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RESEARCH

Insight on Tannin Extraction and Mechanical Changes During Maceration from Skins and Seeds of Italian Red Grape Varieties

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Abstract
This study investigated tannin extraction in four Italian red grape varieties — 'Aglianico', 'Nebbiolo', 'Primitivo', and 'Sangiovese'. The grape initial tannin content was characterized through a potential extraction. Moreover, the extractable phenolic content was evaluated through maceration in wine-like solution of skins, seeds, and their combinations for 10 days, with ethanol concentration incrementally adjusted to simulate fermentation. Texture analysis of grape seeds and skins was conducted before and after the wine-like solution macerations. Results revealed variety-dependent differences in the mechanical and acoustic properties of grape skins and seeds, with seeds showing increased acoustic energy upon breakage. Grape varieties showed differences in skin and seed phenolic pools and extractability. Significant positive correlations were found between potential and extractable tannin content. Smaller, less galloylated flavan-3-ols were well extracted in model wine solutions. Extraction curves were examined from skins, seeds, and combined skins + seeds matrices, evidencing a faster skin phenolics extraction than seeds. Interestingly, tannin extraction from skins + seeds did not correspond to the sum of individual skin and seed extractions. Moreover, the skin-to-seed tannin ratio and tannin structural characteristics also varied between skin and seed joint and separate extractions.

Keywords Grape proanthocyanidins · Tannin extraction curves · Phenolic compounds · Simulated maceration

Introduction

Flavanols (or flavan-3-ols) have an important role in determining wine quality because they are responsible for astringency and bitterness (Paissoni et al., 2023; Vidal et al., 2003). Flavanols are constituted by an aromatic ring (A ring) fused to a pyran ring (C ring) characterized by the presence of a hydroxyl group at the 3-position, and another aromatic ring (B ring) attached to the C ring with a single bond. Four diastereoisomers are possible due to the presence of two chiral centers on the C ring (positions 2 and 3). Various forms of flavanols exist, depending on their stereoisomer, the number of hydroxyl groups on the B ring, and the presence of a gallate ester group on the 3-position of the C ring (Waterhouse, 2002).

Grape flavan-3-ols can be found in wine as monomers, oligomers, and polymers. The latter forms are known as proanthocyanidins (PAs) or condensed tannins and are classified into two groups depending on the type of flavan-3-ol subunits: procyanidins, which contain (+)-catechin and (−)-epicatechin subunits, and prodelphinidins composed of (+)-gallocatechin and (−)-epigallocatechin subunits. They are mostly associated with the astringency perception in wine, due to the formation and subsequent precipitation of tannin-salivary proteins complex that takes place in the mouth (Sarni-Manchado et al., 1999). Condensed tannins' sensory properties depend not only on their concentrations but also on their chemical structure, mean degree of polymerization, degree of galloylation, and B-ring trihydroxylation (Ma et al., 2014). Several compositional differences are present according to tannin origin, i.e., grape seed or skin tannins: while both procyanidins and prodelphinidins are found in grape skins, only procyanidins can be detected in seeds. Moreover, unlike seed tannins, which are characterized by a non-specific terminal and extension subunit

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nature, skin PAs contain (+)-catechin as terminal units and (–)-epicatechin as extension subunits (Downey et al., 2003). In addition, tannins extracted from skins have a higher mean degree of polymerization (mDP) and are less galloylated (i.e., percentage of monomers with gallate esters) compared to those extracted from seeds. For these reasons, seed and skin tannins are associated with different sensory perceptions (Vidal et al., 2003).

Grape skin and seed tannin content, and its extraction during winemaking, is of primary importance in determining the wine tannin composition and concentration. Previous authors studied the contribution of seed and skin tannins to wine tannin content, but contrasting information has been found in the current literature. Some studies indicate a predominance of skin tannins in wine (Busse-Valverde et al., 2012; Peyrot Des Gachons & Kennedy, 2003; Sparrow et al., 2015) and some acknowledge that seeds strongly impact the wine tannin content (Bautista-Ortín et al., 2014; Kovac et al., 1995), while other authors found that seeds may bring more PAs in wine than skins (Kennedy, 2008; Rousserie et al., 2020). According to other studies (Bautista-Ortín et al., 2016a; Bindon et al., 2014), it is difficult to assess the correlation between the wine tannin concentration and the total amount of tannins in skins and seeds; therefore, the role of grape skins and seeds in determining the wine tannin composition remains unclear. Concerning extractability, tannins are differently extracted according to their tissue location: they are extracted faster from skins, while seeds need to be hydrated to release proanthocyanidins (Rousserie et al., 2019). Tannin extraction is also influenced by grape cell wall material, which varies among grape varieties (Ortega-Regules et al., 2008; González-Centeno et al., 2010). Cell wall components, primarily composed of proteins and polysaccharides, can act as a barrier to tannin release or actively bind tannins, thereby limiting their extraction (Bautista-Ortín et al., 2015). Additionally, during red wine maceration, the simultaneous extraction of anthocyanins may further impact tannin extraction efficiency. Anthocyanins compete with tannins for cell-wall adsorption sites, thereby enhancing tannin concentration in the final product (Bautista-Ortín et al., 2016b).

