seeds. 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 standardized by rating its value to the mean of the population, and is related to the mean error of the model (SEC%). A good calibration model should have high R_c and low SEC and SEC%. On the other hand, the predictive accuracy of the calibration models was evaluated from the standard error of cross-validation (SECV). For full cross-validation, the sample set was divided into several groups by leave-one-out splitting and each group was then validated using the calibration model developed with the other samples. Finally, validation errors were combined

into SECV. The goodness of the prediction ability requires minimizing SECV. Furthermore, the coefficient of variation (SECV%) was calculated as the ratio of the SECV value to the mean of the population. The residual predictive deviation (RPD) is the most commonly used statistical index to account for the model reliability and was defined as the ratio between the standard deviation (SD) of the sample set and the SECV value. Another index, the residual predictive interquartile amplitude (RPIQ) based on quartiles, was calculated as the ratio of the interquartile amplitude of the population to the SECV value.[39]

RESULTS AND DISCUSSION

Chemical Analysis. The necessity of using analytical methods that are reliable for a wide range of concentration for phenolic compounds, particularly flavanols in seeds, has conducted to perform the study on six red winegrape varieties. This variety variability was also combined with the agroclimatic variability by collecting grape samples in two growing zones. Furthermore, three harvest dates were considered to cover the natural variability associated with the ripening process. The reference values for the spectrophotometric indices, monomeric and dimeric flavanol composition and proanthocyanidin composition of the seeds, determined by chemical methods, are shown in Tables 1, 2 and 3, respectively. For each chemical parameter and factor, the absorbance value, content or percentage represents the mean value of all data related, regardless of the other factors. For example, the A_{280} value reported for Cabernet Sauvignon seeds is expressed as the mean value of the variation range for this cultivar, regardless of the growing zone or ripening stage. The same is applicable to other cultivars, growing zones and ripening stages. For each case, the heterogeneity of the samples considered determines the variability of the results obtained. Despite this variability, some significant differences were found among cultivars or growing zones, which are of relevance in obtaining robust calibration models for wider ranges of the chemical parameters,

and therefore they are discussed below. Nevertheless, no chemical parameter permitted the discrimination of ripening stages from the results combined of all cultivars and growing zones.

Tables 1-3 show significantly lower values of the spectrophotometric indices (A_{280}, TP, TF, PRO, FRV) and of the extractable content of total monomeric flavanols (TM) and total polymeric proanthocyanidins (TPP) in the seeds of the Sangiovese cultivar, followed by Merlot, whereas the higher were associated with Cabernet Sauvignon and Freisa seeds. The compounds C and EC were by far the main constituents of seed monomeric flavanols because GC, EGC and ECG occurred only at low concentrations (0.6-7.1% of total monomers according to the cultivar). Monomeric flavanols like GC and EGC, which are usually not detectable using other analytical protocols, were found even though at the lowest contents in accordance with other works published.[40,41] The extractable contents determined of monomeric flavanols comprised the range reported for different red winegrape cultivars from Trentino.[6] In agreement with a previous work, the most abundant monomeric flavanol in the seeds of the Sangiovese cultivar was EC, accounting for a mean percentage of 52.4% of total monomers, followed by C (43.5%).[15] In Cabernet Sauvignon, Freisa, Nebbiolo and Teroldego seeds, C was the predominant flavanol representing 52.1-69.6% of the extractable concentration of all monomeric flavanols. Similar mean percentages of C and EC were found in Merlot seeds (50.2% vs 46.4%). Although Merlot seeds did not display higher extractable concentrations of EC than Cabernet Sauvignon ones as reported in other works,[5,16,20] the relative percentages of each compound agreed with others published for Cabernet Sauvignon and Merlot.[5,42,43] A possible reason is the vineyard and vintage effect on the flavanolic composition of the seeds.[20,43] In fact, the growing zone affected the monomeric flavanol composition (Table 2). Significantly higher extractable contents were observed for most of these monomeric compounds in Trentino, being ECG the only exception with lower values.

On the other hand, the extractable content of EC, GC, EGC and ECG in the seeds agreed for the six winegrape cultivars studied.

The highest amount of dimeric flavanols in the seeds occurred in the Cabernet Sauvignon cultivar, and the lowest corresponded to Nebbiolo and Sangiovese. Procyanidins B_1 and B_2 were equally present in the seeds of Cabernet Sauvignon, Freisa, Merlot and Nebbiolo cultivars, whereas procyanidin B_2 was the major dimer in Sangiovese and Teroldego (52.0-72.1% of total dimers). Some authors reported that procyanidin B_2 is the main dimer in winegrape seeds.[4,16,20,42] This is common to most studies but not to all.[5,15,43]

Polymeric proanthocyanidins were more abundant in the seeds than monomeric flavanols and consisted of terminal and extension units.[1,16,42] The terminal units were mainly composed of C and EC (75.4-90.2%) with predominance of C, excepting for the Sangiovese cultivar. The mean percentage of ECG was also important as evidenced by the %G value ($\%G_t$ in Table 3). The most abundant constituent of the extension units was EC with percentages ranging from 74.7% to 91.6%. These results were quite similar to those observed in other studies.[6,42,44] The %G value is related to the proportion of ECG subunits in polymeric flavanols, and Sangiovese seeds are characterized by a higher percentage of galloylated subunits in both terminal and extension units (Table 3). The mean value of mDP varied between 2.1 and 2.5 depending on the cultivar (whole mDP range from 1.7 to 3.1), and therefore the seeds of the cultivars investigated were mainly rich in monomers and small oligomers (mDP around 2-3). This can be supported by a poorer extraction of large proanthocyanidins in the wine-like solution used, and/or by possible depolymerization as a consequence of the acidity of the hydroalcoholic solution buffered at pH 3.2.[6] The hardening of the seed, caused by the intensive dehydration of the outer integument, prevents flavanolic compounds of being extracted during ethanolic maceration.[19,44] In fact, other works showed mDP values ranging from 1.5 to 4.3 when a wine-like solution was used for the extraction of

phenolic compounds from the seeds.[16,42] It is important to consider that the growing zone was a factor influencing the mDP value because significant differences were observed. Further research is required to understand how the environmental factors determine this variability.

Instrumental Acoustic Properties. The crunchiness of the grape seeds was evaluated by a recently developed method based on the recording of the acoustic emission produced during the compression test.[33] The use of six different red winegrape varieties harvested at three ripening stages in two growing zones is expected to cover a wide variation range of the instrumental acoustic properties. Tables 4, 5 and 6 show the mean values of the acoustic parameters measured at gain 0 and 24 dB in the intact seeds, as defined for the chemical parameters. Very few significant varietal differences were found in the acoustic related attributes measured before seed breaking, whereas all of them determined after breaking were significantly different among cultivars. The higher discriminating power of the acoustic parameters measured after seed breaking, if compared to those measured before breaking, could be due to the occurrence of major structural breakdown and larger acoustic events.[33] As can be observed in Table 5, the seeds of the Teroldego cultivar are characterized by high values of AE, LD and N_{pk} measured at gain 0, and of AV_{pk} measured at any gain. On the other hand, Merlot seeds had high values of AE, LD and N_{pk} measured at gain 24 dB but low ones at gain 0, and low values of AV_{pk} at any gain. Sangiovese seeds showed low values of AE, LD and N_{pk} measured at any gain. This was confirmed using total values (sum of before and after seed breaking values) of the acoustic parameters during the compression test (Table 6). The acoustic pressure level at the breakage and the maximum acoustic pressure level permitted to differentiate Nebbiolo from Freisa, Merlot and Teroldego seeds, for which the lower and higher values of the two parameters were obtained, respectively. With few exceptions, the results obtained agreed with those reported for Cabernet Sauvignon and Merlot cultivars.[32,33]

The effect of the growing zone and the ripening stage on the texture parameters of the seeds was small, particularly after breaking. In Trentino, there were significantly lower values of the acoustic pressure level at the breakage and the maximum acoustic pressure level. During ripening, the values of LD and $N_{pk>5\ dB}$ measured at gain 24 dB before breaking decreased significantly, whereas the values of $AV_{pk>5\ dB}$ increased at the same gain setting (Table 4). When total values were assessed at gain 24 dB, the same trend was undergone for $N_{pk>5\ dB}$ and $AV_{pk>5\ dB}$. Under similar operative conditions, the same ripening effect was experimented on these instrumental acoustic properties in Merlot seeds.[33]

Crunchiness is a very complex sensory textural attribute. Some authors evidenced that the number of acoustic peaks can be successfully used as an instrumental indicator of the sensory crispness/crunchiness of foods.[45] The more the acoustic peaks there are, the crispier/crunchier the product is.[28,29] Another commonly used instrumental descriptor of crunchiness is the mean amplitude of the acoustic events, which also increases with the crunchiness.[28,29] Nevertheless, this last parameter depends on the stress level in the source of acoustic emission and on the attenuation of the elastic waves from the source to the sensor. In fact, the hardening of the tissue causes the decrease in the attenuation of the elastic waves.[28] According to Tables 5 and 6, this indicates that the Teroldego cultivar has the crunchiest seeds, whereas Merlot and Sangiovese seeds are less crunchy. Furthermore, the crunchiness of the seeds should increase during ripening because of hardening.

