# 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

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## Abstract

**Aims :** Fourier Transform-Near Infrared (FT-NIR) spectrum and instrumental texture parameters were assessed as total phenol content and extractability predictors in intact grape seeds.

**Methods and results :** The study was carried out on Cabernet-Sauvignon seeds from grapes harvested at six different advanced physiological stages throughout ripening and calibrated by flotation to reduce the in-field heterogeneity inside each sample. Among the instrumental mechanical properties tested (i. e., break force, break energy, Young's modulus of elasticity and deformation index), the seed Young's modulus of elasticity showed an increase during the first four weeks of ripening. This parameter also showed significant correlations with phenol content and extractability, although with low R coefficients. These correlations highlighted that the springier seed tissues greatly increase phenol extractability. Nevertheless, the best prediction of seed phenol content, performed directly on intact seeds, was found using FT-NIR spectroscopy in transmittance mode. The standard error of prediction for total phenol content was less than 8 %, while that for phenol extractability was worse.

**Conclusion**: On the basis of these results, the two analytical methods could be applied in oenology for the rapid monitoring of seed phenolic maturity.

**Significance and impact of the study :** The phenolic composition of grapes at the harvest time is a key factor determining their quality, and thus the quality of the finished wine. The chemical methods used for the determination of seed phenol content and extractability are generally slow because they require a preliminary extraction. Therefore, a rapid evaluation of these parameters could be highly interesting for the oenological sector.

**Key words :** instrumental texture properties, FT-NIR spectrum, grape seeds, phenol content, extractability

## Résumé

**Objectifs :** Le spectre FT-NIR (Fourier Transform-Near Infrared) et les paramètres instrumentaux de la texture sont assumés comme indices de la teneur en phénols et de leur extractibilité de pépins entiers.

**Méthodes et résultats :** L'étude concerne les pépins de raisins de Cabernet-Sauvignon cueillis pendant la maturation des baies. Parmi les propriétés mécaniques instrumentales testées (résistance des pépins à la rupture, force de rupture, formule d'élasticité de Young, indice de déformation), la formule d'élasticité de Young a montré une augmentation pendant les quatre premières semaines de maturation. Ce paramètre a également montré une corrélation significative avec la teneur en phénols et avec l'extractibilité, bien qu'avec un faible coefficient R. Ces corrélations soulignent une extractibilité des phénols plus accentuée dans les tissus des pépins plus élastiques. Néanmoins, la meilleure prédiction de la teneur en phénols, évaluée directement sur les pépins intacts, a été celle obtenue par l'emploi de la spectrophotométrie FT-NIR en transmittance. L'erreur standard pour la prédiction de la teneur en phénols totaux était inférieure à 8 % tandis que celle pour l'extractibilité était moins fiable.

**Conclusion :** Sur la base de ces résultats, les deux techniques peuvent être proposées pour la détermination rapide de la maturité phénolique des pépins.

**Signification et impact de l'étude :** La composition phénolique des raisins à la récolte est un élément clé pour leur qualité et donc pour la qualité du vin produit. Les méthodes chimiques pour la détermination de la teneur en phénols des pépins et de leur extractibilité sont généralement longues, parce qu'une extraction préliminaire est requise. Une évaluation rapide de ces paramètres peut donc être très utile pour le secteur œnologique.

**Mot-clés :** propriétés instrumentales de la texture, spectre FT-NIR, pépins de raisin, teneur en phénols, extractibilité

*manuscript received 29th November 2011 - revised manuscript received 16 March 2012*

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*J. Int. Sci. Vigne Vin*, 2012, **46**, n°1, 29-40
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## INTRODUCTION

One of the key factors affecting red wine quality is the phenolic ripeness of berry seeds at harvest time because their phenolic composition is responsible for some wine sensory properties, such as astringency and bitterness (VIDAL *et al.*, 2004 ; McRAE and KENNEDY, 2011). Moreover, the seeds are rich in flavan-3-ols, which are better anthocyanin copigments than those found in the skins, and therefore contribute to improve long-term colour stability (GONZALEZ-MANZANO *et al.*, 2004 ; CHEYNIER *et al.*, 2006). The phenolic composition of seeds changes during fruit development, with flavan-3-ols reaching their highest concentration at veraison, after which they decline slowly until close to grape ripeness and remain relatively constant thereafter (KENNEDY *et al.*, 2000a ; MATEUS *et al.*, 2001 ; DOWNEY *et al.*, 2003 ; CADOT *et al.*, 2006 ; BARBAGALLO *et al.*, 2011 ; LORRAIN *et al.*, 2011).

Phenolic compounds are extracted from berry skins and seeds into the wine during the maceration/fermentation step. The histological and histochemical modifications occurring in seeds during the fruit development affect the ability to release phenols because the solidification of the cells rich in tannins, before harvest, can negatively affect the extractability of these compounds (CADOT *et al.*, 2006). This decreasing trend in seed phenol extractability during ripening (MATEUS *et al.*, 2001) may be consistent with an oxidative process of flavan-3-ols (KENNEDY *et al.*, 2000b), which favours the association with cell-wall components. The oxidation products would likely be flavan-3-ols cross-linked with other phenols, polysaccharides or proteins (KENNEDY *et al.*, 2000a ; DOWNEY *et al.*, 2003).

Wine phenolic composition depends on the original grape phenolic profile but also on the extraction conditions and the winemaking technique employed (SACCHI *et al.*, 2005). The reliable estimation of the content of these compounds in the finished wine, from the determination of certain chemical and mechanical parameters of the grapes, represents a valuable tool for winemakers. For example, it provides important information for harvest date selection and winemaking management. The contribution of seed flavan-3-ols to the wine phenolic composition progressively increases with maceration time and thus becomes highly predominant when post-fermentation maceration practices are used (GONZALEZ-MANZANO *et al.*, 2004 ; CANALS *et al.*, 2005 ; DEL LLAUDY *et al.*, 2008).

