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Optimization of a Method Based on the Simultaneous Measurement of Acoustic and Mechanical Properties of Winegrape Seeds for the Determination of the Ripening Stage

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ABSTRACT

An instrumental texture analysis method has been optimized for the differentiation of grape ripening stages based on the simultaneous determination of mechanical and acoustic parameters of the seeds. Two factorial Central Composite Design was used to optimize the most influencing operative conditions (speed and deformation) on mechanical and acoustic measurements. This experimental design in combination with Response Surface Methodology showed that the most responsive parameters to changes in seeds during ripening were Young's modulus of elasticity, many acoustic parameters measured with instrumental gain set to 0 (acoustic energy, linear distance and number of peaks) and others measured at 24 dB gain (linear distance, number of peaks and average pressure level). However, the optimal operative conditions depended on the texture parameter used. A correlation study between texture parameters and phenolic compounds of the seeds revealed that acoustic parameters like the average pressure level could be proposed as phenolic maturity indices.

Keywords: instrumental texture analysis; acoustic emission; mechanical properties; grape seeds; phenolic maturity index; Response Surface Methodology.

INTRODUCTION

Grape seeds represent an important source of phenolic compounds, and contain approximately 60–70 % of total extractable content of grape phenolic compounds,[1,2] mainly gallic acid, monomeric catechins (catechin, epicatechin and epicatechin-3-*O*-gallate) and their polymers.[3–5] They are mostly located in the epidermis, the outer integument and the inner layer of inner integument.[6] This relatively high abundance of phenolic compounds in grape seeds provides functional and nutritive benefits on human health that derive from the wine consumption and are related to the well-known antibacterial and antioxidant activity of these compounds.[7,8] The phenolic composition of the seeds depends on multiple factors, such as grape variety, environmental conditions, viticulture practices and degree of grape ripeness among others.[9–11]

A decrease in the flavan-3-ol content of the seeds occurs gradually during grape ripening,[6,9,12-15] and is accompanied by a reduction in their tannic intensity and astringency.[16] These changes also comprise an increase in the hardness of the seeds[16] because of histological and histochemical modifications, such as the solidification of the cells rich in tannins, that can negatively affect the ability to release phenols during winemaking.[6] The decrease in the easiness for the extraction of seed phenols during ripening[12] may be consistent with an oxidative process of flavan-3-ols,[17] which favors their association with cell-wall components.[9,13] Generally, these changes do not comprise all clusters and all berries at the same time.

Winegrape tasting is a well-recognized tool that is used by many wine professionals to support harvesting decisions. Many descriptors have been proposed for the sensory evaluation of berry seeds, such as sourness, astringency, hardness and cracking. They may be of great relevance to discriminate degrees of ripeness. The first two sensory attributes decrease during grape ripening, whereas the latter two increase.[18,19] Instrumental texture measurements permit to reduce the variability associated with the subjectivity of sensory analysis, and sensory descriptors related to the texture of the berry are highly correlated with compression parameters.[18] Furthermore, the mechanical properties of berry skins have successfully been used to assess the phenolic ripeness of red grapes. In fact, the berry skin break force can be considered the best mechanical attribute to estimate anthocyanin extraction kinetics with adequate reliability.[20] On the other hand, the berry skin thickness has been proposed as predictor of the anthocyanin extractability.[21] In the seeds, Young's modulus of elasticity is the instrumental texture parameter best correlated with the phenol extractability.[22]

Acoustic vibration techniques have been widely used to monitor the changes in the firmness and hardness of many kinds of fruits during the ripening process.[23-25] The acoustic emissions produced during force/deformation measurements can be simultaneously recorded, and are well correlated with sensory characteristics associated with crispness.[26,27] In the seeds, there is only one work published to date on the objective changes in acoustic and mechanical properties during ripening.[28] In particular, the deformation index, acoustic energy and average acoustic pressure of berry seeds are influenced by the developmental changes occurring in the last stages of grape ripening.[28]

The aim of this work was to optimize the experimental conditions of the instrumental texture test that permit the best monitoring of the changes in the mechanical and acoustic parameters of the seeds during grape ripening. Many factors that could influence the ability of this methodology to discriminate ripening stages were also evaluated in terms of variability.

The development/optimization of this methodology is supported by the necessity of replacing the time-consuming chemical methods commonly used in the wine industry by simpler, faster, reliable, economically reasonable and environmentally friendly ones as routine analytical tools. This work could suppose an important progress in the research on rapid analysis methodologies for determining the extractable phenol content of grape seeds during ripening, and therefore for making harvesting decisions and winemaking management.

MATERIALS AND METHODS

Grape samples. The study was carried out on the Merlot red cultivar (*Vitis vinifera* L.), grown at a vineyard of 0.5 ha located in Piedmont (North-West Italy), in 2011. The 200 vines selected were homogeneous according to age (12 years), clone (181F) and cultural practices. They were grafted onto the S.O.4 rootstock, planted at 2.2 m × 0.9 m, Guyot-pruned and vertically trained. The yield was approximately 8.4 t/ha.

The grape samples were collected at two advanced ripening stages, and the study was carried out separately on the samples collected at each date. The sampling dates were 1st September (P1) and 20th September (P2). For each date, the bunches (n = 25) were randomly harvested from the vines selected. Once in the laboratory, a sub-sample of approximately 0.5 kg of grapes (ca. 350-400 berries) was randomly selected by picking berries from different positions in the cluster (shoulder, middle and bottom). One seed per berry was carefully separated from the pulp and cleaned with absorbent paper before analysis up to achieve the sufficient number of seeds. Afterwards, for the optimization of the sample size required for instrumental texture analysis, the mechanical and acoustic properties were determined in 80 intact seeds using standard conditions of the texture test.[28,29] For the estimation of the variability in the instrumental texture parameters within and between clusters, the seeds (n = 5) of the berries located in a given position of the cluster were separately analyzed from those of the berries located in the other two positions,[30] with a total of 150 seeds per 10 clusters. One set of 30 seeds (3 replicates of 10 seeds) from grape berries harvested in four different commercial vineyards at each sampling date was used for the correlation study between the phenolic composition of the seed hydroalcoholic extracts and the texture parameters directly measured on the intact seeds. Finally, the remaining berries of the sub-sample initially selected (distributed into 3 replicates) were used for determining standard physicochemical parameters in the grape must obtained by manual crushing and centrifugation. All of the measurements were performed in the same day as picking to avoid changes.

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). Phenol standards ((+)-catechin, cyanidin chloride) were supplied from Extrasynthèse (Genay, France).

Technological ripeness parameters. Total soluble solids concentration (°Brix, as SSC) was measured with an Atago 0–32 °Brix temperature compensating refractometer (Atago Corporation, Tokyo, Japan), and pH was determined by potentiometry using a Crison electrode (Carpi, Italy). Titratable acidity (TA), expressed as g/L tartaric acid, was estimated using the OIV method.[31] Organic acids (malic acid, tartaric acid and citric acid) and reducing sugars (glucose and fructose) were quantified (as g/kg berries) using a P100-AS3000 HPLC system (Thermo Electron Corporation, Waltham, MA, USA), equipped with an UV detector (UV3000) set to 210 nm and a refractive index detector (Waters 2414, Waters Corporation, Milford, MA, USA), respectively. The analyses were performed isocratically at 0.8 mL/min flow-rate and 65 °C column temperature with a 300 × 7.8 mm i.d. Aminex HPX-87H cation exchange column and a Cation H^+ Microguard cartridge (Bio-Rad Laboratories, Hercules, CA, USA). The mobile phase was 0.0013 mol/L H_2SO_4.[32] The data analysis was carried out using the ChromQuest chromatography data system (ThermoQuest, Inc., San Jose, CA, USA).

Phenol extraction and determination. The 24 replicates of 10 seeds, after immersion in 25 mL of a hydroalcoholic buffer at pH 3.2 containing 5 g/L tartaric acid, 2 g/L $Na_2S_2O_5$ and 12 % ethanol, were placed in a controlled temperature room at 25 ºC for one week.[33-35] Spectrophotometric methods were used to determine total polyphenols (mg (+)-catechin/kg grape, as TP_s), total flavonoids (mg (+)-catechin/kg grape, as TF_s), proanthocyanidins (mg cyanidin chloride/kg grape, as PRO_s) and flavanols reactive to vanillin (mg (+)-catechin/kg grape, as FRV_s).[33,34] An UV-1800 spectrophotometer (Shimazdu Scientific Instruments Inc., Columbia, MD, USA) was used. The relative standard deviation (RSD) based on repeated analysis (n = 20) of the same sample was 1.58, 0.93, 1.74 and 2.80 % for TP, TF, PRO and FRV, respectively.[34]

Instrumental mechanical properties. The mechanical properties of the intact seeds were determined by a compression test using a TA-XT Plus Texture Analyzer (SMS-Stable Micro Systems, Surrey, UK) equipped with a SMS HDP/90 platform, a SMS P/35 probe and a 50 kg load cell.[29] Each one of the intact grape seeds was individually compressed, and the following instrumental mechanical parameters were measured or calculated: the seed break force (N, as F_s), the seed break energy (mJ, as W_s), the seed Young's modulus of elasticity (N/mm, as E_s) and the seed deformation index (%, as DI_s). This last index was calculated as the distance of the seed break point/seed height × 100.[29,35] Before each test session, the instrument was calibrated for force and distance.