Relationships between instrumental acoustic parameters and phenolic composition of the seeds. In order to develop calibration models that predict with adequate reliability the phenolic composition of the seeds from direct instrumental measurement of the acoustic parameters, a correlation study was performed using the mean values for each sample. Tables 7 and 8 show only the significant correlation coefficients (R) obtained at gain 0 and 24 dB, respectively. The significant correlation coefficients at $p < 0.05$ were between 0.334 and

0.426, at $p < 0.01$ ranged from 0.433 to 0.523, and at $p < 0.001$ varied between 0.537 and 0.713. At gain 0, most of these correlations affected the spectrophotometric index PRO, the extractable content of monomeric flavanols, the mDP value, the G_t percentage and the composition of terminal units. Although the acoustic pressure level at the breakage and the maximum acoustic pressure level had the largest number of significant correlations, the highest correlation coefficients corresponded to total LD and $N_{pk>15\ dB}$ with the G_t percentage ($R > 0.700$). Significant correlations were also found at gain 24 dB for the spectrophotometric index TF, the extractable content of monomeric and B_1 procyanidin, the mDP value, the G_t percentage and the composition of terminal and extension units, but the coefficients were relatively small ($R < 0.600$). In this case, the most significant and strongest correlations corresponded to the extractable content of EC with LD and $N_{pk>5\ dB}$ before seed breaking, as well as to the mDP value with LD, $N_{pk>5\ dB}$ and $AV_{pk>5\ dB}$ before breaking and total LD, $N_{pk>5\ dB}$ and $AV_{pk>5\ dB}$ ($R > 0.530$). At gain 0, almost all chemical parameters were negatively correlated with the instrumental texture parameters, with the exception of the percentage of C in the terminal and extension units. Instead at gain 24 dB, the correlations obtained for LD and N_{pk} were generally positive, whereas AE and AV_{pk} were always negatively correlated with the chemical parameters. Therefore, the negative or positive trend depended on the chemical parameter at gain 0 but on the texture property at gain 24 dB.

The following step was to construct linear regression calibration models for those most significant and strongest correlations between the phenolic composition of the seeds, determined by the reference methods, and the acoustic parameters instrumentally determined at gain 0 and 24 dB ($R > 0.530$, $p < 0.001$). Table 9 shows that the best statistical parameters of calibration corresponded to the relationships between the G_t percentage and total LD or total $N_{pk>15\ dB}$ measured at gain 0 ($R_c > 0.700$, SEC% = 14.4), followed by those between the G_t percentage and LD after breaking, N_{pk} after breaking or total $N_{pk>5\ dB}$ measured at gain 0

(R_c = 0.660-0.684, SEC% ≤ 15.5). Regarding the calibrations carried out using the acoustic parameters measured at gain 24 dB, the statistical parameters were not very satisfactory (R_c < 0.600), particularly for the EC content with SEC% values higher than 38.0.

A full cross-validation was performed to assess the robustness of the linear regression calibration models, and the chemical parameters determined in the seeds by the reference method were compared with those predicted by the calibration models obtained (Table 9). Because of the wide range of samples analyzed to provide adequate variability in the parameters evaluated, the variation range effect (measurement range or mean of this range) on the SECV value was removed by standardizing the predictive accuracy of each calibration model using three statistical parameters (SECV%, RPD and RPIQ). The SECV% values lower than 20 are considered acceptable for most of analytical purposes,[46] which evidences the suitability of the calibration models developed to predict the G_t percentage and the mDP value in intact berry seeds. The differences found between the reference values and those predicted by the calibration models were smaller for the determination of the mDP value from LD, $N_{pk>5\ dB}$ and $AV_{pk>5\ dB}$ before breaking or total LD, $N_{pk>5\ dB}$ and $AV_{pk>5\ dB}$ measured at gain 24 dB, and of the G_t percentage from total LD and $N_{pk>15\ dB}$ measured at gain 0 (SECV% < 16.0), followed by those obtained for the determination of the G_t percentage from AE, LD and N_{pk} after breaking or total AE and $N_{pk>5\ dB}$ measured at gain 0 (SECV% = 16.3-18.4). However, the differences associated with the EC content were too high (SECV% > 40.0).

A better standardization of the SECV value is provided by the RPD and RPIQ indices (Table 9). Taking into account that a small SECV value if compared to the population spread of a certain chemical parameter gives a relatively high index, the higher the RPD value the greater the predictive accuracy. Some authors established standards referring the RPD values higher than 2.0 to very satisfactory calibration models for prediction purposes, whereas the values ranging between 1.4 and 2.0 were indicative of fair models.[47] Nevertheless, no

statistical basis was used to determine these thresholds. In this sense, some researchers have begun to criticize this statistical index, and even proposed the use of the RPIQ index to a better evaluation of the predictive ability of the calibration models.[48] According to this criterion, the calibration models developed for total LD and total $N_{pk>15\ dB}$ measured at gain 0 were satisfactory for prediction purposes of the G_t percentage (RPIQ ≈ 2.1). On the other hand, the predictive accuracy of the different acoustic parameters measured at gain 24 dB was unreliable for quantitative purposes, but acceptable for screening of the mDP value (RPIQ = 1.55-1.62). The remaining chemical parameters cannot be reliably predicted from the acoustic parameters evaluated.

In winery, a fast estimation of the G_t percentage and the mDP value could facilitate making harvesting decisions and winemaking management because of their incidence on sensory attributes of the seeds (astringency and bitterness), and therefore of the wine. The first parameter is closely related to galloylated flavanolic compounds, which are directly involved in astringency. The second one is strongly related to the chain length for low molecular weight flavanols, extractable during the winemaking process, and influences both astringency and bitterness. Bitterness is restricted to monomers and low molecular weight oligomers,[8,10,11] whereas astringency is strongly affected by the stereochemistry and molecular conformation of proanthocyanidins, which are related to the composition of the terminal and extension units.[49] The larger, more water-soluble tannins are perceived as more astringent than the smaller, more hydrophobic and pigmented tannins, which are perceived as hotter and more bitter.[50]

In an attempt to reduce the high SEC and SECV values obtained for the prediction of the extractable content of EC in the seeds, exponential regression calibration models were developed from both LD and $N_{pk>5\ dB}$ before breaking measured at gain 24 dB. The R_c values increased slightly (0.649 and 0.595, respectively), but SEC% and SECV% decreased up to

values less than 10, and the RPIQ index achieved values close to 2.0. Therefore, the EC content may be predicted from the instrumental acoustic parameters using exponential calibration models for screening purposes.