Few studies have been performed to define the best indices to evaluate the phenol extractability from berry seeds. The most widely used methods to determine the phenolic ripeness of grapes are the Glories method, the Cromoenos method, and the two methods implemented by the Australian Wine Research Institute (AWRI) and the Institut Technique de la Vigne et du Vin (ITV) (FRAGOSO *et al.*, 2010 ; KONTOUDAKIS *et al.*, 2010). The tannin content in the grape berry at harvest is not a good indicative of the extracted amount of tannins into the wine (JENSEN *et al.*, 2008 ; GONZALEZ-NEVES *et al.*, 2010). By contrast, wine total phenols are well correlated with grape total phenols, the correlation factor being higher than 0.80 (JENSEN *et al.*, 2008 ; GONZALEZ-NEVES *et al.*, 2010 ; KONTOUDAKIS *et al.*, 2010). Given that the extraction efficiency of phenolic compounds is strongly dependent on the extraction method used, the seed maturity index (Mp) has been proposed to predict those provided by berry seeds in the resulting wines (CAGNASSO *et al.*, 2008, 2011 ; FRAGOSO *et al.*, 2010 ; GONZALEZ-NEVES *et al.*, 2010 ; ZANONI *et al.*, 2010). However, these chemical methods are tedious and time consuming.

Harvest and winemaking decisions are highly influenced by the desired wine style and quality. Therefore, winemakers demand the development of inexpensive, fast, simple, reliable and environmentally friendly methods to be used for routine monitoring of the changes occurring in grape phenolic composition during ripening. Since phenol extraction is usually the most critical step, the direct analysis methods of whole berry, skin or/and seed, based on instrumental texture analysis or near infrared (NIR) spectroscopy, represent powerful analytical tools in this research field, replacing the time-consuming chemical methods.

The structural properties of grape cell-walls may determine the mechanical resistance, the texture and the ease of processing berries (BARNAVON *et al.*, 2000). Very good results have been reported in the literature for the assessment of anthocyanin extractability using instrumental texture analysis parameters. In particular, berry skin break force can be considered the best mechanical attribute to estimate anthocyanin extraction kinetics (ROLLE *et al.*, 2012), whereas berry skin thickness has been proposed as predictor of anthocyanin extractability (RIO SEGADE *et al.*, 2011). By contrast, the knowledge of the mechanical properties of berry seeds is scarce and no relationship has been established with the phenol content and extractability (ROLLE *et al.*, 2009b ; TORCHIO *et al.*, 2010).

Near Infrared (NIR) spectroscopy is an accurate, fast and non-destructive technique that has been used to determine phenolic compounds in wines (COZZOLINO *et al.*, 2004a, 2006a ; DI EGIDIO *et al.*, 2010 ; CASALE *et al.*, 2010) and in grape homogenates (COZZOLINO *et al.*, 2005, 2006b ; DAMBERGS *et al.*, 2006 ; COZZOLINO *et al.*, 2008). Moreover, it has been used directly in intact grape berries in order to determine total


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anthocyanins (COZZOLINO *et al*., 2006b, 2008), as well as extractable anthocyanins (at pH 1.0 and 3.2) and total phenols (KEMPS *et al*., 2010). Using NIR spectroscopy, FERRER-GALLEGO *et al*. (2011) have recently determined the concentrations of the main phenolic families (flavanols, anthocyanins, flavonols and phenolic acids) and total phenolic compounds in grape skins and intact red grapes during ripening. The same authors have also published an interesting study on the possibility of using NIR to evaluate the monomeric and oligomeric flavanol composition of seeds (FERRER-GALLEGO *et al*., 2010).

The aim of this work was to evaluate the potential of instrumental mechanical properties and FT-NIR (Fourier Transform - NIR) spectrum to predict total phenol content and phenol extractability in intact grape seeds. The study was carried out on Cabernet sauvignon, one of the world's most studied and widely recognized red grape varieties. To our knowledge, this is the first time that instrumental texture analysis and NIR spectroscopy have been used for estimating the total content and extractability of phenolic compounds in grape seeds. The real prediction performance of the different methods proposed was determined according to relationships with reference methods in order to ensure their application as routine analytical tools for optimizing harvest decisions, as well as for assessing and managing phenol extraction during the winemaking process, particularly in grape varieties containing high amounts of seed flavanols.

## MATERIALS AND METHODS

### 1. Grape samples

Grape samples of Cabernet-Sauvignon red cultivar (*Vitis vinifera* L.) were collected at different advanced physiological stages from a vineyard located in Piedmont (North-West Italy) during six consecutive weeks in 2010. About 5 kg of grape berries for each sampling date were randomly picked with attached pedicels. Three sub-samples of 50 berries were used for determining technological maturity parameters in the grape must obtained by manual crushing and filtration (UnS = non-sorted grapes).

The remaining grape berries were immediately sorted into six density classes (A-F) by flotation in different saline solutions (from 100 to 190 $g.L^{-1}$ sodium chloride) (FOURNAND *et al*., 2006 ; KONTOUDAKIS *et al*., 2011 ; ROLLE *et al*., 2011b). The density of these solutions ranged from 1069 to 1125 $kg.m^{-3}$. The berries were first introduced into the less dense solution and « floating » berries were considered to have the same density as the solution. These berries were weighted and the others were removed and introduced into the next denser solution. The same process was successively applied to all saline solutions. This allowed to reduce the in-field heterogeneity of the physiological characteristics within each sample and thus to assess the real potential of the method proposed. One berry density class per sampling date was selected after density sorting for the determination of chemical parameters, mechanical properties and FT-NIR spectra. The selected density class was not necessarily the predominant one. Rather, the grape physiological differences between the first and the last sampling date were emphasized by selecting the less dense class A (1081 $kg.m^{-3}$) for the first ripening stage, and then increasing density of the berry classes selected throughout ripening up to the selection of the denser class E (1107 $kg.m^{-3}$) for the last two weeks.