Instrumental acoustic properties. An Acoustic Envelope Detector (AED, Stable Micro Systems) equipped with a 12.7-mm diameter Brüel & Kjær 4188-A-021 microphone (Nærum, DK) was used for the acoustic emission measurements during the compression test. The microphone was positioned at a 20 mm distance from the sample at an angle of 45º and connected to the TA-XT Plus Texture Analyzer. The measurements were carried out at two different instrumental gain SPL values, which was set to 0 and 24 dB. The calibration was performed before each measurement session using an Acoustic Calibrator Type 4231 (94 dB and 114 dB-1000 Hz). The AED operates by integrating all of the frequencies within the band pass range and generating a voltage proportional to the acoustic pressure level. All of the tests were performed in a laboratory with no special soundproofed facilities and at room temperature.

For each gain, the following instrumental acoustic parameters were acquired: the acoustic pressure level at the breakage (dB), the maximum acoustic pressure level (dB), the acoustic energy (dB × mm or dB × s, 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 ≥ than 15 dB (dB, as $AV_{pk>15\ dB}$) and the average acoustic pressure level for peaks with threshold ≥ than 5 dB (dB, as $AV_{pk>5\ dB}$).[36] 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) for the simultaneous force and acoustic emission measurements involving the Texture Exponent software.

Experimental design and statistical analysis. Two factorial 2^2 Central Composite Design (CCD) comprising 13 experiments was used to optimize the operative conditions in the mechanical and acoustic measurements.[37] The independent variables selected were the speed (X_1) and the deformation applied (X_2) during the compression test, which varied from 0.20 to 6.00 mm/s and from 25 to 75 %, respectively, at five different levels.[29] The coded values of the variables ranged between +1.414 and −1.414, taking the zero value as central point. Table 1 shows the factorial design matrix with the variable values in both coded and non-coded form for each experiment. Five replicates of the central point were carried out. The average values of the variation between the thirty determinations performed at the two sampling dates studied were fitted to the following second-order polynomial model:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2$$

where Y is the predicted response, X_1 and X_2 correspond to the independent variables, b_0 is the value in the central point, b_1 and b_2 represent the principal effects associated with each variable, b_{11} and b_{22} are the squared effects, and b_{12} is the interaction effect. The second-degree polynomial equations obtained were represented as surface plots using Response Surface Methodology (RSM) to highlight the optimized operative conditions. Statistical analysis was performed to predict models through regression analysis (R^2). The regression models were highly significant ($p < 0.01$) with a satisfactory value of the determination coefficient ($R^2 \geq 0.80$), indicating that at least 80 % of the variability in the response could be explained by the second-order equations.

Statistical analyses were performed using the software package Statistica version 7.0 (Statsoft Inc., Tulsa, OK, 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 texture parameters and the phenolic composition of the seeds.

RESULTS AND DISCUSSION

Assessment of technological ripening. The chemical parameters of Merlot grapes were determined at the two harvest dates, and the results obtained are shown in Table 2. As expected, the values of total soluble solids content increased significantly from P1 to P2 sampling date, whereas contrariwise titratable acidity and malic acid content decreased significantly. These differences, together with the significant reduction in the glucose/fructose ratio that resulted in values lower than 1 at the second harvest date, agreed with a noticeable advance of grape ripening between the two harvest dates studied.

Sample size. The first step to optimize the experimental conditions that permit the best monitoring of the changes in the instrumental mechanical and acoustic parameters of berry seeds during grape ripening was to evaluate the influence of the sample size on the variability in the measurements.[38] Therefore, the optimum sample size was assessed representing the RSD values against the number of seeds for each texture parameter (mechanical and acoustic)[38] measured at the initial operative conditions (1 mm/s speed and 50 % deformation).[28] A representative profile, force-time curve and acoustic emission-time curve, of the force and the sound simultaneously recorded during the compression test on the berry seeds was reported in a previous work.[28]

At gain 0 dB, the RSD values were higher and the variation in the RSD values with the increase in the number of seeds was also higher than at gain 24 dB. Figures 1 and $2_{[a\text{-}c]}$ show these representations for the most influenced mechanical properties and the acoustic attributes, respectively, measured at gain 0. The RSD values became stable when at least 30 seeds were analyzed. Therefore, one sample of 30 seeds was used in the next experiments. Sample sizes comprised between 15 and 30 berries are usually used for the compression and puncture tests on the whole berry, and also for the puncture test on the berry skin.[29,30,38]

A high variability was observed in the values of texture parameters of the seeds, it being higher than in those referred on the whole berry.[38] This variability is particularly high for the instrumental acoustic parameters measured at gain 0 before breaking, which have RSD values higher than 70 % for a sample size higher than 6. When these parameters were measured after breaking and during the entire compression test, the intra-sample variability was lower than 35 % for a number of seeds higher than 30. The acoustic pressure level at the breakage, the maximum acoustic pressure level, the $AV_{pk>5\ dB}$ and $AV_{pk>15\ dB}$ after breaking, and total $AV_{pk>5\ dB}$ and $AV_{pk>15\ dB}$ of berry seeds showed lower intra-sample variability with RSD values lower than 12 % for any sample size even at gain 0. Regarding the mechanical properties of berry seeds, the RSD values were higher for the W_s (RSD < 36 %) than for the F_s, E_s and DI_s (RSD < 25 %). This variability inside each sample is due to the heterogeneity of the seed texture. When 40 berry seeds were texturally analyzed at gain 0, Rolle et al. (2012) also reported RSD values up to approximately 45 % for the AE and for the W_s, as well as a lower intra-sample variability for all remaining mechanical parameters (RSD < 28 %), and for the maximum and average acoustic pressure levels (RSD < 12 %).[28]

Variability inside and among clusters. After verifying that the RSD values for the texture properties of the seeds from the same berry are lower than those of the seeds from different berries, a study was carried out to assess if the variability in the mechanical and acoustic parameters at harvest can be due to differences in the tissue texture of the seeds belonging to grape berries from different clusters, and even from different positions within the same cluster. The results obtained showed that there were no significant differences ($p < 0.05$) in the instrumental parameters of the seeds from grape berries sampled in different positions of the cluster. This agreed with other study previously performed on berry skins, which reported no influence of the position of the grape berry within the cluster on the skin hardness assessed by a puncture test.[30] Contrariwise, some significant differences were found in the seed acoustic parameters for berries belonging to different clusters, particularly in the AE after breaking ($p < 0.01$), total AE ($p < 0.01$), the $AV_{pk>5\ dB}$ and $AV_{pk>15\ dB}$ after breaking ($p < 0.05$) and total $AV_{pk>5\ dB}$ and $AV_{pk>15\ dB}$ ($p < 0.05$) at gain 0, and in the AE before and after breaking ($p < 0.01$), total AE ($p < 0.01$), the LD before breaking ($p < 0.05$), the LD after breaking ($p < 0.001$), total LD ($p < 0.001$), the $N_{pk>5\ dB}$ before breaking ($p < 0.01$), the $N_{pk>15\ dB}$ after breaking ($p < 0.001$), total $N_{pk>15\ dB}$ ($p < 0.01$) and total $AV_{pk>5\ dB}$ ($p < 0.01$) at gain 24 dB. Therefore, the variability among clusters could partially explain the differences observed in the acoustic parameters of the seeds but not completely. Firstly, the variability among clusters did not explain the high intra-sample variability in the acoustic properties measured at gain 0 before seed breaking. Secondly, some acoustic parameters with high intra-sample variability showed no significant differences between clusters, whereas others with low intra-sample variability showed significant differences between clusters. On the other hand, the higher significance of the differences found between clusters in the W_s ($p < 0.001$) if compared with the F_s, E_s and DI_s ($p < 0.01$) could explain the higher intra-sample variability in the former mechanical parameter.

Experimental design. Once defined instrumental conditions, such as platform, probe, load cell, microphone, frequency of data acquisition and also sample size, the operative conditions of the compression test such as the speed and the deformation to apply were optimized. In the few studies published to date on the determination of the mechanical properties of grape seeds, the same operative conditions were used (1 mm/s speed and 50 % deformation).[28,34] The scientific literature shows a wide range of values for the speed used in grape studies, which varied from 0.2 mm/s[29] to 100 mm/min[39] in compression tests on the whole berry and berry skin. The breakage of the seeds requires higher deformation than that of the whole berry or the berry skin. For some grape varieties, a 25 % deformation applied on the whole berry can cause skin breakage.[29]

At the second sampling date (P2), the F_s ranged from 42.5 to 46.8 N, the W_s ranged from 8.43 to 10.94 mJ, the E_s ranged from 87.7 to 101.9 N/mm, and the DI_s ranged from 15.5 to 17.6 %. These values were intermediate to those reported for other red winegrape varieties cultivated in Italy.[29,34] In the full series of experiments (1-13), ANOVA analyses of the variation percentage between determinations performed at the two sampling dates studied for the mechanical parameters revealed that the E_s, which is related to the stiffness or rigidity of the seed tissue, permitted the most significant discrimination of the two ripening stages in any tested experiment. This variation was always positive, which agreed with the progressive increase in the seed stiffness for Cabernet-Sauvignon grapes during the ripening process.[28] Essentially, grape ripening does not tend to increase the resistance of the seed to rupture but to stiffen.[22] The W_s had only significant differences ($p < 0.05$) between the two sampling dates studied in the experiment 7. The high variability in the W_s at advanced physiological stages of the grapes could hinder the differentiation of the ripening stages.[28]

At gain 0, if compared with the Cabernet-Sauvignon cultivar also grown in Piedmont,[28] the maximum acoustic pressure level was higher for Merlot seeds with values that varied from 102 to 107 dB at the P2 sampling date. Total AE and total LD had a high variability among different operative conditions with values ranging from 2.84 to 77.72 dB × mm and from 556 to 2958, respectively.