The extraction of seed tannins does not necessarily require ethanol in the media, although its presence can enhance the extraction rate by damaging the seed's lipidic layers (Hernández-Jiménez et al., 2012). For this reason, previous studies highlighted that long macerations may result in wine with a higher seed PA concentration due to the more intense seed hydration taking place (Busse-Valverde et al., 2012). Conversely, the simultaneous extraction of tannins and polysaccharides during maceration can reduce tannin levels over time, as these compounds may bind together and precipitate (Jones-Moore et al., 2022). Red grape varieties show differences in the tannin concentration of grape seeds and skins (Mattivi et al., 2009); thus, the variety is certainly one of the most important factors in determining the tannin content and composition of wine. Therefore, understanding varietal differences in potential grape tannin content and phenolic extraction curves during maceration may provide valuable information to adapt winemaking techniques for producing monovarietal wines, as well as blend products obtained from them. This study aimed to better understand tannin extraction processes from four red winegrape varieties known for their peculiar tannin content expressed in monovarietal wines (Giacosa et al., 2021), by first assessing the potential tannin extraction from the individual grape components (skins, seeds) and then evaluating how the extraction from skins and seeds can influence each other during simultaneous maceration in a model wine solution. Furthermore, the mechanical properties of skins and seeds were evaluated before and after maceration to understand possible textural changes in relation to the phenolic extraction. This study was performed as a part of a national project aimed at understanding the tannin diversity of Italian monovarietal wine (D-Wines collaboration) concerning spectroscopic, sensory, volatile, and macromolecular traits, as well as their extraction from grapes.

Materials and Methods

Sample Collection, Preparation, and Density Sorting

Ten kilograms each of grapes cv. 'Aglianico', 'Nebbiolo', 'Primitivo', and 'Sangiovese' (*Vitis vinifera* L.) from the 2019 vintage were harvested at ripeness from the ampelographic collection of Grinzane Cavour (Piemonte, northwestern Italy) on September 29, October 17, September 26, and September 23, respectively. The juice composition at harvest (unsorted berries) was evaluated, after manual crushing of grapes and centrifugation of the resulting juice (2700 × g at 20 °C for 15 min; Hettich 32R, Tuttlingen, Germany), using a refractometer for total soluble solids (TSS, °Brix) quantification (Palette 0–32, Atago Co. Ltd., Tokyo, Japan), by titration for total acidity determination (OIV-MA-AS313-01 official method; OIV, 2016), and by potentiometry for pH determination using an InoLab 730 calibrated pH meter (WTW, Weilheim, Germany), according to the OIV-MA-AS313-15 method (OIV, 2016). The grape juices showed TSS values of 21.0, 24.2, 21.8, and 22.2°Brix, respectively, for 'Aglianico', 'Nebbiolo', 'Primitivo', and 'Sangiovese', 11.3, 7.4, 6.5, and 5.1 g/L as tartaric acid, respectively, for total acidity, and pH 2.94, 3.14, 3.27, and 3.48, respectively.

The sample used for the following determinations (instrumental mechanical properties and phenolic extractions) derived from a grape selection based on density: this operation allows to limit variability thus obtaining homogeneous

batches in terms of grape ripeness, with an effect also on mechanical and phenolic traits (Rolle et al., 2012). The bunches were manually destemmed and the resulting berries sorted according to the method proposed by Fournand et al. (2006). To cover the typical density range of berries at harvest, eight saline solutions were prepared containing from 130 to 190 g of sodium chloride/L; then, berries were sorted according to their density. For each cultivar, the berries of the most represented class (1092 kg/m^3 for 'Aglianico', 'Primitivo', and 'Sangiovese'; 1100 kg/m^3 for 'Nebbiolo') were selected corresponding to the 23.8%, 25.2%, 35.5%, and 23.3% for 'Aglianico', 'Primitivo', 'Sangiovese', and 'Nebbiolo', respectively, of the total grape berries weight (w/w). The selected berries were washed and then used for the following procedures.