A sub-sample of 40 berries was selected from each selected density class per sampling date. One seed per berry was selected in order to cover a wider range of phenol content with the same number of seeds. The seeds were carefully removed from the pulp, cleaned with adsorbent paper and weighted before analysis. Firstly, the instrumental mechanical properties and FT-NIR spectrum were determined in these 40 intact grape seeds. Afterwards, these same seeds were used for the determination of phenol content and relative phenol extractability. The remaining berries, sub-divided in three replicates, were used for determining the technological maturity parameters in the sorted grape must obtained by manual crushing and filtration. Once the different sub-samples were prepared, they were immediately analyzed.

### 2. Reagents

All 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).

### 3. Technological maturity parameters

ºBrix, pH and titratable acidity were determined according to International Organization of Vine and Wine (OIV) methods (OIV, 2008).

### 4. Phenol content and extractability

Each seed was quickly immersed in 1.5 mL of a buffer solution containing 12 % (v/v) ethanol, 200 $mg.L^{-1}$ sodium metabisulphite to limit the oxidation of phenolic compounds and 5 $g.L^{-1}$ tartaric acid. The pH was adjusted to 3.2 by the addition of 1 M sodium hydroxide (TORCHIO *et al*., 2010). The sample was then incubated in a temperature-controlled room at 25 ºC for 7 days, after which the extract was used for determining extractable phenols (solution $A_1$). Residual berry seeds were rinsed with a hydroalcoholic solution and quickly immersed in 1.5 ml of a new hydroalcoholic buffer containing higher


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ethanol (IACOPINI *et al.*, 2008) and sodium metabisulphite concentrations (70 % v/v and 2 $g.L^{-1}$, respectively). After a 15-day soaking period in a temperature-controlled room at 25 ºC, the extract was used for determining non-extractable phenols (solution $A_2$). Total phenol content in berry seeds was calculated as Atot = $A_1 + A_2$, while the extractability yield was calculated as $A_1/(A_1 + A_2)$ and expressed as percentage (%). The phenol content in the berry seed extracts was determined by spectrophotometric measurement at 280 nm (A280 nm) (RIBÉREAU-GAYON, 1970) using a UV-1800 spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA).

### 5. Instrumental mechanical properties

For the assessment of seed mechanical properties, a compression test was carried out using an Universal Testing Machine TAxT2i Texture Analyzer (SMS-Stable Micro Systems, Surrey, United Kingdom) equipped with a SMS HDP/90 platform, a SMS P/35 probe and a 50 kg load cell (LETAIEF *et al.*, 2008a). The test speed was 1 $mm.s^{-1}$ and the deformation applied was 50 %. All the data were acquired at 400 Hz and evaluated using the Texture Expert Exceed software package (version 2.54 for Windows).

The following instrumental texture parameters were acquired: seed break force (N, as $F_s$), seed break energy (mJ, as $W_s$), seed Young's modulus of elasticity ($N.mm^{-1}$, as $E_s$) and seed deformation index (%, as $DI_s$). The latter parameter was calculated as distance of seed break point/seed height x 100 (LETAIEF *et al.*, 2008a; ROLLE *et al.*, 2009a).

### 6. FT-NIR analysis

Intact berry seeds were scanned in a NIR-Flex N500 spectrophotometer equipped with Solids Transmittance modules (Buchi, Flawil, Switzerland), as shown in Figure 1. The FT-NIR spectra were collected in transmittance mode in the wavelength range of 4000-12000 $cm^{-1}$ using a NIR-Operator software (Buchi, Flawil, Switzerland). For each seed, 64 scans were acquired, with a nominal resolution of 4 $cm^{-1}$, on the lateral side of the seed. All NIR analyses were performed at room temperature (20 ± 1 °C). The mean spectrum of the data set is shown in Figure 2.

All spectra were randomly sub-divided into two sets: the first one (about 2/3) was used for calibration and the second one (about 1/3) for validation. Principal component analysis (PCA) was performed before regression and was used to provide information about the latent structure of the spectral data and to detect spectral outliers that were eliminated at this stage. The FT-NIR spectra were pre-treated and a spectral region was selected to minimize

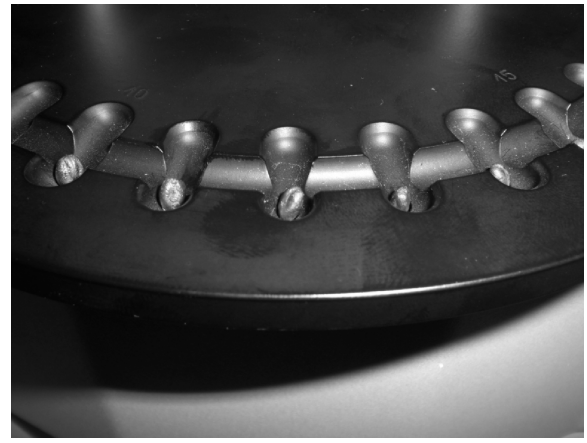


**Figure 1. Positioning of intact berry seeds on the Solids Transmittance modules of the NIR-Flex N500 spectrophotometer.**

the standard error of prediction (SEP) (ELFADL *et al.*, 2010). The calibrations were performed by using a partial least square (PLS) regression for each parameter with NIRCAL 5.2 software (Buchi, Flawil, Switzerland). Table 1 shows the spectral pre-treatments applied, the number of principal components (PCs), the outliers eliminated and the wavelengths chosen using the automatic optimization of the calibration of the NIRCAL software.

### 7. Statistical analysis

Statistical analyses were performed using the SPSS statistical software package (version 17.0; SPSS Inc., Chicago, IL, USA). The Tukey-b test for $p < 0.05$ was used in order to establish statistical differences by one-way analysis of variance (ANOVA). Pearson correlation coefficients were calculated to determine significant correlations.