The spectrophotometric indices, such as the A_{280} value and the extractable content of TP, TF, PRO and FRV, are more usually used in the wine industry to assess the phenolic maturity of the seeds. Because of the weakness of the relationships found between the spectrophotometric indices and the instrumental acoustic parameters of the seeds working with the six winegrape cultivars together ($R < 0.400$, $p < 0.05$, Tables 7 and 8), the predictive ability of the texture attributes better correlated with these indices was evaluated but working with the Nebbiolo cultivar individually. The variability in the reference values for the phenolic composition of Nebbiolo seeds, determined by spectrophotometric methods, was suitable for developing calibration models because the agroclimatic and ripening effects were considered by harvesting samples in ten different vineyards at two different ripening stages. A correlation study confirmed the results reported in a preliminary work performed on Merlot seeds.[33] At gain 24 dB, the strongest and most significant correlations ($R = 0.576$-0.696, $p < 0.01$) were obtained between the TF content and total $N_{pk>5\ dB}$, $AV_{pk>5\ dB}$ before and after breaking, $AV_{pk>15\ dB}$ after breaking or total $AV_{pk>15\ dB}$. At gain 0, the FRV content was best correlated with the maximum acoustic pressure level ($R = 0.584$, $p < 0.01$). For these last acoustic parameters, the linear regression calibration models were developed, and their predictive accuracy was evaluated. As can be seen in Table 10, the best performance parameters of calibration corresponded to the prediction of the extractable content of TF from AV_{pk} measured at gain 24 dB after seed breaking ($R_c \approx 0.7$, SEC% < 10). On the other hand, SECV% values around 12 together with RPIQ values higher than 2 evidenced an acceptable robustness of the calibration models for quantification purposes. Regarding the extractable content of FRV, the analytical performance of the calibration model was not good, so that the

maximum acoustic pressure level measured at gain 0 was not a reliable predictor and could be only applied for poor screening. The determination of the extractable content of FRV in the seeds provides relevant information because this spectrophotometric index is sensitive to the presence of monomeric flavanols, and is partially related with the concentration of low molecular weight proanthocyanidins with a mDP value from 2 to 4.[51] Oligomeric flavanols represent the main phenolic fraction released from intact seeds during winemaking.

In conclusion, this study on the assessment of the instrumental acoustic parameters as possible predictors of the phenolic maturity of the seeds revealed that the use of common calibration models for different winegrape cultivars and growing zones provides a satisfactory prediction of complex chemical parameters related to the flavanolic composition like the G_t percentage, the EC content and the mDP value. This is of great relevance considering that highly informative chemical methods, such as the determination of the flavanols amount and profile by LC-MS before and after phloroglucinolysis, are rather time consuming and expensive. In terms of the spectrophotometric indices commonly used in the wine industry, the construction of separate calibration models for each cultivar is recommended for quantitative or screening purposes depending on the index. Therefore, the fast, simple and economically reasonable instrumental determination of the texture parameters, associated with the acoustic response to mechanical loading of the grape seeds, could be a valuable tool in making harvesting decisions and winemaking management, particularly when a large number of samples need to be analyzed.

Acknowledgements. The authors thank Marco Stefanini and Graziano Murada for kindly providing the grape samples from Trentino and Valtellina, respectively.

References

(1) Cáceres, A.; Peña-Neira, A.; Galvez, A.; Obreque-Slier, E.; López-Solís, R.; Canals, J.M. Phenolic compositions of grapes and wines from cultivar Cabernet Sauvignon produced in Chile and their relationship to commercial value. *J. Agric. Food Chem.* **2012**, *60*, 8694−8702.

(2) Kallithraka, S.; Garcia-Viguera, C.; Bridle, P.; Bakker, J. Survey of solvents for the extraction of grape seed phenolics. *Phytochem. Anal.* **1995**, *6*, 265–267.

(3) Nawaz, H.; Shi, J.; Mittal, G.S.; Kakuda, Y. Extraction of polyphenols from grape seeds and concentration by ultrafiltration. *Sep. Purif. Technol.* **2006**, *48*, 176–181.

(4) Monagas, M.; Gómez-Cordovés, C.; Bartolomé, B.; Laureano, O.; Ricardo da Silva, J.M. Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from *Vitis vinifera* L. cv. Graciano, Tempranillo, and Cabernet Sauvignon. *J. Agric. Food Chem.* **2003**, *51*, 6475–6481.

(5) Rodríguez Montealegre, R.; Romero Peces, R.; Chacón Vozmediano, J.L.; Martínez Gascueña, J.; García Romero, E. Phenolic compounds in skins and seeds of ten grape *Vitis vinifera* varieties grown in a warm climate. *J. Food Comp. Anal.* **2006**, *19*, 687–693.

(6) Mattivi, F.; Vrhovsek, U.; Masuero, D.; Trainotti, D. Differences in the amount and structure of extractable skin and seed tannins amongst red grape varieties. *Aust. J. Grape Wine Res.* **2009**, *15*, 27–35.

(7) Ferrer-Gallego, R.; García-Marino, M.; Hernández-Hierro, J.M.; Rivas-Gonzalo, J.C.; Escribano-Bailón, M.T. Statistical correlation between flavanolic composition, colour and sensorial parameters in grape seed during ripening. *Anal. Chim. Acta* **2010**, *660*, 22–28.

(8) Peleg, H.; Gacon, K.; Schlich, P.; Noble, A.C. Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. *J. Sci. Food Agric.* **1999**, *79*, 1123–1128.

(9) McRae, J.M.; Falconer, R.J.; Kennedy, J.A. Thermodynamics of grape and wine tannin interaction with polyproline: Implications for red wine astringency. *J. Agric. Food Chem.* **2010**, *58*, 12510–12518.

(10) Obreque-Slier, E.; Peña-Neira, A.; López-Solís, R. Precipitation of low molecular weight phenolic compounds of grape seeds cv. Carménère (*Vitis vinifera* L.) by whole saliva. *Eur. Food Res. Technol.* **2011**, *232*, 113–121.

(11) Cheynier, V.; Dueñas-Paton, M.; Salas, E.; Maury, C.; Souquet, J.M.; Sarni-Manchado, P.; Fulcrand, H. Structure and properties of wine pigments and tannins. *Am. J. Enol. Vitic.* **2006**, *57*, 298–305.

(12) Puškaš, V.; Miljić, U. The application of D-optimal design for modelling the red wine ageing process. *Food Control* **2012**, *28*, 362–367.

(13) Núñez, V.; Gómez-Cordovés, C.; Bartolomé, B.; Hong, Y.-J.; Mitchell, A.E. Non-galloylated and galloylated proanthocyanidin oligomers in grape seeds from *Vitis vinifera* L. cv. Graciano, Tempranillo and Cabernet Sauvignon. *J. Sci. Food Agric.* **2006**, *86*, 915–921.

(14) Fanzone, M.; Zamora, F.; Jofré, V.; Assof, M.; Peña-Neira, A. Phenolic composition of Malbec grape skins and seeds from Valle de Uco (Mendoza, Argentina) during ripening. Effect of cluster thinning. *J. Agric. Food Chem.* **2011**, *59*, 6120–6136.

(15) Kotseridis, Y.; Georgiadou, A.; Tikos, P.; Kallithraka, S.; Koundouras, S. Effects of severity of post-flowering leaf removal on berry growth and composition of three red *Vitis vinifera* L. cultivars grown under semiarid conditions. *J. Agric. Food Chem.* **2012**, *60*, 6000−6010.

(16) Obreque-Slier, E.; López-Solís, R.; Castro-Ulloa, L.; Romero-Díaz, C.; Peña-Neira, A. Phenolic composition and physicochemical parameters of Carménère, Cabernet

Sauvignon, Merlot and Cabernet Franc grape seeds (*Vitis vinifera* L.) during ripening. *LWT - Food Sci. Technol.* **2012**, *48*, 134–141.

(17) Kennedy, J.A.; Matthews, M.A.; Waterhouse, A.L. Changes in grape seed polyphenols during fruit ripening. *Phytochemistry* **2000**, *55*, 77–85.

(18) Downey, M.O.; Harvey, J.S.; Robinson, S.P. Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. *Aust. J. Grape Wine Res.* **2003**, *9*, 15–27.

(19) Cadot, Y.; Miñana-Castelló, M.T.; Chevalier, M. Anatomical, histological, and histochemical changes in grape seeds from *Vitis vinifera* L. cv Cabernet franc during fruit development. *J. Agric. Food Chem.* **2006**, *54*, 9206–9215.

(20) Lorrain, B.; Chira, K.; Teissedre, P.-L. Phenolic composition of Merlot and Cabernet-Sauvignon grapes from Bordeaux vineyard for the 2009-vintage: Comparison to 2006, 2007 and 2008 vintages. *Food Chem.* **2011**, *126*, 1991–1999.

(21) Kennedy, J.A.; Troup, G.J.; Pilbrow, J.R.; Hutton, D.R.; Hewitt, D.; Hunter, C.R.; Ristic, R.; Iland, P.G.; Jones, G.P. Development of seed polyphenols in berries from *Vitis vinifera* L. cv. Shiraz. *Aust. J. Grape Wine Res.* **2000**, *6*, 244–254.