Regarding ANOVA analyses of the variation percentage for the acoustic parameters of berry seeds measured at gain 0, most of the acoustic properties that showed significant changes increased with the sampling date in any experimental condition. Few exceptions were observed for the LD and the N_{pk} depending on the operative conditions. The results obtained agreed with the significant increase in the N_{pk}, the AE and the AV_{pk} for Cabernet-Sauvignon seeds during grape ripening.[28] At gain 24 dB, the significant changes were always positive only for the AV_{pk}, whereas those for the AE were always negative. Furthermore, no significant variation was observed in the acoustic pressure level at the breakage and the AE after breaking in all of the experiments performed at gain 24 dB. Therefore, the same parameter could have different evolution with the advance in grape ripening depending on the experimental conditions used.[38] At any gain, the most responsive acoustic parameters ($p < 0.001$) to the ripening effect were the AE before breaking, the LD after breaking, total LD, the $N_{pk>5\ dB}$ after breaking, and the $AV_{pk>5\ dB}$ and $AV_{pk>15\ dB}$ after breaking. The AE after breaking and total AE were also potentially differentiating parameters at gain 0. Instead, the $N_{pk>15\ dB}$ before breaking, total $N_{pk>5\ dB}$, and total $AV_{pk>5\ dB}$ and $AV_{pk>15\ dB}$ were strongly influenced at gain 24 dB. The higher discriminating power of the acoustic parameters measured after seed breaking, if compared with those measured before breaking, could be due to the occurrence of major structural breakdown and larger acoustic events.

Based on the results of these experiments, regression analysis was performed to provide an accurate mathematical description of the effect of the two independent variables (speed and deformation applied during the compression test) on the variation in the responsive instrumental mechanical properties and acoustic parameters of the seeds to grape ripening. The second-order polynomial equations are shown in Table 3. The test speed had a more noticeable influence on the changes in the instrumental texture parameters, excepting for total AE expressed in space and measured at gain 24 dB, and total $AV_{pk>15\ dB}$ measured at gain 0. The test speed showed little quadratic effects, excepting for the AE before breaking and total AE expressed in space and measured at gain 24 dB, whereas the deformation applied had only linear effects. The two independent variables showed no interaction effect.

Regarding the mechanical parameters, the test speed showed the highest positive effect on the changes in the F_s and E_s. This last texture property also was the most positively influenced one by the deformation applied during the compression test. The two independent variables had a negative effect on the DI_s.

Regarding the seed acoustic parameters, the test speed had the most noticeable positive linear effect on the variation in the LD before breaking, and the $AV_{pk>5\ dB}$ and $AV_{pk>15\ dB}$ before breaking at gain 0. The LD after breaking and total LD were the most negatively affected parameters by this independent variable. Furthermore, the deformation applied during the compression test also showed the most important negative effect on the LD before breaking and total LD. In any way, the highest positive coefficients for this second independent variable were associated with the LD after breaking.

When at least 80 % of the variability in the response can not be explained by the second-order equations, those operative conditions (experiments 1-13, Table 1) giving a more significant change in the texture parameters of berry seeds between ripening stages were selected (Table 3). For the highly significant regression models ($p < 0.01$; $R^2 \geq 0.80$), response surface methodology plots (Figures 3, 4 and 5) show more clearly how these two independent variables (speed and deformation applied during the seed compression test) influence the changes. Three-dimensional graphics represent smoothed surfaces, where the variation in a texture parameter between the two sampling dates studied is plotted against the two independent variables. The maximum point, or even the minimum one for negative responses, on the surface represents the best operative conditions for the differentiation of ripening stages according to each texture property selected of the seeds. The surface plots in Figure 3 indicate that the maximum variation between ripening stages corresponded to the E_s parameter in accordance with ANOVA analyses, and was achieved at the lowest values of the two independent variables (negative) or either at the highest ones of the deformation applied for a test speed of 2.39 mm/s (positive). The best response predicted (positive) could be achieved according to the F_s for the highest values of an independent variable but the lowest ones of the other variable. Figure 3 confirmed the little power of the DI_s to discriminate grape ripening stages. The results obtained for Cabernet-Sauvignon seeds evidenced that the mechanical parameter showing the greatest differences among the harvest dates was the DI_s measured at 1 mm/s test speed and 50 % deformation applied.[28] This reveals the importance of selecting the operative conditions (speed/deformation).

Figure 4 shows the surface plots for the seed acoustic parameters measured at gain 0. The most important variation between ripening stages was associated with the LD after breaking, expressed in space or time, and corresponded to the lowest values of the test speed at any deformation applied, particularly at 75 %. This same positive response to the changes during ripening was also observed, but lower, for the $N_{pk>15\ dB}$ after breaking and total $N_{pk>15\ dB}$. The acoustic pressure level at the breakage had the better positive response predicted at the highest values of the test speed and the lowest ones of the deformation applied. The maximum acoustic pressure level showed the stronger variation (positive) for a test speed of 4.24 mm/s at any deformation applied, particularly at 25 %. In the last two cases, the lowest values of the two independent variables also provided similar values of the response predicted but negative. The $AV_{pk>5\ dB}$ after breaking showed a response predicted with higher variation between ripening stages at a test speed of 5.22 mm/s for 25 % deformation applied. On the other hand, the higher variation in the $AV_{pk>15\ dB}$ after breaking was achieved at the highest values of the deformation applied for 5.38 mm/s test speed.

The surface plots in Figure 5 represent the variation between ripening stages for the seed acoustic parameters, when the measurements were carried out at gain 24 dB. The maximum positive response to the changes was achieved for the LD after breaking, expressed in space or time, at the lowest values of the test speed and 31 % deformation applied. Although with very low variability between ripening stages, the maximum acoustic pressure level showed the better response predicted at the highest values of the two independent variables. The optimal conditions for each mechanical/acoustic parameter are summarized in Table 3.

Application of the method proposed to evaluate the phenolic composition. A first attempt to evaluate the phenolic maturity of the seeds was done through correlation studies. Table 4 shows the significant Pearson's correlation coefficients between the texture properties and the phenolic composition of Merlot seeds. The protocol used for the extraction of phenols from intact fresh seeds was suitable to estimate the extractable content into the wine during winemaking, according to the scientific literature.[40] Among polyphenols determined by spectrophotometric assays, TP and PRO are closely correlated between them, and the latter can be mainly related with the concentration of high molecular weight proanthocyanidins (>5 units). FRV is sensitive to the presence of monomeric phenols, and is partially related with the concentration of low molecular weight proanthocyanidins with a polymerization degree from 2 to 4.[41]

The only mechanical parameter of the seeds significantly correlated with their phenolic composition was the F_s, which showed a low positive correlation coefficient with the content of FRV_s. For the acoustic attributes measured at gain 0, the maximum acoustic pressure level was the parameter most significantly correlated with FRV_s (0.584; $p < 0.01$), followed by the $AV_{pk>5\ dB}$ and $AV_{pk>15\ dB}$ before and after breaking, total $AV_{pk>5\ dB}$ and $AV_{pk>15\ dB}$, and total LD. Therefore in berry seeds, simple monomeric and low molecular weight oligomer flavanols were the only phenolic compounds significantly correlated with the mechanical properties and the acoustic parameters measured at gain 0. These results are reasonable. During ripening, the lignification process that induces changes in the mechanical properties of the seeds also directly influences the extractability of low molecular weight phenols, which are the main fraction of polyphenols released from intact seeds during winemaking.

Regarding the seed acoustic parameters measured at gain 24 dB, the highest and most significant correlations (ca. -0.700; $p < 0.01$) were achieved between TF_s content and the $AV_{pk>5\ dB}$ and $AV_{pk>15\ dB}$ after breaking. These correlation coefficients were negative as those found between TF_s and the AE before breaking, the $AV_{pk>5\ dB}$ before breaking, and total $AV_{pk>5\ dB}$ and $AV_{pk>15\ dB}$. However, TF_s content of the seeds was positively correlated with the LD after breaking, total LD, the $N_{pk>5\ dB}$ after breaking and total $N_{pk>5\ dB}$. TP_s showed similar correlations to TF_s, but lower, with total $N_{pk>5\ dB}$, the $AV_{pk>5\ dB}$ and $AV_{pk>15\ dB}$ after breaking, and total $AV_{pk>15\ dB}$. The LD after breaking, the $N_{pk>15\ dB}$ after breaking and total $N_{pk>15\ dB}$ also were positively correlated with FRV_s. PRO_s had no significant correlation with the mechanical and acoustic parameters of the seeds. Other study reported very low correlation coefficients between mechanical parameters and the content of TP_s for Cabernet-Sauvignon seeds.[22] Therefore, TP_s content is not the main factor related to the texture properties but the seed phenolic profile.

Scientific literature reports a evidence of correlation between the seed coat color and TP_s, many flavanols or extractable tannins during ripening indicating that the external color may be used as an indicator of the seed maturity.[42-44] However, it is important to emphasize that this is the first study on the assessment of the instrumental acoustic parameters of the seeds as ripeness predictors and on the evaluation of their relationship with the phenolic composition.

In conclusion, the optimum operative conditions (speed/deformation couple) depend on the mechanical and acoustic parameter measured in berry seeds, whose selection requires a strong and significant correlation with the chemical parameter to determine. Based on maximum variation between ripening stages, some mechanical parameters (F_s, E_s), acoustic properties measured at gain 0 (AE before and after breaking, total AE, LD after breaking, total LD, $N_{pk>5\ dB}$ after breaking, $N_{pk>15\ dB}$ after breaking, total $N_{pk>15\ dB}$, $AV_{pk>5\ dB}$ and $AV_{pk>15\ dB}$ after breaking, acoustic pressure level at the breakage, maximum acoustic pressure level),and acoustic properties measured at gain 24 dB (AE before breaking, LD after breaking, total LD, $N_{pk>15\ dB}$ before breaking, $N_{pk>5\ dB}$ after breaking, total $N_{pk>5}$

$_{dB}$, total $AV_{pk>5\ dB}$ and $AV_{pk>15\ dB}$, $AV_{pk>5\ dB}$ and $AV_{pk>15\ dB}$ after breaking) could be considered ripeness indices. Particularly, the latter acoustic parameter could be proposed as predictor of TF_s and TP_s extractable at pH 3.2. On the other hand, the maximum acoustic pressure level at gain 0 was the best acoustic parameter to predict the extractable content of oligomer flavanols in the seeds.