## RESULTS AND DISCUSSION

The distribution, expressed as percentage, of Cabernet-Sauvignon grape berries in different density classes at six sampling dates is reported in Figure 3. The contribution of each density class varied, depending on the grape ripening stage. As expected, the lower density classes (≤ B) made up the majority (> 86 %) in the less ripe grapes (harvest dates I and II), whereas the contribution of the higher density classes increased with grape ripeness and, therefore, with harvest date. The last two ripening stages (V and VI) showed a similar distribution, with a contribution of the grapes belonging to the density class E of 23.1 and 27.1 %, respectively.

Environmental variables within vineyards, such as the location of the vines, the position of the clusters in the vine, the position of the berries in the cluster, let alone the


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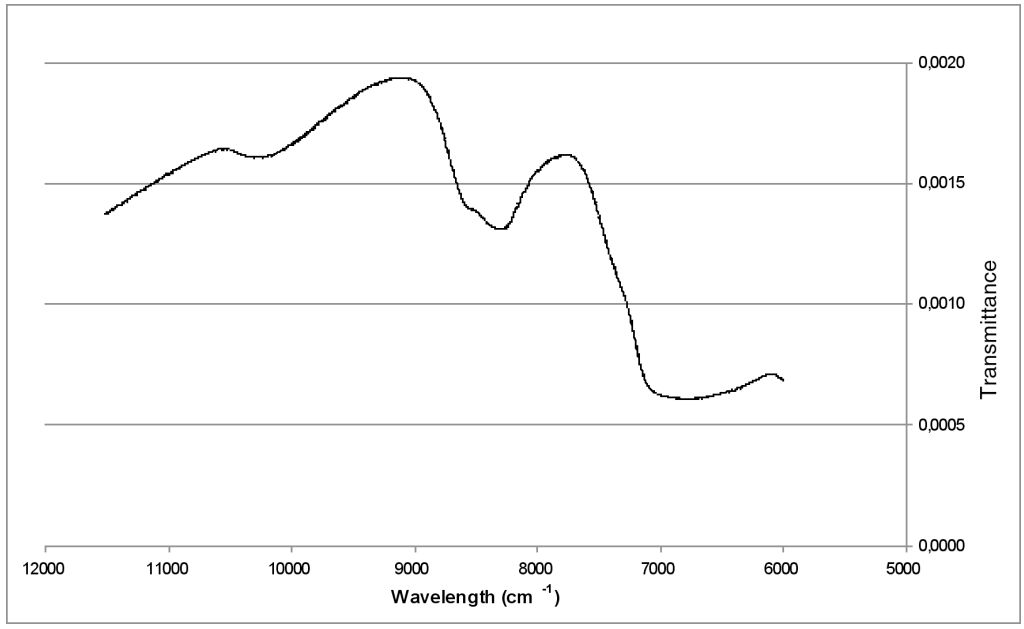



**Figure 2. Mean spectrum of the data set for intact grape seeds.**

**Table 1. Spectral pre-treatments applied, number of principal components (PCs), number of outliers eliminated and wavelengths chosen for NIR models in intact berry seeds for seed phenol content and extractability of sorted Cabernet-Sauvignon grapes harvested at different ripening stages.**

| Chemical parameter | Spectral pre-treatments | Outliers | PCs | Wavelength ($cm^{-1}$) |
|---|---|---|---|---|
| $A_{1,280nm}$ | ilg | 7 | 16 | 6000-7144, 7404-11520 |
| $A_{2,280nm}$ | ncl | 11 | 12 | 6000-7144, 7404-11520 |
| $A_{tot,280nm}$ | SNV | 16 | 18 | 6000-11520 |
| $A_{1,280nm}$/seed weight ($mg^{-1}$) | mf | 4 | 17 | 6000-9000 |
| $A_{2,280nm}$/seed weight ($mg^{-1}$) | ncl | 13 | 11 | 6000-11520 |
| $A_{tot,280nm}$/seed weight ($mg^{-1}$) | mf | 5 | 17 | 6000-9000 |
| Extractability (%) | db1,SNV | 10 | 15 | 6000-9000 |

$A_{1,280}$ nm = extractable phenol content, $A_{2,280}$ nm = non-extractable phenol content, $A_{tot,280nm} = A_{1,280nm} + A_{2,280nm}$, ilg = inverse absorbance 1/(10^x), ncl = normalization by closure, SNV = standard normal variate, mf = full multiplicative scatter correction, db1 = first derivative BCAP.

berry size, may induce important differences in the chemico-physical characteristics of grapes (FAILLA *et al.*, 2004 ; LETAIEF *et al.*, 2008b ; CHORTI *et al.*, 2010). The distribution of the berries in the vineyard according to the density changes during the ripening process (ROLLE *et al.*, 2011b). Therefore, a non-negligible heterogeneity occurs throughout the ripening process.

## 1. TECHNOLOGICAL MATURITY PARAMETERS

Table 2 shows the technological maturity parameters for the non-sorted Cabernet sauvignon grape berries sampled at six ripening stages and for the densitometrically sorted grape class selected in each sample. As reported by FOURNAND *et al.* (2006) in Shiraz berries, the difference in the sugar content of berries belonging to two consecutive density classes is ~ 17 $g.L^{-1}$ (i. e., 1 % v/v potential alcohol). In this study on Cabernet-Sauvignon grapes, the differences ranged from 8 to 15 $g.L^{-1}$. The most relevant decrease in titratable acidity was detected between the berries belonging to the two lowest density classes (A and B). Furthermore, titratable acidity slightly increased and pH decreased at the last harvest date in sorted and non-sorted grape berries. The values of the technological maturity parameters obtained at harvest were those usually found for the Cabernet-Sauvignon cultivar in the Piedmont region (ROLLE *et al.*, 2011a).