(22) Boido, E.; García-Marino, M.; Dellacassa, E.; Carrau, F.; Rivas-Gonzalo, J.C.; Escribano-Bailón, M.T. Characterisation and evolution of grape polyphenol profiles of *Vitis vinifera* L. cv. Tannat during ripening and vinification. *Aust. J. Grape Wine Res.* **2011**, *17*, 383–393.

(23) Rustioni, L.; Bedgood Jr., D.R.; Failla, O.; Prenzler, P.D.; Robards, K. Copigmentation and anti-copigmentation in grape extracts studied by spectrophotometry and post-column-reaction HPLC. *Food Chem.* **2012**, *132*, 2194–2201.

(24) Olarte Mantilla, S.M.; Collins, C.; Iland, P.G.; Johnson, T.E.; Bastian, S.E.P. Review: Berry Sensory Assessment: concepts and practices for assessing winegrapes' sensory attributes. *Aust. J. Grape Wine Res.* **2012**, *18*, 245–255.

(25) Le Moigne, M.; Maury, C.; Bertrand, D.; Jourjon, F. Sensory and instrumental characterisation of Cabernet Franc grapes according to ripening stages and growing location. *Food Qual. Pref.* **2008**, *19*, 220–231.

(26) Letaief, H., Maury, C., Simoneaux, R., Siret, R. Sensory and instrumental texture measurements for assessing grape seed's parameters during fruits development. *J. Sci. Food Agric.* **2013**, in press, DOI 10.1002/jsfa.6071.

(27) Duizer, L. A review of acoustic research for studying the sensory perception of crisp, crunchy and crackly textures. *Trends Food Sci. Technol.* **2001**, *12*, 17–24.

(28) Zdunek, A.; Konopacka, D.; Jesionkowska, K. Crispness and crunchiness judgment of apples based on contact acoustic emission. *J. Texture Stud.* **2010**, *41*, 75–91.

(29) Saeleaw, M.; Schleining, G. A review: Crispness in dry foods and quality measurements based on acoustic-mechanical destructive techniques. *J. Food Eng.* **2011**, *105*, 387–399.

(30) Van Vliet, T.; Primo-Martín, C. Interplay between product characteristics, oral physiology and texture perception of cellular brittle foods. *J. Texture Stud.* **2011**, *42*, 82–94.

(31) Rolle, L.; Torchio, F.; Lorrain, B.; Giacosa, S.; Río Segade, S.; Cagnasso, E.; Gerbi, V.; Teissedre, P.-L. Rapid methods for the evaluation of total phenol content and extractability in intact grape seeds of Cabernet-Sauvignon: instrumental mechanical properties and FT-NIR spectrum. *J. Int. Sci. Vigne Vin* **2012**, *46*, 29–40.

(32) Rolle, L.; Giacosa, S.; Torchio, F.; Río Segade, S. Changes in acoustic and mechanical properties of Cabernet Sauvignon seeds during ripening. *Am. J. Enol. Vitic.* **2012**, *63*, 413–418.

(33) Torchio, F.; Giacosa, S.; Río Segade, S.; Mattivi, F.; Gerbi, V.; Rolle, L. Optimization of a method based on the simultaneous measurement of acoustic and mechanical

properties of winegrape seeds for the determination of the ripening stage. *J. Agric. Food Chem.* **2012**, *60*, 9006−9016.

(34) Mattivi, F.; Zulian, C.; Nicolini, G.; Valenti, L. Wine, biodiversity, technology, and antioxidants. *Ann. N. Y. Acad. Sci.* **2002**, *957*, 37–56.

(35) Di Stefano, R.; Cravero, M.C. Metodi per lo studio dei polifenoli dell'uva. *Riv. Vitic. Enol.* **1991**, *44*, 37–45.

(36) Torchio, F.; Cagnasso, E.; Gerbi, V.; Rolle, L. Mechanical properties, phenolic composition and extractability indices of Barbera grapes of different soluble solids contents from several growing areas. *Anal. Chim. Acta* **2010**, *660*, 183–189.

(37) Fortes Gris, E.; Mattivi, F.; Ferreira, E.A.; Vrhovsek, U.; Curi Pedrosa, R.; Bordignon-Luiz M.T. Proanthocyanidin profile and antioxidant capacity of Brazilian *Vitis vinifera* red wines. *Food Chem.* **2011**, *126*, 213–220.

(38) Kennedy, J.A.; Jones, G.P. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J. Agric. Food Chem.* **2001**, *49*, 1740–1746.

(39) Bellon-Maurel, V.; Fernandez-Ahumada, E.; Palagos, B.; Roger, J.-M.; McBratney, A. Critical review of chemometric indicators commonly used for assessing the quality of the prediction of soil attributes by NIR spectroscopy. *Trends Anal. Chem.* **2010**, *29*, 1073–1081.

(40) Bucić-Kojić, A.; Planinić, M.; Tomas, S.; Jakobek, L.; Šeruga, M. Influence of solvent and temperature on extraction of phenolic compounds from grape seed, antioxidant activity and colour of extract. *Int. J. Food Sci. Technol.* **2009**, *44*, 2394–2401.

(41) Yilmaz, E.E.; Özvural, E.B.; Vural, H. Extraction and identification of proanthocyanidins from grape seed (*Vitis Vinifera*) using supercritical carbon dioxide. *J. Supercritical Fluids* **2011**, *55*, 924–928.

(42) Obreque-Slier, E.; Peña-Neira, A.; López-Solís, R.; Zamora-Marín, F.; Ricardo-Da Silva, J.M.; Laureano, O. Comparative study of the phenolic composition of seeds and skins from Carménère and Cabernet Sauvignon grape varieties (*Vitis vinifera* L.) during ripening. *J. Agric. Food Chem.* **2010**, *58*, 3591–3599.

(43) Chira, K.; Schmauch, G.; Saucier, C.; Fabre, S.; Teissedre, P.-L. Grape variety effect on proanthocyanidin composition and sensory perception of skin and seed tannin extracts from Bordeaux wine grapes (Cabernet Sauvignon and Merlot) for two consecutive vintages (2006 and 2007). *J. Agric. Food Chem.* **2009**, *57*, 545–553.

(44) Bautista-Ortín, A.B.; Rodríguez-Rodríguez, P.; Gil-Muñoz, R.; Jiménez-Pascual, E.; Busse-Valverde, N.; Martínez-Cutillas, A.; López-Roca, J.M.; Gómez-Plaza, E. Influence of berry ripeness on concentration, qualitative composition and extractability of grape seed tannins. *Aust. J. Grape Wine Res.* **2012**, *18*, 123–130.

(45) Varela, P.; Chen, J.; Fiszman, S.; Povey, M.J.W. Crispness assessment of roasted almonds by an integrated approach to texture description: Texture, acoustic, sensory and structure. *J. Chemometrics* **2006**, *20*, 311–320.

(46) Cozzolino, D.; Cynkar, W.U.; Dambergs, R.G.; Mercurio, M.D.; Smith, P.A. Measurement of condensed tannins and dry matter in red grape homogenates using near infrared spectroscopy and partial least squares. *J. Agric. Food Chem.* **2008**, *56*, 7631–7636.

(47) Chang, C.-W.; Laird, D.A.; Mausbach, M.J.; Hurburgh, Jr. C.R. Near-Infrared reflectance spectroscopy–principal components regression analyses of soil properties. *Soil Sci. Soc. Am. J.* **2001**, *65*, 480–490.

(48) Cozzolino, D.; Cynkar, W.; Shah, N.; Smith, P. Quantitative analysis of minerals and electric conductivity of red grape homogenates by near infrared reflectance spectroscopy. *Comput. Electron. Agric.* **2011**, *77*, 81–85.

(49) Quijada-Morín, N.; Regueiro, J.; Simal-Gándara, J.; Tomás, E.; Rivas-Gonzalo, J.C.; Escribano-Bailón, M.T. Relationship between the sensory-determined astringency and the flavanolic composition of red wines. *J. Agric. Food Chem.* **2012**, *60*, 12355−12361.