Therefore, this work opens new possibilities in the wine industry for determining the phenolic ripeness of the seeds by means of an inexpensive, rapid and reliable instrumental method, although a specific predictive model must be still developed. Thus, the direct texture analysis of intact berry seeds could provide a rapid estimation of the extractable content of many phenolic compounds, which is of great importance for the harvest date selection and winemaking management. Nevertheless, further studies are necessary for different grape varieties, growing areas and vintages to obtain sufficiently robust predictions.

Supporting Information

This information shows the results obtained in each experiment for the mechanical and acoustic parameters of intact seeds for Merlot grapes. This material is available free of charge via the Internet at http://pubs.acs.org.

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FIGURE CAPTIONS

Figure 1. Relative standard deviation (%) versus number of samples for the mechanical parameters of the seeds: (○) break force, (□) break energy, (Δ) Young's modulus of elasticity and (◊) deformation index.

Figure 2. Relative standard deviation (%) versus number of samples for the acoustic parameters of the seeds measured at gain 0 (a) before breaking, (b) after breaking and (c) during the entire compression test: (○) acoustic energy expressed in space, (□) linear distance and (Δ) number of acoustic peaks higher than 5 dB.

Figure 3. Response surface curves for the mechanical parameters of the seeds versus independent variables (X_1) speed and (X_2) deformation: break force (F_s), Young's modulus of elasticity (F_s) and deformation index (DI_s).

Figure 4. Response surface curves for the acoustic parameters of the seeds versus independent variables (X_1) speed and (X_2) deformation measured at gain 0: acoustic pressure level at the breakage, maximum acoustic pressure level, linear distance after breaking, number of acoustic peaks after breaking higher than 15 dB, total number of acoustic peaks higher than 15 dB, and average acoustic pressure level for peaks after breaking higher than 5 and 15 dB.

Figure 5. Response surface curves for the acoustic parameters of the seeds versus independent variables (X_1) speed and (X_2) deformation measured at gain 24 dB: maximum acoustic pressure level and linear distance after breaking.