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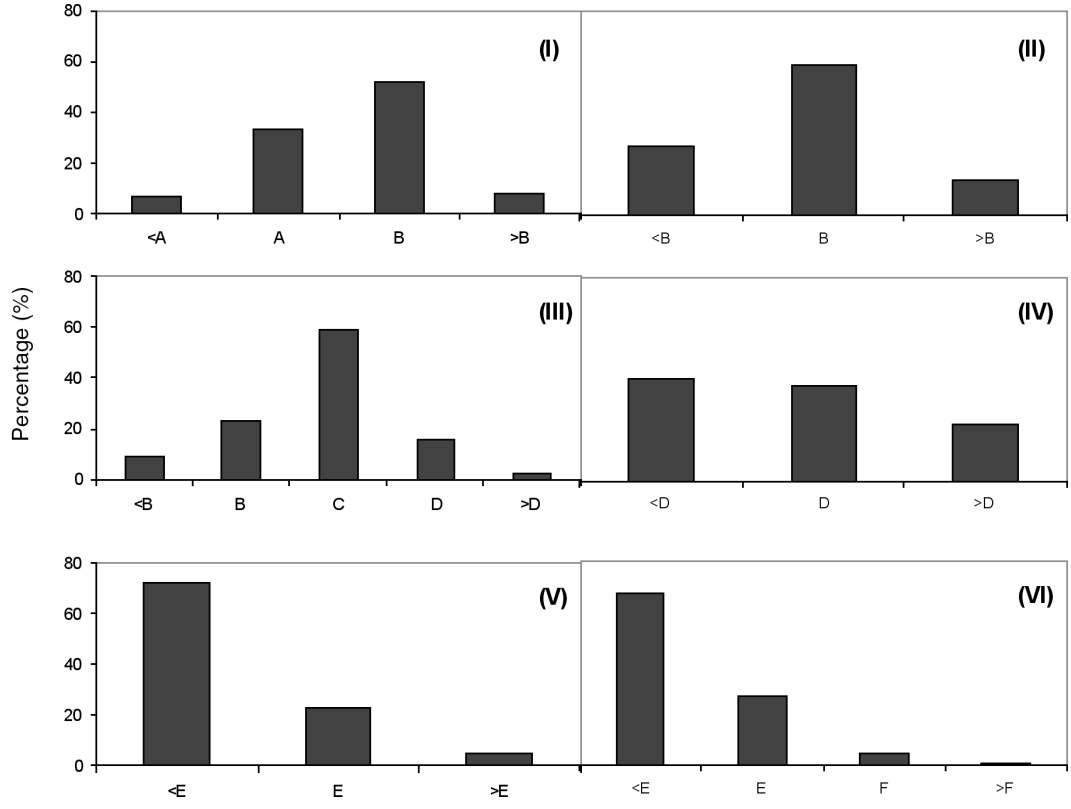



**Figure 3. Distribution (in percentage) of Cabernet-Sauvignon grapes in different density classes (A-F) at six sampling dates (I-VI). A = 1081 kg.m$^{-3}$, B = 1088 kg.m$^{-3}$, C = 1094 kg.m$^{-3}$, D = 1100 kg.m$^{-3}$, E = 1107 kg.m$^{-3}$, and F = 1115 kg.m$^{-3}$.**

**Table 2. Technological maturity parameters (average values) of non-sorted (UnS) and density-sorted (A to E) Cabernet-Sauvignon grapes harvested at different ripening stages.**

| Harvest date | Density class | °Brix | Titratable acidity (g.L$^{-1}$ tartaric acid ) | pH |
|---|---|---|---|---|
| I | UnS | 20.2 | 9.05 | 3.14 |
| | A | 19.8 | 9.99 | 3.13 |
| II | UnS | 21.0 | 8.80 | 3.13 |
| | B | 20.8 | 7.13 | 3.24 |
| III | UnS | 21.5 | 6.14 | 3.31 |
| | C | 21.6 | 6.78 | 3.29 |
| IV | UnS | 22.9 | 5.69 | 3.42 |
| | D | 23.1 | 5.95 | 3.36 |
| V | UnS | 22.4 | 5.13 | 3.56 |
| | E | 24.2 | 4.92 | 3.55 |
| VI | UnS | 22.7 | 5.92 | 3.34 |
| | E | 24.3 | 6.08 | 3.36 |

All data are expressed as average value (n = 3). Density class : A = 1081 kg.m$^{-3}$, B = 1088 kg.m$^{-3}$, C = 1094 kg.m$^{-3}$, D = 1100 kg.m$^{-3}$ and E = 1107 kg.m$^{-3}$.


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## 2. Phenol content and extractability

Berry seeds were characterized in terms of extractable and non-extractable phenol content. The results obtained for the sorted Cabernet-Sauvignon grapes harvested at six different ripening stages are shown in Table 3. For seed extractable phenol content (using solution $A_1$ containing 12 % ethanol and 7-day maceration ; $A_{1,280nm}$), no significant difference was found among the different density classes. The extended maceration of the same seeds (15 days more using solution $A_2$ containing a higher ethanol content (70 %) ; $A_{2,280nm}$) permitted the extraction of a lower "remaining phenol content" for the less dense grape berries. Although no significant differences were observed in total phenol content among density classes after the seed treatment with both solutions $A_1$ and $A_2$ ($A_{tot,280nm}$), an increasing trend was found with the ripening process. On the other hand, the results were also expressed as phenol content per seed weight and, in that case, the increasing trend was more clearly evidenced in the extractable, non-extractable and total phenol content. Furthermore, the same evolution was observed for phenol content per berry weight (data not shown). This disagrees with the phenolic composition changes reported by some authors during seed development (KENNEDY *et al*., 2000a ; MATEUS *et al*., 2001 ; DOWNEY *et al*., 2003 ; CADOT *et al*., 2006 ; LORRAIN *et al*., 2011). They suggested that flavan-3-ols reach their highest concentration at veraison, after which they decline slowly until close to grape ripeness but thereafter remain relatively constant. However, these last studies were performed using non-sorted grape berries and, therefore, it is possible that significant differences were not observed during ripening as consequence of the physiological heterogeneity within each grape sample. Some authors confirmed that the berry size is related with the seed phenolic composition : the larger berries, apart from the higher contribution of seeds to total berry weight, have a higher content of seed total flavonoids (BARBAGALLO *et al*., 2011).