(50) McRae, J.M.; Schulkin, A.; Kassara, S.; Holt, H.E.; Smith, P.A. Sensory properties of wine tannin fractions: implications for in-mouth sensory properties. *J. Agric. Food Chem.* **2013**, *61*, 719−727.

(51) Vrhovsek, U.; Mattivi, F.; Waterhouse, A.L. Analysis of red wine phenolics: comparison of HPLC and spectrophotometric methods. *Vitis* **2001**, *40*, 87–91.

Funding

Part of this study was funded by Fondazione Fojanini di Studi Superiori (Sondrio, Italy).

Table 1. Phenolic Composition of Seeds from Several Winegrape Varieties Sampled in Two Growing Zones at Different Ripening Stages[a]

Factor	A_{280}/kg grape [b,f]	mg/kg grape [b,f]			
		TP	TF	PRO	FRV
Variety[c]					
Cabernet Sauvignon	23.5±4.5b,c	1449±315b	1519±342b	1434±288b	1360±313b,c
Freisa	25.8±3.7c	1535±382b	1694±310b	1407±229b	1502±270c
Merlot	17.3±4.7a,b	1073±248a,b	1238±332a,b	1031±208a	980±273a,b
Nebbiolo	20.1±1.5b,c	1158±266a,b	1309±135a,b	1127±86a,b	1096±94a,b,c
Sangiovese	13.6±3.8a	863±165a	836±242a	782±224a	901±346a
Teroldego	21.5±4.5b,c	1461±230b	1470±421b	1109±243a,b	1205±238a,b,c
Sign[g]	***	***	***	***	**
Growing zone[d]					
Piedmont	20.4±4.4	1151±226	1346±317	1154±277	1215±247
Trentino	19.9±6.6	1346±442	1314±486	1122±348	1115±401
Sign	ns	ns	ns	ns	ns
Ripening stage[e]					
S1	21.9±5.8	1428±408	1540±418	1254±312	1286±356
S2	18.5±4.6	1154±343	1188±331	1053±308	1096±303
S3	20.6±5.9	1192±259	1309±386	1142±289	1135±334
Sign	ns	ns	ns	ns	ns

[a] A_{280} = absorbance measured at 280 nm, TP = total polyphenols, TF = total flavonoids, PRO = proanthocyanidins, FRV = flavanols reactive to vanillin. [b] Data are expressed as mean value ± standard deviation. [c] n = 18 for winegrape variety, [d] n = 54 for growing zone, [e] n = 36 for ripening stage. [f] Different Latin letters within the same column indicate significant differences according to Tukey-b test ($p < 0.05$). [g] Sign: **,*** and ns indicate significance at $p < 0.01$, 0.001 and not significant, respectively.

Table 2. Monomeric Flavanol Composition of Seeds from Several Winegrape Varieties Sampled in Two Growing Zones at Different Ripening Stages[a]

Factor	mg/kg grape [b,f]					
	C	EC	GC	EGC	ECG	TM
Variety[c]						
Cabernet Sauvignon	126.1±53.1b	70.1±32.2	0.61±0.46	1.12±0.85	1.89±1.43	199.8±85.7b
Freisa	126.6±71.0b	79.2±37.7	0.47±0.08	1.08±0.56	3.08±2.26	210.5±107.4b
Merlot	57.3±18.7a,b	53.1±18.9	0.37±0.09	0.72±0.16	2.34±2.36	113.8±36.3a,b
Nebbiolo	101.7±35.5a,b	45.0±17.7	0.47±0.10	0.94±0.34	1.66±1.01	149.7±53.3a,b
Sangiovese	33.3±14.6a	40.0±15.8	0.40±0.10	0.80±0.16	1.72±1.75	76.2±30.5a
Teroldego	106.3±46.2b	65.9±27.6	0.41±0.08	0.88±0.31	1.20±0.71	174.8±71.6a,b
Sign[g]	**	ns	ns	ns	ns	*
Growing zone[d]						
Piedmont	64.3±32.9	41.2±16.5	0.36±0.06	0.66±0.16	3.07±1.53	109.5±47.6
Trentino	117.8±59.7	76.5±26.5	0.55±0.25	1.19±0.50	0.82±0.87	196.9±84.6
Sign	**	***	**	***	***	***
Ripening stage[e]						
S1	118.8±56.8	73.7±25.5	0.45±0.09	1.00±0.39	2.45±2.07	196.5±78.9
S2	76.0±38.9	52.8±23.4	0.42±0.08	0.80±0.27	1.40±1.36	131.5±59.1
S3	81.7±57.8	51.5±30.4	0.50±0.34	0.99±0.63	2.02±1.59	136.7±87.2
Sign	ns	ns	ns	ns	ns	ns

[a]C = catechin, EC = epicatechin, GC = gallocatechin, EGC = epigallocatechin, ECG = epicatechin gallate, TM = total monomers. [b]Data are expressed as mean value ± standard deviation. [c]n = 18 for winegrape variety, [d]n = 54 for growing zone, [e]n = 36 for ripening stage. [f]Different Latin letters within the same column indicate significant differences according to Tukey-b test ($p < 0.05$). [g]Sign: *,**,*** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively.

Table 3. Dimer and Polymer Flavanolic Composition of Seeds from Several Winegrape Varieties Sampled in Two Growing Zones at Different Ripening Stages[a]

Factor	mg/kg grape [b,f]				mDP [b,f]	% [b,f]	
	B_1	B_2	TD	TPP		G_t	G_e
Variety[c]							
Cabernet Sauvignon	49.0±18.4b	56.7±26.9b	105.7±43.6b	916.8±281.5b	2.4±0.3	15.5±4.3a,b	10.7±4.8
Freisa	45.5±8.5b	44.0±7.5a,b	89.5±9.7a,b	866.4±110.5b	2.5±0.5	17.5±1.7b	10.2±3.6
Merlot	36.6±16.7a,b	33.4±12.0a	70.0±26.3a,b	596.2±169.9a,b	2.4±0.4	18.0±3.1b	10.4±1.1
Nebbiolo	32.0±8.4a,b	31.4±5.8a	63.4±13.9a	747.9±189.6a,b	2.3±0.4	16.1±2.6a,b	9.2±2.1
Sangiovese	23.4±6.5a	34.6±7.7a,b	58.0±12.1a	473.2±229.6a	2.1±0.3	18.3±3.9b	11.5±1.4
Teroldego	32.4±9.7a,b	44.7±10.7a,b	77.1±19.7a,b	623.8±123.4a,b	2.3±0.5	12.4±2.1a	11.4±1.8
Sign[g]	**	*	*	**	ns	*	ns
Growing zone[d]							
Piedmont	39.8±11.8	44.4±8.3	84.3±18.6	735.8±244.4	2.1±0.3	17.2±3.2	11.0±3.2
Trentino	32.2±15.9	36.6±19.9	68.8±33.1	657.6±238.0	2.6±0.4	15.5±3.7	10.1±1.9
Sign	ns	ns	ns	ns	***	ns	ns
Ripening stage[e]							
S1	39.7±14.6	41.7±11.7	81.4±23.8	771.1±249.0	2.6±0.4	16.8±2.9	10.4±3.8
S2	33.6±11.9	34.2±9.6	67.8±19.4	659.6±218.7	2.2±0.4	14.7±3.3	10.6±2.1
S3	36.8±16.0	46.2±21.6	82.9±36.3	694.1±245.5	2.3±0.4	17.2±4.0	10.6±2.1
Sign	ns	ns	ns	ns	ns	ns	ns

[a] B_1 = procyanidin B_1, B_2 = procyanidin B_2, TD = total dimers, TPP = total polymers, mDP = mean degree of polimerization, G_t = galloylation in terminal units, G_e = galloylation in extension units. [b] Data are expressed as mean value ± standard deviation. [c] n = 18 for winegrape variety, [d] n = 54 for growing zone, [e] n = 36 for ripening stage. [f] Different Latin letters within the same column indicate significant differences according to Tukey-b test ($p < 0.05$). [g] Sign: *,** and ns indicate significance at $p < 0.05$, 0.01 and not significant, respectively.