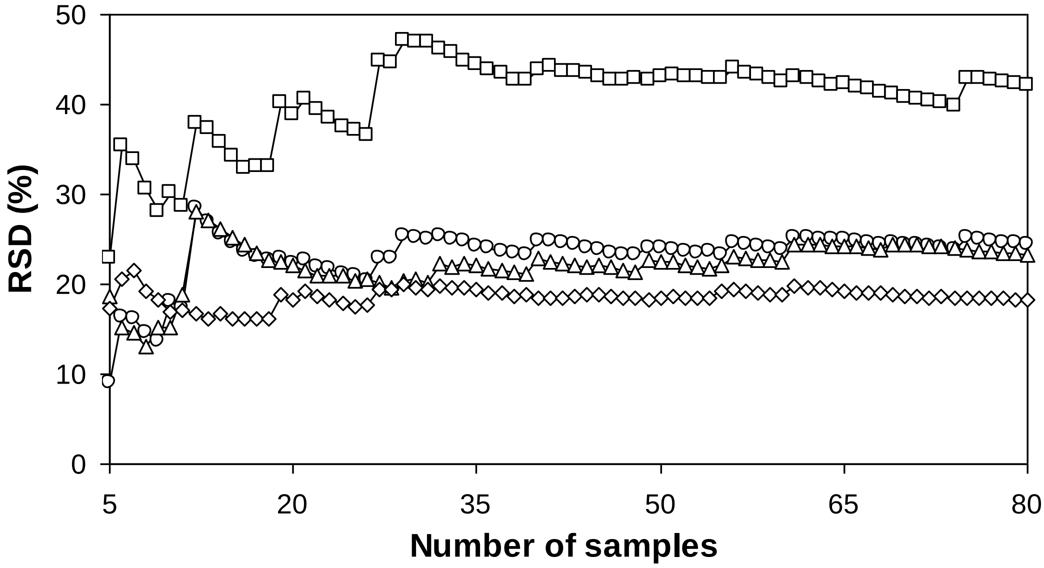


Figure 1

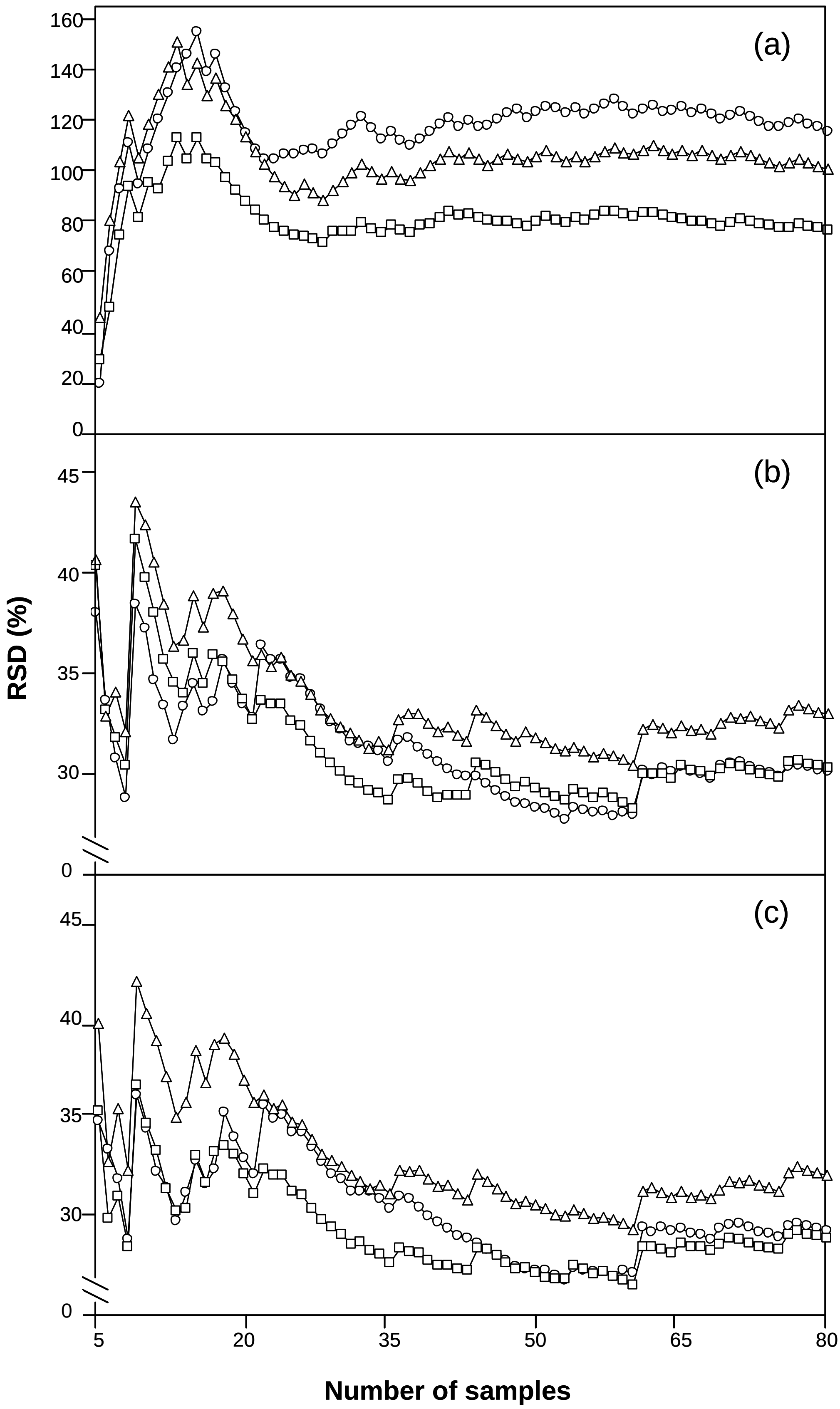
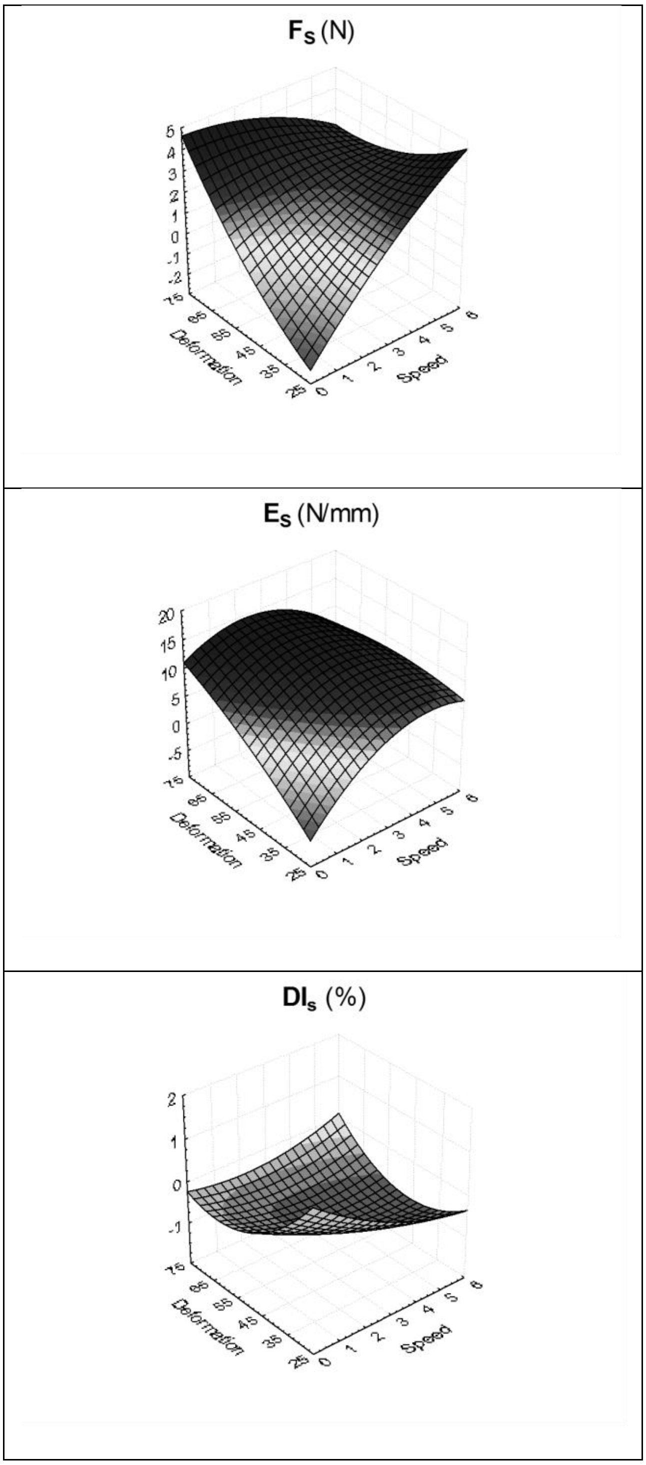
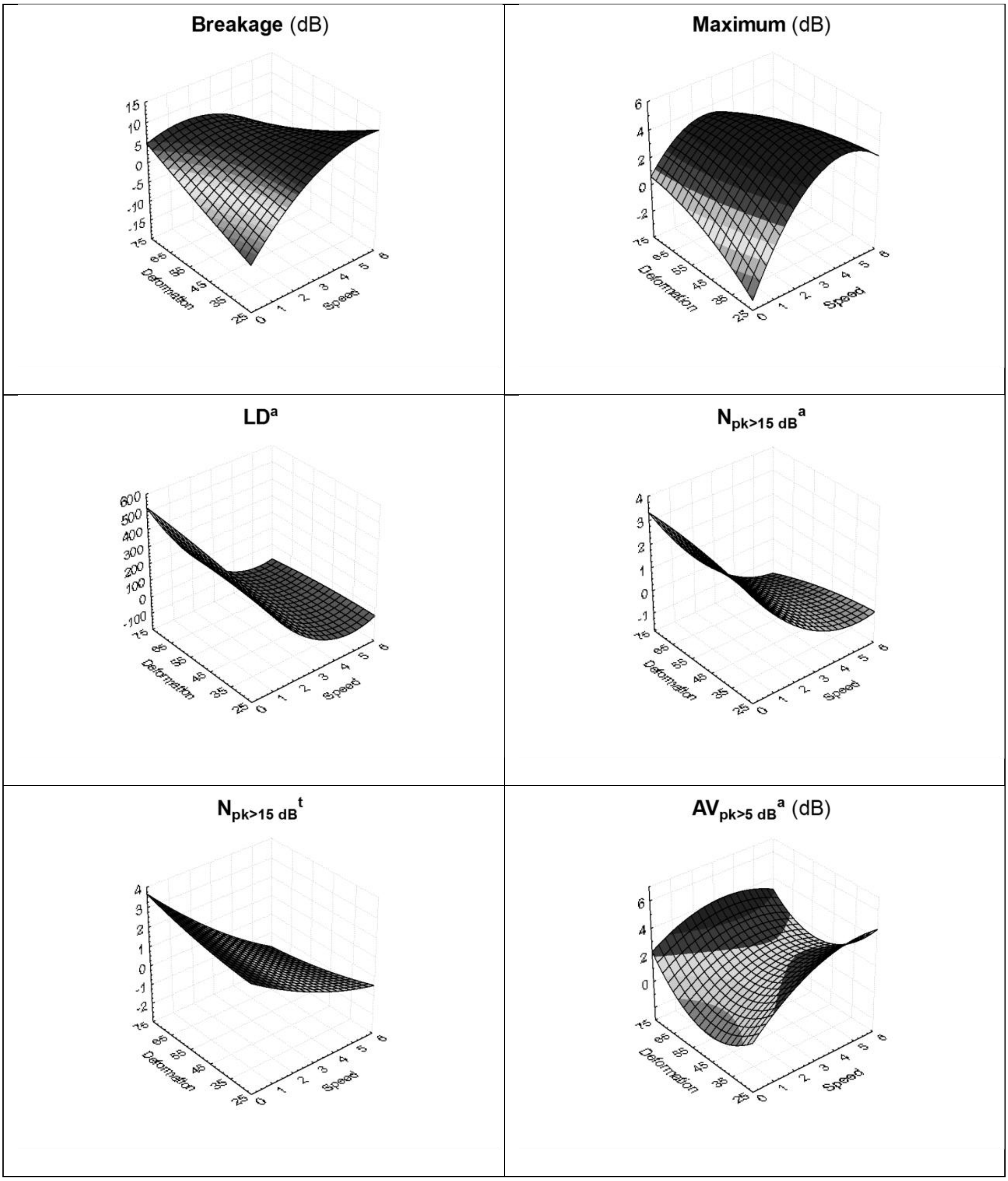

(a)
(b)
(c)
RSD (%)
Number of samples
160
140
120
100
80
60
40
20
0
45
40
35
30
0
45
40
35
30
0
5
20
35
50
65
80

Figure 2



F_S (N)
Deformation
Speed
E_S (N/mm)
Deformation
Speed
DI_s (%)
Deformation
Speed

Figure 3



Breakage (dB)
Maximum (dB)
LD^{a}
$N_{pk>15\ dB}{}^{a}$
$N_{pk>15\ dB}{}^{t}$
$AV_{pk>5\ dB}{}^{a}$ (dB)
Deformation
Speed

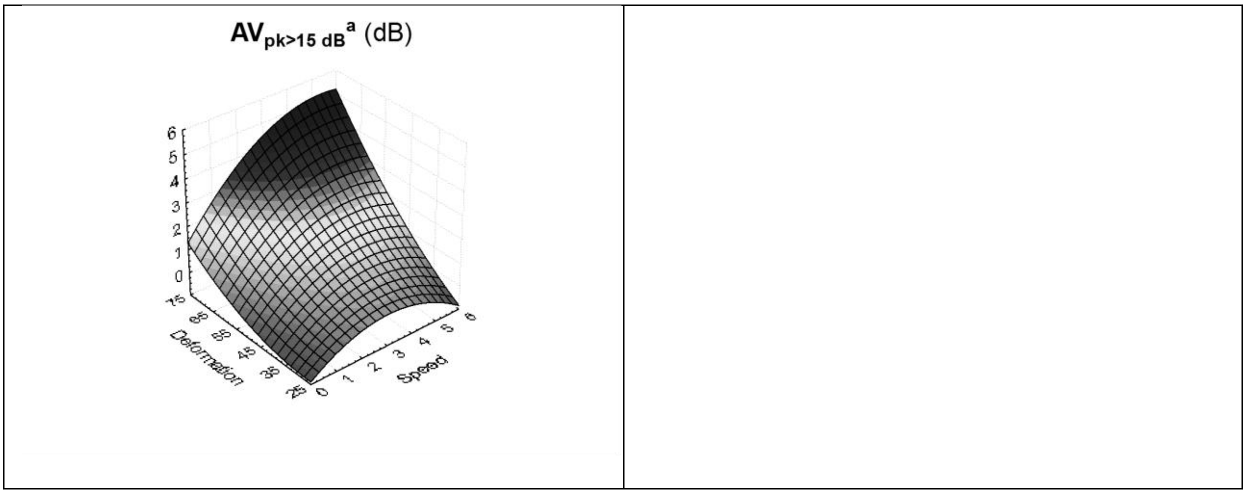


Figure 4

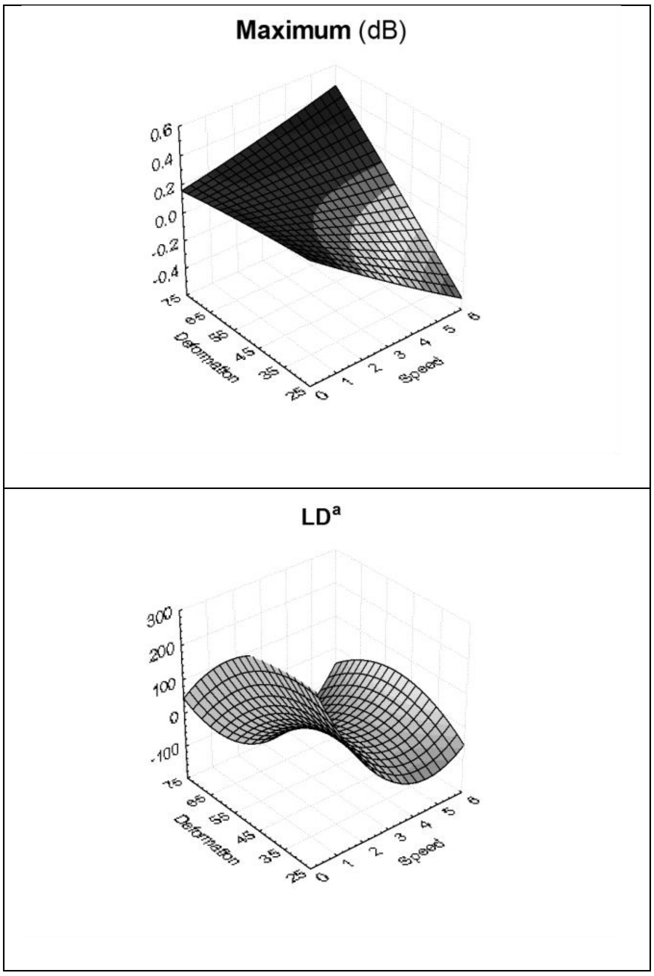


Figure 5

Table 1. Experimental Matrix of CCD

Experiment	Speed (coded value) (x)	Deformation applied (coded value)	Speed (real value) (X, mm/s)	Deformation applied (real value)
1	-1	-1	1.05	32
2	1	-1	5.15	32
3	-1	1	1.05	68
4	1	1	5.15	68
5	-1.4141	0	0.20	50
6	1.4141	0	6.00	50
7	0	-1.4141	3.10	25
8	0	1.4141	3.10	75
9	0	0	3.10	50
10	0	0	3.10	50
11	0	0	3.10	50
12	0	0	3.10	50
13	0	0	3.10	50