The seed phenol extractability showed no trend with the ripening process. KONTOUDAKIS *et al*. (2011) also reported an increase in the contribution of seeds to wine proanthocyanidin (condensed tannins) concentration when lower density grapes were used. Unripe seeds generally release longer tannins and, therefore, produce wines that are more astringent (DEL LLAUDY *et al*., 2008).

Proanthocyanidins, which are constituted of flavan-3-ol monomer sub-units, attract much attention from a red wine type and quality perspective. Seed proanthocyanidins have a low mean degree of polymerization (mDP), and they are characterized by a relatively high proportion of (+)-catechin, (-)-epicatechin, and (-)-epicatechin-3-O-gallate, while (-)-epigallocatechin is generally not found (KENNEDY *et al*., 2000a ; OBREQUE-SLIER *et al*., 2010). Since cultural and environmental factors influence flavanol accumulation and decreasing galloylation in seed tannins favours a higher or quicker tannin extraction, the extractability may be also improved by managing the cell-wall composition and thickness in the vineyard (MATTIVI *et al*., 2009 ; HANLIN *et al*., 2010).

## 3. Instrumental mechanical properties

The instrumental mechanical properties of berry seeds for the sorted Cabernet-Sauvignon grapes harvested at six different dates during ripening are shown in Table 4. No significant change in the parameters characterizing the seed hardness ($F_s$ and $W_s$) was observed with increasing berry density. During ripening, the colour of grape seeds changes from green to brown with a concomitant hardening of the seed coat (KENNEDY *et al*., 2000b ; CADOT *et al*., 2006). No hardening of the grape seeds was reported in Barbera grapes during the sugar accumulation from 235 to 269 $g.L^{-1}$ (TORCHIO *et*

**Table 3. Seed weight and seed phenol content and extractability of sorted Cabernet-Sauvignon grapes harvested at different ripening stages.**

| Harvest date | Density class[a] | Seed weight (mg) | $A_{1,280nm}$ | $A_{2,280nm}$ | $A_{tot,280nm}$ | Phenol extractability (%) | $A_{1,280nm}$ / seed weight ($mg^{-1}$) | $A_{2,280nm}$ / seed weight ($mg^{-1}$) | $A_{tot,280nm}$ / seed weight ($mg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| I | A | $38.4\pm4.1^{b}$ | 34.8±5.0 | 7.5±1.2 | 42.3±5.4 | $82.2\pm2.8^{b}$ | $0.90\pm0.11^{ab}$ | $0.19\pm0.03^{a}$ | $1.10\pm0.12^{ab}$ |
| II | B | $38.9\pm6.1^{b}$ | 32.4±7.2 | 8.5±2.4 | 41.9±8.8 | $79.1\pm4.3^{a}$ | $0.84\pm0.14^{a}$ | $0.22\pm0.04^{b}$ | $1.05\pm0.16^{a}$ |
| III | C | $38.6\pm5.3^{b}$ | 33.7±6.2 | 8.5±1.8 | 42.2±6.9 | $79.7\pm4.6^{a}$ | $0.88\pm0.16^{ab}$ | $0.22\pm0.03^{b}$ | $1.10\pm0.15^{ab}$ |
| IV | D | $38.5\pm3.3^{b}$ | 33.5±3.2 | 7.8±1.3 | 41.2±3.5 | $81.1\pm2.8^{ab}$ | $0.87\pm0.08^{ab}$ | $0.20\pm0.03^{ab}$ | $1.07\pm0.08^{ab}$ |
| V | E | $34.9\pm5.0^{a}$ | 34.7±5.3 | 8.4±2.5 | 43.1±7.0 | $80.6\pm3.9^{ab}$ | $1.01\pm0.15^{c}$ | $0.24\pm0.05^{c}$ | $1.24\pm0.16^{c}$ |
| VI | E | $38.2\pm5.2^{b}$ | 35.7±5.0 | 7.9±1.6 | 43.6±6.2 | $81.9\pm2.1^{b}$ | $0.93\pm0.08^{bc}$ | $0.21\pm0.03^{ab}$ | $1.14\pm0.09^{b}$ |
| | | ** | ns | ns | ns | *** | *** | *** | *** |

All data are expressed as average value ± standard deviation (n = 40). Different letters within the same column indicate significant differences among harvest dates (Tukey-b test ; $p < 0.05$). **,*** and ns indicate significance at $p < 0.01$, 0.001 and not significant, respectively.
$A_{1,280nm}$ = extractable phenol content, $A_{2,280nm}$ = non-extractable phenol content, $A_{tot,280nm} = A_{1,280nm} + A_{2,280nm}$. [a]A = 1081 $kg.m^{-3}$, B = 1088 $kg.m^{-3}$, C = 1094 $kg.m^{-3}$, D = 1100 $kg.m^{-3}$, E = 1107 $kg.m^{-3}$.


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**Table 4. Seed mechanical properties of sorted Cabernet-Sauvignon grapes harvested at different ripening stages.**

| Harvest date | Density class[a] | $F_s$ (N) | $W_s$ (mJ) | $E_s$ ($N.mm^{-1}$) | $DI_s$ (%) |
|---|---|---|---|---|---|
| I | A | 44.5±7.8 | 11.9±3.2 | 68.7±15.4[a] | 31.4±5.7 |
| II | B | 42.2±9.1 | 11.8±4.0 | 70.1±13.1[ab] | 29.7±5.9 |
| III | C | 45.7±7.9 | 12.6±3.5 | 77.2±13.9[bc] | 30.2±4.9 |
| IV | D | 47.5±9.4 | 12.6±3.4 | 83.2±13.5[c] | 28.2±4.2 |
| V | E | 44.6±9.2 | 12.3±5.3 | 81.0±14.0[c] | 28.9±7.6 |
| VI | E | 46.1±7.3 | 12.7±3.6 | 77.4±10.9[bc] | 29.8±4.2 |
| | | ns | ns | *** | ns |

All data are expressed as average value ± standard deviation (n = 40). Different letters within the same column indicate significant differences among harvest dates (Tukey-b test; $p < 0.05$). *** and ns indicate significance at $p < 0.001$ and not significant, respectively. $F_s$ = berry seed break force, $W_s$ = berry seed break energy, $E_s$ = berry seed Young´s modulus of elasticity, $DI_s$ = berry seed deformation index (distance of seed break point/seed height x 100). [a]A = 1081 $kg.m^{-3}$, B = 1088 $kg.m^{-3}$, C = 1094 $kg.m^{-3}$, D = 1100 kg.m-3, and E = 1107 $kg.m^{-3}$.