Table 4. Instrumental Acoustic Parameters Determined Before Breakage of Intact Seeds from Several Winegrape Varieties Sampled in Two Growing Zones at Different Ripening Stages[a]

Factor	AE (dB×mm) [b,f]		LD [b,f]		$N_{pk>5\ dB}$ [b,f]		$N_{pk>15\ dB}$ [b,f]		$AV_{pk>5\ dB}$ (dB) [b,f]		$AV_{pk>15\ dB}$ (dB) [b,f]	
	G=0	G=24	G=0	G=24	G=0	G=24	G=0	G=24	G=0	G=24	G=0	G=24
Variety[c]												
Cabernet Sauvignon	1.0±0.2b	20.5±2.4a,b	221.8±41.4	602.2±129.5	1.4±0.2	14.6±5.5	1.4±0.3	2.3±0.7	53.4±6.5	46.2±5.5	53.7±6.3	58.3±7.5
Freisa	1.0±0.3a,b	23.1±0.8b,c	232.2±65.1	774.3±110.8	1.6±0.5	20.0±4.1	1.5±0.5	3.7±0.6	56.0±8.0	44.7±3.1	55.8±7.6	63.2±4.7
Merlot	0.6±0.3a,b	20.0±2.7a,b	146.6±57.8	698.2±203.6	1.0±0.5	18.0±7.1	0.9±0.4	3.2±2.0	42.8±11.7	44.5±7.8	42.8±11.6	57.0±8.3
Nebbiolo	0.7±0.3a,b	19.6±1.7a	190.3±41.2	658.5±158.5	1.2±0.3	16.6±5.4	1.2±0.3	2.7±1.4	46.4±7.6	44.0±4.1	46.1±7.8	59.0±5.9
Sangiovese	0.6±0.4a	24.4±1.3c	154.1±59.6	711.7±125.0	1.1±0.4	18.2±4.6	1.1±0.4	3.0±0.5	43.1±13.3	45.5±3.0	43.2±13.3	60.5±3.6
Teroldego	0.8±0.2a,b	22.5±2.1a,b,c	189.2±34.6	765.9±141.6	1.1±0.3	19.3±5.5	1.1±0.2	3.7±0.9	47.7±4.0	44.4±4.6	46.4±4.0	59.4±1.9
Sign[g]	*	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Growing zone[d]												
Piedmont	0.8±0.3	22.0±2.7	184.3±58.8	674.6±146.8	1.3±0.4	17.3±5.4	1.2±0.4	2.8±0.9	47.5±11.8	45.4±4.1	47.3±11.7	59.5±6.0
Trentino	0.8±0.3	21.5±2.5	192.1±57.6	731.1±150.0	1.2±0.4	18.3±5.3	1.2±0.4	3.4±1.3	48.7±7.7	44.4±5.2	48.4±7.9	59.7±5.5
Sign	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Ripening stage[e]												
S1	0.8±0.3	21.5±2.4	168.8±63.4	809.4±98.2b	1.1±0.4	22.0±3.8b	1.0±0.4	3.3±0.7	44.6±11.2	41.9±2.4a	44.7±11.2	57.1±5.7
S2	0.9±0.3	21.5±3.0	214.2±49.2	676.3±154.7a	1.4±0.4	16.7±4.8a	1.4±0.4	3.2±1.5	53.6±6.1	45.3±4.6a,b	52.8±6.4	60.6±7.0
S3	0.7±0.3	22.1±2.5	186.3±53.9	635.2±134.8a	1.2±0.4	15.2±4.8a	1.2±0.4	2.8±1.1	47.0±10.2	47.0±5.1b	46.9±10.4	61.2±3.4
Sign	ns	ns	ns	**	ns	**	ns	ns	ns	*	ns	ns

[a]AE = acoustic energy, LD = linear distance, $N_{pk>5\ dB}$ = number of acoustic peaks higher than 5 dB, $N_{pk>15\ dB}$ = number of acoustic peaks higher than 15 dB, $AV_{pk>5\ dB}$ = average pressure level for peaks higher than 5 dB, $AV_{pk>15\ dB}$ = average pressure level for peaks higher than 15 dB, G = gain setting (dB). [b]Data are expressed as mean value ± standard deviation. [c]n = 180 for winegrape variety, [d]n = 540 for growing zone, [e]n = 360 for ripening stage. [f]Different Latin letters within the same column indicate significant differences according to Tukey-b test ($p < 0.05$). [g]Sign: *,**,*** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively.

Table 5. Instrumental Acoustic Parameters Determined After Breakage of Intact Seeds from Several Winegrape Varieties Sampled in Two Growing Zones at Different Ripening Stages[a]

Factor	AE (dB×mm) [b,f]		LD [b,f]		$N_{pk>5\ dB}$ [b,f]		$N_{pk>15\ dB}$ [b,f]		$AV_{pk>5\ dB}$ (dB) [b,f]		$AV_{pk>15\ dB}$ (dB) [b,f]	
	G=0	G=24	G=0	G=24	G=0	G=24	G=0	G=24	G=0	G=24	G=0	G=24
Variety[c]												
Cabernet Sauvignon	11.7±1.1a	45.8±2.0c	1874.4±193.9b	1581.5±56.0b	14.7±1.6b	31.4±1.0b,c	13.5±1.5b	17.6±0.5b	72.0±1.2a	65.6±1.7a,b	72.1±1.3a	73.6±1.2a,b
Freisa	11.3±1.5a	41.5±2.5a,b	1570.9±99.0a	1410.9±35.0a,b	12.5±1.0a	28.6±1.2b,c	11.2±0.7a	15.7±0.6a,b	73.7±1.0a,b	65.2±1.9a,b	74.1±1.1a,b	73.6±1.1a,b
Merlot	9.8±1.4a	46.5±2.7c	1530.1±246.2a	1619.8±236.5b	11.9±1.7a	32.0±3.7c	11.0±1.7a	17.3±2.4b	71.9±1.6a	62.7±2.8a	72.2±1.5a	72.0±2.1a
Nebbiolo	12.3±1.5a,b	40.8±1.6a,b	1868.2±177.2b	1449.6±140.2a,b	14.7±1.5b	27.7±2.0a,b	13.2±1.2b	16.1±1.5a,b	73.7±1.2a,b	66.9±2.5b,c	74.1±1.2a,b	75.4±1.5b,c
Sangiovese	10.3±1.2a	38.8±1.9a	1515.1±137.1a	1295.8±122.3a	11.8±0.8a	24.4±2.1a	10.6±0.9a	14.0±1.5a	74.0±1.3a,b	66.5±1.5b,c	74.3±1.3a,b	74.9±1.1b,c
Teroldego	14.1±1.8b	43.0±3.2b,c	2089.7±168.2b	1463.0±165.1a,b	16.7±1.3b	28.4±3.0b,c	14.8±1.1b	16.6±2.0b	74.3±1.7b	69.6±1.4c	74.7±1.8b	77.0±1.3c
Sign[g]	***	***	***	**	***	***	***	**	**	***	**	***
Growing zone[d]												
Piedmont	11.8±2.2	42.6±3.7	1682.7±231.7	1421.1±165.0	13.2±1.8	28.0±3.6	11.9±1.6	15.7±1.8	73.5±1.8	66.4±3.1	73.9±1.9	74.7±2.4
Trentino	11.3±1.8	42.7±3.5	1790.8±307.4	1512.1±172.1	14.1±2.5	29.3±3.1	12.7±2.2	16.6±2.0	73.1±1.4	65.8±2.5	73.3±1.4	74.2±1.7
Sign	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Ripening stage[e]												
S1	11.1±2.3	41.2±3.3	1630.8±288.0	1454.6±123.0	12.9±2.3	28.5±2.9	11.7±2.0	15.9±1.3	73.1±1.8	64.7±2.9	73.5±1.9	73.9±2.0
S2	12.3±1.8	43.2±3.4	1827.8±219.4	1486.1±231.3	14.2±1.9	28.9±4.2	12.9±1.5	16.5±2.4	73.9±1.3	66.9±2.7	74.1±1.4	74.8±2.2
S3	11.2±1.7	43.8±3.7	1756.6±299.7	1474.4±152.6	13.9±2.4	28.9±3.0	12.4±2.2	16.3±1.9	72.7±1.5	66.7±2.6	73.1±1.6	74.5±2.2
Sign	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

[a]AE = acoustic energy, LD = linear distance, $N_{pk>5\ dB}$ = number of acoustic peaks higher than 5 dB, $N_{pk>15\ dB}$ = number of acoustic peaks higher than 15 dB, $AV_{pk>5\ dB}$ = average pressure level for peaks higher than 5 dB, $AV_{pk>15\ dB}$ = average pressure level for peaks higher than 15 dB, G = gain setting (dB). [b]Data are expressed as mean value ± standard deviation. [c]n = 180 for winegrape variety, [d]n = 540 for growing zone, [e]n = 360 for ripening stage. [f]Different Latin letters within the same column indicate significant differences according to Tukey-b test ($p < 0.05$). [g]Sign: **,*** and ns indicate significance at $p < 0.01$, 0.001 and not significant, respectively.