Table 2. Technological Ripening Parameters for Merlot Grape Berries Harvested at Two Different Dates

Ripening parameter	Harvest date		Sign
	1st September	20th September	
SSC (Brix)	21.6 ± 0.1	23.3 ± 0.1	**
TA (g/L tartaric acid)	6.46 ± 0.02	5.47 ± 0.04	**
pH	3.46 ± 0.01	3.50 ± 0.03	ns
Tartaric acid (g/kg)	7.47 ± 0.20	6.80 ± 0.33	ns
Malic acid (g/kg)	2.37 ± 0.05	1.33 ± 0.03	**
Citric acid (g/kg)	0.26 ± 0.01	0.26 ± 0.07	ns
Glucose/Fructose	1.01 ± 0.00	0.94 ± 0.00	***

All data are expressed as average value ± standard deviation. n = 3. Sign: **,*** and ns indicate significance at $p < 0.01$, 0.001 and not significant, respectively. SSC = total soluble solids content, TA = titratable acidity.

Table 3. Second-Order Polynomial Model by Central Composite Design (CCD) for Seed Texture Parameters of Merlot Grapes

Texture parameter	Gain	Equation						R^2	Optimal conditions[#,¤]	
			X_1	X_2	X_1^2	X_2^2	X_1X_2		X_1	X_2
F_s (N)	-	-3.5645	2.3195	0.0193	-0.0630	0.0012	-0.0312	0.88	6.00[¤]	25[¤]
W_s (mJ)	-	0.5546	0.1750	-0.0150	-0.0346	0.0003	-0.0031	0.40	3.10[#]	25[#]
E_s (N/mm)	-	-17.3103	6.9318	0.5353	-0.5995	-0.0021	-0.0542	0.84	2.39[¤]	75[¤]
DI_s (%)	-	4.5370	-0.6683	-0.1570	0.0340	0.0012	0.0070	0.80	0.20[¤]	25[¤]
Breakage (dB)	0	-13.3032	9.3293	0.2066	-0.6360	0.0005	-0.0956	0.84	6.00[¤]	30[¤]
Maximum (dB)	0	-7.6260	4.1806	0.2078	-0.4139	-0.0013	-0.0267	0.85	4.24[¤]	25[¤]
	24 dB	0.3106	-0.2328	-0.0009	0.0015	-1.49E-05	0.0036	0.85	6.00[¤]	75[¤]
AE^b (dB×mm)	0	2.7744	1.2354	-0.1402	-0.2246	0.0013	0.0035	0.47	3.10[#]	25[#]
	24 dB	-1.3411	-0.2982	-0.0170	0.1362	0.0005	-0.0076	0.51	3.10[#]	50[#]
AE^b (dB×s)	0	0.6476	0.4507	-0.0374	-0.0689	0.0004	-0.0007	0.41	3.10[#]	25[#]
	24 dB	-3.3956	3.4806	-0.1645	-0.3763	0.0019	-0.0051	0.45	3.10[#]	50[#]
AE^a (dB×mm)	0	-17.2068	4.7317	0.5164	-0.5173	-0.0032	-0.0203	0.57	0.20[#]	50[#]
AE^a (dB×s)	0	-3.3217	0.4873	0.1792	-0.0220	-0.0007	-0.0172	0.66	0.20[#]	50[#]
AE^t (dB×mm)	0	-14.4324	5.9672	0.3762	-0.7419	-0.0018	-0.0168	0.72	0.20[#]	50[#]

	24 dB	5.3378	-0.0266	-0.3172	0.1204	0.0035	-0.0088	0.76	3.10#	50#
AE^t (dB×s)	0	-2.6741	0.9380	0.1417	-0.0910	-0.0003	-0.0178	0.57	0.20#	50#
	24 dB	-1.6507	2.9873	-0.2017	-0.3100	0.0022	-0.0047	0.52	3.10#	50#
LD^b	0	115.3008	71.6477	-6.8462	-9.6050	0.0776	-0.3174	0.40	3.10#	25#
	24 dB	-12.4079	196.7966	-15.0294	-24.9629	0.1422	0.1185	0.45	6.00#	50#
LD^a	0	350.6943	-202.4500	3.6836	22.4554	-0.0184	-0.4699	0.86	0.20¤	75¤
	24 dB	210.2977	-220.8400	8.8671	21.6837	-0.1477	1.0070	0.82	0.20¤	31¤
LD^t	0	465.9951	-130.8020	-3.1626	12.8504	0.0592	-0.7873	0.79	0.20#	50#
	24 dB	197.8898	-24.0436	-6.1623	-3.2792	-0.0056	1.1255	0.67	6.00#	50#
$N_{pk>5\ dB}{}^b$	0	1.1347	0.5542	-0.0660	-0.0764	0.0008	-0.0022	0.30	3.10#	25#
	24 dB	-0.0516	6.2686	-0.4841	-0.8994	0.0040	0.0207	0.53	3.10#	25#
$N_{pk>15\ dB}{}^b$	0	0.8175	0.5422	-0.0498	-0.0806	0.0006	-0.0019	0.40	3.10#	25#
	24 dB	2.4614	0.3681	-0.1237	-0.0420	0.0013	-0.0028	0.32	3.10#	25#
$N_{pk>5\ dB}{}^a$	0	2.2232	-0.9781	0.0182	0.1567	0.0002	-0.0124	0.78	0.20#	50#
	24 dB	5.9908	-2.4278	-0.0685	0.1268	-0.0012	0.0345	0.69	1.05#	68#
$N_{pk>15\ dB}{}^a$	0	1.8136	-1.1081	0.0422	0.1233	-0.0003	-0.0061	0.84	0.20¤	68¤
	24 dB	2.4462	-3.3838	0.1760	0.3391	-0.0026	0.0142	0.61	6.00#	50#
$N_{pk>5\ dB}{}^t$	0	3.3579	-0.4239	-0.0478	0.0803	0.0009	-0.0146	0.47	0.20#	50#

	24 dB	5.9392	3.8408	-0.5526	-0.7726	0.0027	0.0552	0.75	$6.00^{\#}$	$50^{\#}$
$N_{pk>15\ dB}^{t}$	0	2.6311	-0.5659	-0.0076	0.0427	0.0003	-0.0080	0.83	$0.20^{¤}$	$75^{¤}$
	24 dB	4.9076	-3.0158	0.0523	0.2971	-0.0012	0.0114	0.58	$3.10^{\#}$	$25^{\#}$
$AV_{pk>5\ dB}^{b}$ (dB)	0	-69.0230	31.2243	1.5507	-3.4987	-0.0096	-0.1659	0.64	$3.10^{\#}$	$50^{\#}$
	24 dB	14.6498	3.4437	-0.7116	-0.4261	0.0076	-0.0133	0.50	$1.05^{\#}$	$68^{\#}$
$AV_{pk>15\ dB}^{b}$ (dB)	0	-74.0433	33.9475	1.7680	-4.2065	-0.0127	-0.1364	0.79	$3.10^{\#}$	$50^{\#}$
	24 dB	32.9763	9.9139	-1.8573	-0.8333	0.0237	-0.1371	0.71	$1.05^{\#}$	$68^{\#}$
$AV_{pk>5\ dB}^{a}$ (dB)	0	7.2723	1.8414	-0.3628	-0.1511	0.0039	-0.0105	0.90	$5.22^{¤}$	$25^{¤}$
	24 dB	5.7965	-0.5870	-0.1170	-0.1414	0.0005	0.0263	0.78	$1.05^{\#}$	$32^{\#}$
$AV_{pk>15\ dB}^{a}$ (dB)	0	-0.2410	0.5013	-0.0494	-0.1357	0.0009	0.0128	0.83	$5.38^{¤}$	$75^{¤}$
	24 dB	1.6399	1.9585	-0.1187	-0.2708	0.0015	-0.0093	0.77	$1.05^{\#}$	$68^{\#}$
$AV_{pk>5\ dB}^{t}$ (dB)	0	-7.3058	0.7699	0.2300	-0.0802	-0.0016	0.0002	0.51	$6.00^{\#}$	$50^{\#}$
	24 dB	-2.0789	1.4801	0.0593	-0.1461	-0.0001	-0.0100	0.69	$1.05^{\#}$	$68^{\#}$
$AV_{pk>15\ dB}^{t}$ (dB)	0	-8.1741	0.3516	0.2703	-0.0588	-0.0020	0.0071	0.47	$6.00^{\#}$	$50^{\#}$
	24 dB	-8.3313	0.9489	0.2958	-0.0297	-0.0022	-0.0141	0.36	$1.05^{\#}$	$68^{\#}$

F_s = seed break force, W_s = seed break energy, E_s = seed Young's modulus of elasticity, DI_s = seed deformation index (distance of seed break point/seed height x 100). ^{b}: before breaking. ^{a}: after breaking. ^{t}: total value during the compression test. 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. [#]optimal conditions of the CCD experimental matrix. [¤]optimal conditions calculated from second-order polynomial functions.