**Table 5. Correlation factors between seed mechanical properties and seed phenol content and extractability of sorted Cabernet-Sauvignon grapes harvested at different ripening stages.**

| Parameter | $F_s$ (N) | $W_s$ (mJ) | $E_s$ ($N.mm^{-1}$) | $DI_s$ (%) |
|---|---|---|---|---|
| $A_{1,280nm}$ | 0.104 | 0.108 | 0.001 | 0.024 |
| $A_{2,280nm}$ | 0.158* | 0.037* | 0.257** | -0.178** |
| $A_{tot,280nm}$ | 0.134* | 0.102 | 0.076 | -0.032 |
| $A_{1,280nm}$/seed weight ($mg^{-1}$) | -0.105 | -0.027 | -0.190** | 0.160* |
| $A_{2,280nm}$/seed weight ($mg^{-1}$) | 0.048 | -0.028 | 0.218** | -0.146* |
| $A_{tot,280nm}$/seed weight ($mg^{-1}$) | -0.086 | -0.018 | 0.122 | -0.113 |
| Extractability (%) | -0.090 | -0.036 | -0.260** | 0.196** |

* and ** indicate significance at $p < 0.05$ and 0.01, respectively. $F_s$ = berry seed break force, $W_s$ = berry seed break energy, $E_s$ = berry seed Young´s modulus of elasticity, $DI_s$ = berry seed deformation index (distance of seed break point/seed height x 100), $A_{1,280nm}$ = extractable phenol content, $A_{2,280nm}$ = non-extractable phenol content, $A_{tot,280nm} = A_{1,280nm} + A_{2,280nm}$.

*al*., 2010), and a decrease in the hardness of Mondeuse grape seeds was even observed throughout the on-vine drying process (ROLLE *et al*., 2009a). On the other hand, the Es increased with berry density. In this sense, during ripening the berry seeds do not tend to harden but to stiffen. By contrast, the seed springiness, evaluated by Young's modulus parameters, progressively increases throughout the on-vine drying process (ROLLE *et al*., 2009a).

A correlation study was performed to assess the potential of instrumental mechanical properties of berry seeds to predict the phenol content and extractability during ripening. Although significant correlations were reported at a significance level of $p < 0.01$ (Table 5), correlation factors higher than 0.25 were only found between the $E_s$ and non-extractable phenol content and between the $E_s$ and phenol extractability. These correlations highlighted that springier seed tissues greatly increase phenol extractability, suggesting that a higher amount of seed phenols would be released into the wine. This could provide valuable information on the phenolic quality of the grape berries that arrive at the winery to produce wines with certain organoleptic characteristics.

Instrumental texture parameters are considered predictors of anthocyanin extractability, particularly the berry skin thickness (RIO SEGADE *et al*., 2011). Furthermore, the berry skin break force allows to estimate anthocyanin extraction kinetics with adequate reliability (ROLLE *et al*., 2012). However, this is the first time that the mechanical properties of berry seeds are assessed as predictors of phenol content and extractability.

The correlation factors obtained for phenol extractability in berry seeds were lower than those for anthocyanin extractability in berry skins because total phenol content in the berry seed extracts was globally determined by spectrophotometric measurement at 280 nm. Therefore, it is necessary to study in depth the correlations of the seed mechanical properties with the different chemical compounds belonging to flavanols like proanthocyanidins, oligomers and monomers (catechin,


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epicatechin and epicatechin-3-O-gallate). A deeper knowledge of the changes in the cell-wall composition, which ultimately impacts on the release of many phenolic compounds from seeds into the wine, is also required to maximize the potential of the instrumental texture analysis in the prediction of the seed phenol extractability (BINDON *et al.*, 2010).

## 4. FT-NIR analysis

Table 6 shows the statistical parameters of the calibration equations for predicting the seed phenol content and extractability in sorted Cabernet-Sauvignon grapes harvested at different physiological stages. The calibration equations were developed using PLS regression from berry seeds treated with different extraction solutions ($A_1$ and $A_2$) and analyzed by UV spectroscopy (reference method), and from intact seeds analyzed by NIR spectroscopy. Moderately good calibration statistical descriptors were obtained for the prediction of total phenol content, with correlation coefficients (R) higher than 0.75 and standard errors of calibration (SEC) lower than 9 %. Therefore, the best calibration model corresponded to total phenol content ($R_c$ = 0.81, SEC = 8.0 %). Conversely, the worst one was reported for phenol extractability ($R_c$ = 0.66, SEC = 2.8 %).

An external validation (Table 7) was also performed to assess the robustness of the calibration models using samples that did not belong to the calibration group. The calibration equations obtained were applied and the values determined by the reference method were compared with those predicted by the NIR calibration after the removal of outliers. In general, values of SEP lower than 20 % are considered acceptable for most analytical purposes, which indicate the suitability of the NIR calibrations to predict the phenol content and extractability in intact berry seeds (COZZOLINO *et al.*, 2008). The differences between the reference and NIR methods were smaller for total phenol content per seed weight ($R_v$ = 0.65, SEP ~ 8 %). In the case of the extractable phenol content ($A_1$), minor differences between the two methods were found ($R_v$ = 0.63, SEP ~ 10 %). The highest values of SEP % were associated with the non-extractable phenol content ($A_2$), whereas the lowest ones of $R_v$ corresponded to the phenol extractability prediction.