Table 6. Total Instrumental Acoustic Parameters of Intact Seeds from Several Winegrape Varieties Sampled in Two Growing Zones at Different Ripening Stages[a]

Factor	Breakage (dB) [b,f]		Maximum (dB) [b,f]		AE (dB×mm) [b,f]		LD [b,f]		$N_{pk>5\ dB}$ [b,f]		$N_{pk>15\ dB}$ [b,f]		$AV_{pk>5\ dB}$ (dB) [b,f]		$AV_{pk>15\ dB}$ (dB) [b,f]	
	G=0		G=0		G=0	G=24	G=0	G=24	G=0	G=24	G=0	G=24	G=0	G=24	G=0	G=24
Variety[c]																
Cabernet Sauvignon	95.0±3.3a,b		98.7±1.6a,b		12.7±1.3a,b	66.3±4.1	2096.3±224.4b,c	2183.6±157.2	16.1±1.8b,c	46.0±5.9	14.8±1.6b,c	19.9±1.0	71.7±1.1	59.4±4.1	71.8±1.1	72.7±2.2
Freisa	100.8±4.6c		102.3±3.8b		12.3±1.8a,b	64.6±3.2	1803.1±150.1a,b	2185.2±132.6	14.1±1.4a,b	48.6±4.6	12.7±1.0a,b	19.5±1.0	72.7±0.9	56.7±3.0	73.0±1.0	71.8±1.7
Merlot	100.6±2.3b,c		102.4±2.4b		10.5±1.7a	66.5±5.4	1676.7±286.5a	2318.0±419.4	12.9±2.0a	50.0±10.4	11.9±2.0a	20.4±4.1	71.5±1.9	56.0±5.6	71.8±1.9	69.9±4.0
Nebbiolo	94.5±2.3a		97.8±1.2a		13.0±1.7a,b	60.4±3.0	2058.5±192.9b,c	2108.1±274.7	15.9±1.5b,c	44.3±6.7	14.3±1.3b,c	18.8±2.6	73.0±1.1	58.3±4.2	73.4±1.2	73.6±2.2
Sangiovese	98.5±3.8a,b,c		100.1±3.0a,b		10.8±1.4a	63.3±1.2	1669.2±167.7a	2007.5±156.9	12.9±0.9a	42.6±4.9	11.7±1.1a	17.0±1.5	72.7±1.6	57.4±2.9	72.9±1.5	72.9±1.4
Teroldego	100.5±3.5b,c		101.7±3.0b		14.9±2.0b	65.5±3.7	2278.9±176.5c	2228.9±256.8	17.8±1.4c	47.7±7.1	15.9±1.1c	20.3±2.8	73.9±1.6	59.4±4.0	74.2±1.7	74.3±2.2
Sign[g]	**		*		***	ns	***	ns	***	ns	***	ns	ns	ns	ns	ns
Growing zone[d]																
Piedmont	100.1±4.2		101.7±3.1		12.5±2.3	64.5±3.3	1867.0±257.8	2095.7±225.3	14.5±1.9	45.2±7.0	13.1±1.7	18.5±1.9	72.7±1.8	58.3±4.0	73.0±1.8	73.0±2.6
Trentino	96.4±3.3		99.2±2.5		12.1±1.9	64.3±4.6	1982.9±335.8	2243.2±264.9	15.3±2.7	47.7±6.7	13.9±2.4	20.0±2.9	72.5±1.2	57.4±3.9	72.7±1.3	72.1±2.7
Sign	**		**		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Ripening stage[e]																
S1	97.2±3.4		99.2±2.7		11.9±2.6	62.6±3.3	1799.5±331.8	2264.0±129.1	14.0±2.6	50.5±4.5b	12.8±2.2	19.2±1.3	72.5±1.8	54.7±2.6a	72.9±1.8	71.5±1.9
S2	99.7±4.9		102.0±3.3		13.2±1.8	64.8±4.0	2042.0±216.0	2162.4±356.5	15.7±1.9	45.5±8.2a,b	14.3±1.5	19.7±3.6	73.1±1.1	58.9±3.6b	73.3±1.2	73.0±3.3
S3	97.8±4.0		100.3±2.8		11.9±1.9	65.8±4.2	1942.9±320.0	2109.6±193.5	15.1±2.4	44.1±5.3a	13.6±2.3	19.2±2.3	72.0±1.6	59.6±3.8b	72.3±1.6	73.1±2.4
Sign	ns		ns		ns	ns	ns	ns	ns	*	ns	ns	ns	**	ns	ns

[a]AE = acoustic energy, LD = linear distance, $N_{pk>5\ dB}$ = number of acoustic peaks higher than 5 dB, $N_{pk>15\ dB}$ = number of acoustic peaks higher than 15 dB, $AV_{pk>5\ dB}$ = average pressure level for peaks higher than 5 dB, $AV_{pk>15\ dB}$ = average pressure level for peaks higher than 15 dB, G = gain setting (dB). [b]Data are expressed as mean value ± standard deviation. [c]n = 180 for winegrape variety, [d]n = 540 for growing zone, [e]n = 360 for ripening stage. [f]Different Latin letters within the same column indicate significant differences according to Tukey-b test ($p < 0.05$). [g]Sign: *,**,*** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively.

Table 7. Significant Pearson's Correlation Coefficients between Instrumental Acoustic Parameters Measured at Gain Setting 0 and Phenolic Composition of Seeds from Several Winegrape Varieties Sampled in Two Growing Zones at Different Ripening Stages[c]

Acoustic parameter[d,e]	mg/kg grape							%			
	PRO	C	GC	EGC	ECG	TM	mDP	G_t	C_t	EGC_t	C_e
Breakage (dB)		-0.389*	-0.498**	-0.457**	0.356*	-0.342*	-0.409*			-0.491**	
Maximum (dB)		-0.403*	-0.461**	-0.492**		-0.370*	-0.457**			-0.487**	
AE[b] (dB×mm)								-0.334*	0.421*		
LD[b]								-0.433**	0.352*		
$AV_{pk>5\ dB}$[b] (dB)								-0.369*			
$AV_{pk>15\ dB}$[b] (dB)								-0.337*			
AE[a] (dB×mm)								-0.554***	0.459**		
LD[a]					-0.395*			-0.683***	0.378*		
$N_{pk>5\ dB}$[a]					-0.361*			-0.660***	0.383*		
$N_{pk>15\ dB}$[a]					-0.376*			-0.684***	0.398*		
$AV_{pk>5\ dB}$[a] (dB)	-0.387*										0.357*
$AV_{pk>15\ dB}$[a] (dB)	-0.370*										0.337*
AE[t] (dB×mm)								-0.560***	0.480**		
LD[t]					-0.356*			-0.713***	0.416*		
$N_{pk>5\ dB}$[t]								-0.671***	0.414*		
$N_{pk>15\ dB}$[t]					-0.338*			-0.706***	0.426*		

[b]: before breaking. [a]: after breaking. [t]: total value during compression test. [c]PRO = proanthocyanidins, C = catechin, GC = gallocatechin, EGC = epigallocatechin, ECG = epicatechin gallate, TM = total monomers, mDP = mean degree of polimerization, G_t = galloylation in terminal units, C_t = catechin in terminal units, EGC_t = epigallocatechin in terminal units, C_e = catechin in extension units. AE = acoustic energy, LD = linear distance, $N_{pk>5\ dB}$ = number of acoustic peaks higher than 5 dB, $N_{pk>15\ dB}$ = number of acoustic peaks higher than 15 dB, $AV_{pk>5\ dB}$ = average pressure level for peaks higher than 5 dB, $AV_{pk>15\ dB}$ = average pressure level for peaks higher than 15 dB. [d]n = 36. [e]Sign: *,**,*** indicate significance at $p < 0.05$, 0.01, 0.001, respectively.