Table 4. Significant Pearson's Correlation Coefficients Between Texture Parameters and Phenolic Composition of Merlot Seeds

Texture parameter	Gain	TP_s (mg (+)-catechin/kg)	TF_s (mg (+)-catechin/kg)	PRO_s (mg cyanidin/kg)	FRV_s (mg (+)-catechin/kg)
F_s (N)	-	ns	ns	ns	0.452*
Maximum (dB)	0	ns	ns	ns	0.584**
AE^b (dB×mm)	24 dB	ns	-0.518*	ns	ns
AE^b (dB×s)	24 dB	ns	-0.519*	ns	ns
LD^a	24 dB	ns	0.525*	ns	0.540*
LD^t	0	ns	ns	ns	0.470*
	24 dB	ns	0.561*	ns	ns
$N_{pk>5\ dB}{}^a$	24 dB	ns	0.467*	ns	ns
$N_{pk>15\ dB}{}^a$	24 dB	ns	ns	ns	0.524*
$N_{pk>5\ dB}{}^t$	24 dB	0.476*	0.576**	ns	ns
$N_{pk>15\ dB}{}^t$	24 dB	ns	ns	ns	0.504*
$AV_{pk>5\ dB}{}^b$ (dB)	0	ns	ns	ns	0.553*
	24 dB	ns	-0.584**	ns	ns
$AV_{pk>15\ dB}{}^b$ (dB)	0	ns	ns	ns	0.540*
$AV_{pk>5\ dB}{}^a$ (dB)	0	ns	ns	ns	0.556*

	24 dB	-0.514**	-0.696**	ns	ns
$AV_{pk>15\ dB}{}^{a}$ (dB)	0	ns	ns	ns	0.536*
	24 dB	-0.487*	-0.689**	ns	ns
$AV_{pk>5\ dB}{}^{t}$ (dB)	0	ns	ns	ns	0.560*
	24 dB	ns	-0.554*	ns	ns
$AV_{pk>15\ dB}{}^{t}$ (dB)	0	ns	ns	ns	0.539*
	24 dB	-0.501*	-0.609**	ns	ns

n = 24. Sign: *,** and ns indicate significance at $p < 0.05$, 0.01 and not significant, respectively. F_s = seed break force, TP_s = seed total polyphenols, TF_s = seed total flavonoids, FRV_s = seed flavanols reactive to vanillin. [b]: before breaking. [a]: after breaking. [t]: total value during compression test. 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.

Table S1. Seed Mechanical Parameters for Merlot Grapes

Experiment/	1			2			3			4			5			6			7			8			9-13		
Parameter	AV	Δ%	sign	AV	Δ%	sign	AV	Δ%	sign	AV	Δ%	sign	AV	Δ%	sign	AV	Δ%	sign	AV	Δ%	sign	AV	Δ%	sign	AV	Δ%	sign
F_s (N)	43.0	-0.3	ns	43.5	6.5	ns	46.8	10.0	**	44.0	7.1	ns	42.5	-1.0	ns	44.3	6.5	ns	45.6	4.8	*	45.7	6.2	*	44.8	6.2	**
W_s (mJ)	10.04	0.8	ns	8.61	-1.4	ns	10.94	10.2	ns	9.03	5.1	ns	9.54	8.3	ns	8.43	-10.5	ns	10.28	6.2	*	9.81	2.6	ns	9.62	5.6	ns
E_s (N/mm)	87.7	4.1	ns	90.6	7.3	*	96.1	12.2	***	92.4	7.4	*	92.9	4.3	**	93.6	12.0	*	95.0	6.8	**	101.9	15.9	***	98.7	15.4	***
DI_s (%)	17.6	1.5	ns	16.5	-1.7	ns	16.9	-1.4	ns	16.6	1.4	ns	16.0	-1.9	ns	15.5	-9.8	ns	16.5	0.8	ns	15.8	-6.2	ns	15.6	-10.1	**

All data are expressed as average value at P2 sampling date (AV) and variation percentage between P1 and P2 sampling dates (Δ%). n = 30. Sign: *,**,*** and ns indicate significance in the variation percentage among determinations performed at the two sampling dates at $p < 0.05$, 0.01, 0.001 and not significant, respectively. F_s = seed break force, W_s = seed break energy, E_s = seed Young's modulus of elasticity, DI_s = seed deformation index (distance of seed break point/seed height X 100).

Table S2. Seed Acoustic Parameters for Merlot Grapes with instrumental gain set to 0

Experiment/Parameter	1			2			3			4			5			6			7			8			9-13		
	AV	Δ%	sign	AV	Δ%	sign	AV	Δ%	sign	AV	Δ%	sign	AV	Δ%	sign	AV	Δ%	sign	AV	Δ%	sign	AV	Δ%	sign	AV	Δ%	sign
Breakage (dB)	102	0.9	ns	105	8.3	*	105	4.4	*	100	-1.4	ns	95	-1.7	ns	101	4.1	ns	105	6.5	ns	106	6.5	*	106	4.7	*
Maximum (dB)	104	0.3	ns	105	2.7	*	106	2.8	**	106	1.6	ns	102	0.0	ns	106	2.1	ns	107	4.3	**	106	2.8	**	107	3.1	*
AE^{b} (dB×mm)	0.58	26.0	ns	3.60	27.5	ns	1.05	40.1	*	4.35	40.5	ns	0.08	-20.9	ns	3.23	-42.0	ns	3.97	56.8	***	3.70	48.6	**	2.93	43.5	*
AE^{b} (dB×s)	0.554	26.0	ns	0.783	27.6	ns	1.004	40.1	*	0.937	40.2	ns	0.417	-21.0	ns	0.640	-37.3	ns	1.320	56.6	***	1.230	48.6	**	0.975	44.0	*
AE^{a} (dB×mm)	6.18	23.0	**	22.51	3.0	ns	18.71	21.2	***	73.37	0.4	ns	2.75	30.7	***	57.19	17.3	***	12.17	13.9	ns	64.46	21.4	***	36.69	8.7	ns
AE^{a} (dB×s)	6.24	23.2	**	5.90	3.8	ns	18.10	21.3	***	15.77	0.9	ns	13.82	30.7	***	11.34	16.2	***	5.24	17.0	*	21.70	21.5	***	12.71	8.4	ns
AE^{t} (dB×mm)	6.76	23.3	**	26.11	6.3	ns	19.76	22.2	***	77.72	2.6	ns	2.84	29.2	***	60.42	14.2	***	16.14	24.5	**	68.16	22.9	***	39.62	11.3	ns
AE^{t} (dB×s)	6.79	23.4	**	6.68	6.6	ns	19.10	22.2	***	16.70	3.1	ns	14.24	29.2	***	11.98	13.4	***	6.57	24.9	**	22.93	22.9	***	13.68	11.0	ns
LD^{b}	129	13.8	ns	195	20.4	ns	195	27.2	ns	180	16.1	ns	88	-17.7	ns	154	-37.5	ns	235	44.5	**	269	29.9	*	215	21.0	ns
LD^{a}	789	22.5	*	415	-5.0	ns	2763	7.3	ns	1324	-4.8	ns	2232	24.7	***	694	-18.3	*	320	-4.3	ns	2314	1.6	ns	1258	-11.1	*
LD^{t}	918	21.3	*	610	3.2	ns	2958	8.7	ns	1504	-2.3	ns	2320	23.1	***	848	-21.8	**	555	16.4	ns	2583	4.5	ns	1473	-6.4	ns
$N_{pk>5\ dB}^{b}$	0.800	15.0	ns	1.160	34.5	ns	1.160	37.9	*	1.080	37.0	ns	0.640	-6.3	ns	0.720	-77.8	ns	1.560	48.7	**	1.840	47.8	*	1.286	25.3	ns
$N_{pk>15\ dB}^{b}$	0.800	15.0	ns	0.840	28.6	ns	1.160	37.9	*	0.840	33.3	ns	0.640	-6.3	ns	0.560	-114.3	ns	1.360	52.9	**	1.640	41.5	*	1.286	28.4	ns
$N_{pk>5\ dB}^{a}$	6.04	25.2	*	4.56	9.6	ns	21.52	6.7	ns	13.80	-10.4	*	15.88	24.7	***	8.28	-6.8	ns	3.12	0.0	ns	21.52	4.3	ns	11.79	-13.7	*
$N_{pk>15\ dB}^{a}$	5.60	25.0	*	2.92	-5.5	ns	19.48	5.5	ns	9.48	-14.3	*	15.24	23.4	**	5.40	-20.7	*	2.20	-10.9	ns	16.68	1.4	ns	9.36	-11.1	ns