**Table 6. Calibration statistical descriptors of NIR models in intact berry seeds for seed phenol content and extractability of sorted Cabernet-Sauvignon grapes harvested at different ripening stages.**

| Chemical parameter | n | Mean | Minimum | Maximum | $R_c$ | SEC | SEC (%) |
|---|---|---|---|---|---|---|---|
| $A_{1,280nm}$ | 132 | 34.3 | 17.4 | 51.6 | 0.69 | 3.7 | 10.8 |
| $A_{2,280nm}$ | 125 | 8.1 | 3.7 | 13.8 | 0.80 | 1.1 | 13.4 |
| $A_{tot,280nm}$ | 132 | 42.1 | 23.2 | 60.2 | 0.81 | 3.4 | 8.0 |
| $A_{1,280nm}$/seed weight ($mg^{-1}$) | 131 | 0.914 | 0.582 | 1.466 | 0.75 | 0.090 | 9.8 |
| $A_{2,280nm}$/seed weight ($mg^{-1}$) | 129 | 0.212 | 0.133 | 0.324 | 0.59 | 0.025 | 12.0 |
| $A_{tot,280nm}$/seed weight ($mg^{-1}$) | 124 | 1.126 | 0.771 | 1.731 | 0.75 | 0.094 | 8.3 |
| Extractability (%) | 129 | 81.0 | 72.8 | 89.7 | 0.66 | 2.30 | 2.8 |

n = number of samples, $R_c$ = correlation coefficient of calibration, SEC = standard error of calibration, SEC (%) = (SEC/Mean) x 100, $A_{1,280nm}$ = extractable phenol content, $A_{2,280nm}$ = non-extractable phenol content, $A_{tot,280nm} = A_{1,280nm} + A_{2,280nm}$.

**Table 7 - Validation statistical descriptors of NIR models in intact berry seeds for seed phenol content and extractability of sorted Cabernet-Sauvignon grapes harvested at different ripening stages.**

| Chemical parameter | n | Mean | Minimum | Maximum | $R_v$ | SEP | SEP (%) |
|---|---|---|---|---|---|---|---|
| $A_{1,280nm}$ | 64 | 33.6 | 22.8 | 46.4 | 0.636 | 3.7 | 10.9 |
| $A_{2,280nm}$ | 62 | 8.0 | 5.4 | 13.0 | 0.756 | 1.2 | 14.5 |
| $A_{tot,280nm}$ | 63 | 41.3 | 28.7 | 53.1 | 0.776 | 3.4 | 8.3 |
| $A_{1,280nm}$/seed weight ($mg^{-1}$) | 62 | 0.909 | 0.642 | 1.177 | 0.634 | 0.089 | 9.8 |
| $A_{2,280nm}$/seed weight ($mg^{-1}$) | 60 | 0.212 | 0.165 | 0.297 | 0.532 | 0.024 | 11.3 |
| $A_{tot,280nm}$/seed weight ($mg^{-1}$) | 60 | 1.124 | 0.870 | 1.388 | 0.654 | 0.088 | 7.8 |
| Extractability (%) | 61 | 81.0 | 75.4 | 86.1 | 0.496 | 2.5 | 3.1 |

n = number of samples, $R_v$ = correlation coefficient of validation, SEP = standard error of prediction, SEP (%) = (SEP/Mean) x100, $A_{1,280nm}$ = extractable phenol content, $A_{2,280nm}$ = non-extractable phenol content, $A_{tot,280nm} = A_{1,280nm} + A_{2,280nm}$.


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The number of seeds analyzed was lower than that reported in other previously published work (FERRER-GALLEGO *et al*., 2010) because the use of sorted berries reduces the physiological heterogeneity within each sample.

Best results were found by FERRER-GALLEGO *et al*. (2010) in the assessment of flavanol monomers, dimers, trimers and tetramers of Graciano grape seeds. On the other hand, other authors reported that anthocyanins extractable at pH 1.0 and pH 3.2 are well predicted by the NIR spectra of intact whole berries in the case of Syrah grapes, whereas they cannot be predicted in other varieties like Cabernet-Sauvignon, Merlot and Carmenère (KEMPS *et al*., 2010). Furthermore, COZZOLINO *et al*. (2004b) also showed unsuitable low values of R for the estimation of total anthocyanins (colour) in intact grape berries.

KEMPS *et al*. (2010) did not achieve satisfactory results for the prediction of the phenol content in intact grape berries using NIR spectroscopy when the phenol content was determined as total phenol index ($mg.L^{-1}$). Nevertheless, this was accurately predicted in intact whole berries and skins when the reference values of phenolic compounds were determined by HPLC-DAD-MS (FERRER-GALLEGO *et al*., 2011). As a whole, these studies suggest that the FT-NIR calibration models, proposed to estimate the phenol content and extractability in intact berry seeds, could be improved by using this last analytical approach as reference method. Although more research is necessary in this field, the NIR spectrum can be considered a useful indicator of the phenol content in intact berry seeds.

## CONCLUSIONS

The winemakers need to assess the grape phenolic ripeness with the aim of classifying grape berries in function of their real quality, and of maximizing the profitability of the phenolic potential achieved in the vineyard through an adequate harvest date selection and winemaking technique. The implementation of simple, reliable, fast and reasonable cost analytical procedures to determine the compositional changes occurring in seed phenols during grape ripening is of paramount importance for the quality of the future wine. Since berry seeds become more rigid during grape ripening, instrumental texture analysis could be a way for a timely and cost-efficient prediction of phenol extractability in berry seeds. However, the significant but lower correlation coefficient suggests continuing with the study in order to improve the method. On the other hand, NIR spectroscopy can be used as a rapid, non-destructive and cost-efficient alternative technique for the prediction of the phenol content in intact berry seeds. Since the statistical descriptors were poor in the phenol content range assessed, an effort has yet to be thoroughly made to improve the prediction models and to evaluate the influence of other factors, such as production area and grape variety, on the development of these models.

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