Table 8. Significant Pearson's Correlation Coefficients between Instrumental Acoustic Parameters Measured at Gain Setting 24 dB and Phenolic Composition of Seeds from Several Winegrape Varieties Sampled in Two Growing Zones at Different Ripening Stages[c]

Acoustic parameter[d,e]	mg/kg							mDP	%			
	TF	C	EC	GC	EGC	TM	B_1		G_t	GC_t	C_e	EC_e
AE[b] (dB×mm)								-0.345*				
LD[b]		0.390*	0.589***	0.339*	0.409*	0.476**		0.557***		0.399*		
$N_{pk>5\ dB}$[b]		0.351*	0.537***		0.362*	0.433**		0.542***				
$N_{pk>15\ dB}$[b]			0.448**			0.346*		0.441**		0.493**		
$AV_{pk>5\ dB}$[b] (dB)	-0.339*	-0.357*	-0.435**			-0.399*	-0.386*	-0.579***				
$AV_{pk>15\ dB}$[b] (dB)								-0.338*				
AE[a] (dB×mm)											-0.393*	
LD[a]								0.342*				0.375*
$N_{pk>5\ dB}$[a]							0.418*	0.356*			-0.374*	0.433**
$N_{pk>15\ dB}$[a]											-0.339*	0.384*
$AV_{pk>5\ dB}$[a] (dB)							-0.373*	-0.447**	-0.491**			
$AV_{pk>15\ dB}$[a] (dB)							-0.422*	-0.380*	-0.462**			
AE[t] (dB×mm)					-0.352*			-0.350*				
LD[t]		0.335*	0.462**			0.391*	0.351*	0.546***		0.415*		
$N_{pk>5\ dB}$[t]	0.367*	0.375*	0.523**			0.443**	0.434**	0.582***				
$N_{pk>15\ dB}$[t]								0.413*		0.409*		0.337*
$AV_{pk>5\ dB}$[t] (dB)			-0.475**		-0.349*	-0.368*	-0.364*	-0.586***	-0.364*			
$AV_{pk>15\ dB}$[t] (dB)			-0.364*				-0.376*	-0.506**	-0.337*			

[b]: before breaking. [a]: after breaking. [t]: total value during compression test. [c]TF = total flavonoids, C = catechin, EC = epicatechin, GC = gallocatechin, EGC = epigallocatechin, TM = total monomers, B_1 = procyanidin B_1, mDP = mean degree of polimerization, G_t = galloylation in terminal units, GC_t = gallocatechin in terminal units, C_e = catechin in extension units, EC_e = epicatechin in extension units. AE = acoustic energy, LD = linear distance, $N_{pk>5\ dB}$ = number of acoustic peaks higher than 5 dB, $N_{pk>15\ dB}$ = number of acoustic peaks higher than 15 dB, $AV_{pk>5\ dB}$ = average pressure level for peaks higher than 5 dB, $AV_{pk>15\ dB}$ = average pressure level for peaks higher than 15 dB. [d]n = 36. [e]Sign: *,**,*** indicate significance at $p < 0.05$, 0.01, 0.001, respectively.

Table 9. Analytical Performance of Calibration Models Developed for Some Chemical Parameters Related to the Phenolic Composition of Winegrape Seeds from Instrumental Acoustic Parameters[c]

Acoustic parameter[d]	Chemical parameter[d]	R_c	SEC	SEC%	SECV	SECV%	RPD	RPIQ
Gain setting 0 dB								
AE[a] (dB×mm)	G_t (%)	0.554	2.79	17.0	3.02	18.4	1.13	1.67
LD[a]		0.683	2.47	15.0	2.68	16.3	1.27	1.88
$N_{pk>5\ dB}$[a]		0.660	2.54	15.5	2.77	16.8	1.23	1.82
$N_{pk>15\ dB}$[a]		0.684	2.46	15.0	2.67	16.3	1.28	1.89
AE[t] (dB×mm)		0.560	2.77	16.9	2.99	18.2	1.14	1.68
LD[t]		0.713	2.36	14.4	2.56	15.6	1.33	2.07
$N_{pk>5\ dB}$[t]		0.671	2.50	15.2	2.71	16.5	1.26	1.86
$N_{pk>15\ dB}$[t]		0.706	2.37	14.4	2.57	15.7	1.32	2.06
Gain setting 24 dB								
LD[b]	EC (mg/kg grape)	0.589	21.49	38.4	22.93	41.0	1.16	1.63
$N_{pk>5\ dB}$[b]		0.537	22.44	40.1	23.96	42.8	1.11	1.56
LD[b]	mDP	0.557	0.33	14.3	0.36	15.5	1.11	1.57
$N_{pk>5\ dB}$[b]		0.542	0.34	14.5	0.37	15.7	1.10	1.55
$AV_{pk>5\ dB}$[b] (dB)		0.579	0.33	14.0	0.36	15.2	1.13	1.60
LD[t]		0.546	0.34	14.4	0.36	15.6	1.10	1.56
$N_{pk>5\ dB}$[t]		0.582	0.33	14.0	0.35	15.1	1.14	1.61
$AV_{pk>5\ dB}$[t] (dB)		0.586	0.32	13.9	0.35	15.0	1.15	1.62

[b]: before breaking. [a]: after breaking. [t]: total value during compression test. [c]R_c = correlation coefficient of calibration, SEC = standard error of calibration, SEC% = (SEC/Mean) x 100, SECV = standard error of cross-validation, SECV% = (SECV/Mean) x 100, RPD = residual predictive deviation (SD/SECV), SD = standard deviation, RPIQ = residual predictive interquartile amplitude (IQ/SECV), IQ = interquartile amplitude. G_t = galloylation in terminal units, EC = epicatechin, mDP = mean degree of polimerization. AE = acoustic energy, LD = linear distance, $N_{pk>5\ dB}$ = number of acoustic peaks higher than 5 dB, $N_{pk>15\ dB}$ = number of acoustic peaks higher than 15 dB, $AV_{pk>5\ dB}$ = average pressure level for peaks higher than 5 dB. [d]n = 36.

Table 10. Analytical Performance of Calibration Models Developed for the Spectrophotometric Indices of the Extractable Content of Phenolic Compounds in Nebbiolo Seeds from Instrumental Acoustic Parameters[c]

Acoustic parameter	Mean±SD[d,e]	Chemical parameter	Mean±SD[d,e]	R_c	SEC	SEC%	SECV	SECV%	RPD	RPIQ
Gain setting 0 dB										
Maximum (dB)	101.3±1.4	FRV (mg/kg grape)	735±185	0.585	150.14	20.4	187.77	25.6	0.99	1.09
Gain setting 24 dB										
$AV_{pk>5\ dB}$[b] (dB)	48.0±5.0	TF (mg/kg grape)	1550±211	0.584	170.87	11.0	202.31	13.1	1.04	1.89
$AV_{pk>5\ dB}$[a] (dB)	68.6±1.3			0.696	151.13	9.8	188.88	12.2	1.11	2.07
$AV_{pk>15\ dB}$[a] (dB)	76.8±1.0			0.689	152.67	9.9	186.61	12.0	1.13	2.09
$AV_{pk>15\ dB}$[t] (dB)	76.1±1.4			0.610	166.85	10.8	195.89	12.6	1.07	1.95
$N_{pk>5\ dB}$[t]	63.6±4.8			0.576	172.12	11.1	201.92	13.0	1.04	1.89

[b]: before breaking. [a]: after breaking. [t]: total value during compression test. [c]R_c = correlation coefficient of calibration, SEC = standard error of calibration, SEC% = (SEC/Mean) x 100, SECV = standard error of cross-validation, SECV% = (SECV/Mean) x 100, RPD = residual predictive deviation (SD/SECV), RPIQ = residual predictive interquartile amplitude (IQ/SECV), IQ = interquartile amplitude. FRV = flavanols reactive to vanillin, TF = total flavonoids. $AV_{pk>5\ dB}$ = average pressure level for peaks higher than 5 dB, $AV_{pk>15\ dB}$ = average pressure level for peaks higher than 15 dB, $N_{pk>5\ dB}$ = number of acoustic peaks higher than 5 dB. [d]n = 20. [e]SD = standard deviation.