$N_{pk>5\ dB}{}^{t}$	6.84	24.0	*	5.72	14.7	ns	22.68	8.3	ns	14.88	-7.0	ns	16.52	23.5	**	9.00	-12.4	ns	4.68	16.2	ns	23.36	7.7	*	13.07	-9.9	ns
$N_{pk>15\ dB}{}^{t}$	6.40	23.8	*	3.76	2.1	ns	20.64	7.4	ns	10.32	-10.5	*	15.88	22.2	**	5.96	-29.5	**	3.56	13.5	ns	18.32	5.0	ns	10.64	-6.4	ns
$AV_{pk>5\ dB}{}^{b}$ (dB)	35.0	-12.7	ns	50.2	24.2	ns	61.1	25.8	ns	52.5	15.8	ns	28.9	-52.8	ns	43.1	-17.2	ns	61.6	23.1	ns	54.9	18.1	ns	66.2	42.5	*
$AV_{pk>15\ dB}{}^{b}$ (dB)	35.0	-12.7	ns	42.5	26.1	ns	61.1	25.8	ns	49.0	23.5	ns	28.9	-52.8	ns	36.1	-30.9	ns	59.0	30.5	ns	55.4	18.8	ns	66.2	47.2	**
$AV_{pk>5\ dB}{}^{a}$ (dB)	73.5	-3.2	ns	81.5	-0.5	ns	73.3	2.2	ns	81.6	2.5	*	73.4	1.3	ns	82.6	4.5	**	86.2	9.7	ns	78.7	4.8	***	75.4	2.6	ns
$AV_{pk>15\ dB}{}^{a}$ (dB)	73.7	-4.2	ns	77.5	-6.0	ns	73.9	3.1	*	83.4	3.1	ns	73.4	1.1	ns	85.3	6.1	**	83.7	6.0	ns	79.8	5.6	***	76.2	2.3	ns
$AV_{pk>5\ dB}{}^{t}$ (dB)	72.5	-2.4	ns	78.6	-2.1	ns	73.2	1.9	ns	80.8	1.9	ns	73.2	1.0	ns	81.5	4.6	**	79.9	0.8	ns	77.8	4.1	**	74.9	3.2	ns
$AV_{pk>15\ dB}{}^{t}$ (dB)	72.7	-2.7	ns	77.0	-3.9	ns	73.7	2.6	*	82.3	2.3	ns	73.2	0.8	ns	83.7	6.4	**	80.0	0.7	ns	78.7	4.9	**	75.5	3.0	ns

All data are expressed as average value at P2 sampling date (AV) and variation percentage between P1 and P2 sampling dates (Δ%). n = 30. Sign: *,**,*** and ns indicate significance in the variation percentage among determinations performed at the two sampling dates at $p < 0.05$, 0.01, 0.001 and not significant, respectively. [b]: before breaking. [a]: after breaking. [t]: total value during the compression test. 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.

Table S3. Seed Acoustic Parameters for Merlot Grapes with instrumental gain set to 24 dB

Experiment/Parameter	1			2			3			4			5			6			7			8			9-13		
	AV	Δ%	sign	AV	Δ%	sign	AV	Δ%	sign	AV	Δ%	sign	AV	Δ%	sign	AV	Δ%	sign	AV	Δ%	sign	AV	Δ%	sign	AV	Δ%	sign
Breakage (dB)	89	-1.2	ns	87	-2.0	ns	88	-2.5	ns	87	-0.9	ns	88	1.3	ns	88	1.7	ns	89	0.0	ns	89	0.1	ns	89	0.0	ns
Maximum (dB)	90	0.1	ns	90	-0.5	**	91	0.3	ns	91	0.3	*	91	0.2	ns	91	0.1	ns	90	0.0	ns	91	0.3	**	91	0.2	ns
AE^b (dB×mm)	19.63	-4.9	ns	21.13	-1.0	ns	20.28	-1.4	ns	20.61	-3.1	ns	11.56	- 16.9	*	19.95	-1.5	ns	23.96	-9.6	ns	22.67	-7.2	ns	22.02	- 20.1	***
AE^b (dB×s)	18.810	-4.8	ns	4.598	-0.6	ns	19.422	-1.4	ns	4.496	-2.6	ns	57.887	- 16.9	*	3.874	-4.1	ns	7.984	-9.5	ns	7.552	-7.0	ns	7.349	- 19.6	***
AE^a (dB×mm)	21.96	-3.8	ns	31.00	2.4	ns	74.78	-1.2	ns	99.27	0.5	ns	31.48	1.3	ns	66.86	-0.6	ns	17.38	13.0	ns	107.09	1.6	ns	62.43	1.8	ns
AE^a (dB×s)	22.05	-3.5	ns	8.06	2.2	ns	72.31	-1.1	ns	21.30	0.8	ns	158.03	1.3	ns	13.35	-0.3	ns	7.21	10.8	ns	36.04	1.6	ns	21.66	1.8	ns
AE^t (dB×mm)	41.59	-4.3	ns	52.12	1.0	ns	95.06	-1.2	ns	119.89	-0.1	ns	43.03	-3.6	ns	86.81	-0.8	ns	41.34	-0.1	ns	129.75	0.1	ns	84.44	-3.9	*
AE^t (dB×s)	40.86	-4.1	ns	12.66	1.2	ns	91.73	-1.2	ns	25.80	0.2	ns	215.91	-3.6	ns	17.22	-1.1	ns	15.20	0.1	ns	43.60	0.1	ns	29.01	-3.6	*
LD^b	432	-7.1	ns	165	-6.4	ns	458	- 11.4	ns	158	-9.4	ns	5105	- 10.6	ns	129	- 19.6	**	159	18.0	*	145	1.7	ns	149	11.5	ns
LD^a	599	-9.5	ns	205	5.6	ns	2257	- 11.9	***	666	-8.3	*	8413	6.6	ns	341	- 17.7	***	163	8.5	ns	1083	-6.2	*	620	4.6	ns
LD^t	1031	-8.5	*	370	0.2	ns	2716	- 11.8	***	824	-8.5	**	13518	0.1	ns	471	- 18.3	***	322	13.2	***	1228	-5.2	*	769	6.0	*
$N_{pk>5\ dB}{}^b$	8.600	- 13.0	ns	2.429	- 25.9	*	8.600	- 43.2	**	2.629	-8.7	ns	141.057	- 12.7	ns	1.914	- 32.8	*	2.486	43.7	**	2.143	25.3	ns	1.886	36.4	*

$N_{pk>15\ dB}^{b}$	2.057	4.2	ns	0.971	-8.8	ns	2.343	24.4	ns	0.943	0.0	ns	48.571	-1.1	ns	0.657	-52.2	*	1.486	61.5	***	1.314	45.7	**	1.029	33.3	ns
$N_{pk>5\ dB}^{a}$	12.31	-8.6	ns	4.91	4.1	ns	43.57	-17.3	***	15.23	-8.4	ns	202.43	1.4	ns	7.97	-19.4	**	3.66	7.0	ns	23.60	-11.4	***	14.31	-0.4	ns
$N_{pk>15\ dB}^{a}$	6.29	-10.0	ns	2.26	12.7	ns	24.51	-12.2	*	8.66	-0.3	ns	63.66	13.5	ns	3.97	-19.4	*	1.89	18.2	ns	13.23	-10.8	*	7.74	7.0	ns
$N_{pk>5\ dB}^{t}$	20.91	-10.4	*	7.34	-5.8	ns	52.17	-21.6	***	17.86	-8.5	*	343.49	-4.4	ns	9.89	-22.0	***	6.14	21.9	***	25.74	-8.3	*	16.20	3.9	ns
$N_{pk>15\ dB}^{t}$	8.34	-6.5	ns	3.23	6.2	ns	26.86	-9.0	*	9.60	-0.3	ns	112.23	7.2	ns	4.63	-24.1	**	3.37	37.3	***	14.54	-5.7	ns	8.77	10.1	*
$AV_{pk>5\ dB}^{b}$ (dB)	50.8	1.4	ns	56.5	-0.9	ns	52.5	8.7	**	62.4	2.3	ns	29.3	-6.1	ns	58.8	4.9	ns	67.0	15.9	ns	71.0	10.0	ns	63.4	18.4	ns
$AV_{pk>15\ dB}^{b}$ (dB)	63.7	7.0	ns	54.1	3.8	ns	70.0	21.7	**	50.0	-14.1	ns	32.9	-4.2	ns	45.0	-18.5	ns	55.9	23.1	ns	66.5	31.7	*	57.2	28.9	ns
$AV_{pk>5\ dB}^{a}$ (dB)	68.0	6.4	***	79.3	-0.9	ns	67.7	4.5	***	80.2	2.3	*	37.9	-2.7	ns	81.6	2.0	*	81.8	2.0	ns	78.0	2.5	***	77.5	1.1	ns
$AV_{pk>15\ dB}^{a}$ (dB)	75.9	1.2	ns	76.2	0.8	ns	75.8	3.2	***	84.5	0.9	ns	51.6	-1.4	ns	83.2	-1.7	ns	83.7	3.0	ns	83.8	2.1	***	83.2	0.5	ns
$AV_{pk>5\ dB}^{t}$ (dB)	60.2	3.6	*	72.8	2.2	ns	65.0	5.6	***	77.2	2.2	*	34.3	-2.8	ns	77.6	2.2	*	77.9	1.5	ns	77.5	2.4	**	76.5	0.0	ns
$AV_{pk>15\ dB}^{t}$ (dB)	74.0	1.1	ns	80.9	1.6	ns	75.3	3.1	***	83.5	0.9	ns	43.9	-0.1	ns	84.0	1.8	ns	81.7	-2.2	ns	82.9	1.3	*	82.3	-0.2	ns

All data are expressed as average value at P2 sampling date (AV) and variation percentage between P1 and P2 sampling dates (Δ%). n = 30. Sign: *,**,*** and ns indicate significance in the variation percentage among determinations performed at the two sampling dates at $p < 0.05$, 0.01, 0.001 and not significant, respectively. [b]: before breaking. [a]: after breaking. [t]: total value during the compression test. 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.