Instrumental Mechanical Properties and Acoustic Emission During Seed Compression

Berry skin and seed mechanical properties (texture analysis) were assessed on peeled grape skins obtained from fresh berries, as well as after the 10-day simulated maceration (described in the next sections), considering 30 replicate measurements (skins or seeds) for each sample. Mechanical properties were evaluated with a Universal Testing Machine TA.XTplus Texture Analyzer (Stable Micro System, Godalming, Surrey, UK) equipped with an HDP/90 platform and different probes from the same manufacturer, which were selected based on the specific test being performed. Berry skin break force (F_{sk}) on fresh and macerated material was evaluated by performing a puncture test, using a P/2N needle probe with a test speed of 1 mm/s and a 5 kg load cell (Giacosa et al., 2015; Letaief et al., 2008). F_{sk} corresponded to the maximum force opposed by grape skin tissues to probe penetration and was expressed as N. In addition, seed break force (F_s) was assessed by individually compressing intact grape seeds (either obtained from fresh berries or analyzed after maceration, in all cases cleaned with absorbent paper before analysis) using a P/35 probe and a 50 kg load cell, at a test speed of 1 mm/s. Seed acoustic properties were simultaneously evaluated during the compression test using an acoustic envelope detector (AED, Stable Micro Systems) equipped with a 12.7-mm diameter Brüel and Kjær 4188-A-021 microphone (Nærum, Denmark). The acoustic detection instrumental settings were those reported by Torchio et al. (2012) with the following modifications: gain setting to 24 dB and using envelope corner frequency filter of 2 kHz. For each instrumental acquisition, two acoustic parameters were evaluated at seed breakage: acoustic emissions (dB) and acoustic energy (AE, dB × mm). All data were acquired at 500 points per second and integrated using the Texture Exponent software (Stable Micro Systems).

Skin and Seed Potential Phenolic Extraction

The potential phenolic content (i.e., the maximum extractable amount under winemaking condition) was assessed using an SO_2-rich extracting solution (5 g of tartaric acid/L, 12% v/v ethanol, 2 g of sodium metabisulphite/L, and adjusted to pH 3.20 with NaOH 1 mol/L; Di Stefano & Flamini, 2008) either for skin or seed material. For each variety, three replicates of ten berries each (representative of the average berries weight, Table S1) were randomly selected among density-sorted berries. Skins and seeds were manually separated from pulp, gently removing the residual pulp on the inner face of the peeled skin with a spatula. All the skins recovered from ten selected berries were immediately immersed, one by one in 40 mL of the hydroalcoholic extracting solution, and then frozen at − 20 °C until analysis. Grape skin samples were then macerated for 4 h and thawed and homogenized at 8000 rpm for 1 min with a T25 Ultra-Turrax immersion blender (IKA, Staufen, Germany). After centrifugation (2700 × g for 5 min at 20 °C; Hettich 32R), the supernatant was collected and diluted to 50 mL using the hydroalcoholic extracting solution. All the grape seeds recovered from the ten selected berries were macerated in the extracting solution at 27 °C for 7 days to allow the extraction, and then the seeds were rinsed and discarded. The obtained extract was diluted to a final volume of 50 mL (to allow the possible calculations as concentration on starting grape material) using the hydroalcoholic extracting solution. The seed extracts were then used for the phenolic compositional analysis.

Grape Components Maceration in Model Wine Solution

A simulated maceration was conducted on grape components to assess their extractable phenolic profiles. To accomplish this goal, the grape components were extracted at 27 °C in 100 mL of a wine-like solution (5 g of tartaric acid/L, 100 mg of sodium metabisulphite/L, and adjusted to pH 3.40 with NaOH 1 mol/L; as reported by Paissoni et al., 2020) to mimic the effect of a real maceration. The maceration lasted for 10 days, which reflects a regular duration commonly used in the winemaking industry for red wine production. Skins (Sk), seeds (Se), and a combination of skins and seeds (Sk + Se) were extracted in this way. For each variety, 80 g of density-sorted berries was sampled (extraction ratio taken from Mattivi et al., 2002). Skins and seeds were manually isolated from berries and macerated separately (Sk and Se, respectively). For each variety, other three sets of 80 g of berries were used to obtain skins and seeds that were jointly macerated in the same model wine solution (Sk + Se). Berry skins and seeds were separated from the pulp residues with the aid of a spatula before being added to the model wine solution. The maceration was conducted following the

method reported by Paissoni et al. (2020): at each sampling point, the liquid taken (3 mL) was replaced with the same amount of absolute ethanol to simulate its increase caused by the fermentation process. Samples were taken for further analysis at 48, 72, 96, 144, and 168 h, therefore increasing by 3% v/v the ethanol concentration at each step and reaching a final concentration of 15% v/v. Samples were also taken and analyzed at the end of maceration after 240 h. The extraction took place in dark glass containers that were filled with nitrogen before sealing at each operation to prevent oxygen exposure during the simulated maceration. All extractions were prepared in triplicate.

Compositional Analyses of Phenolic Compounds

Phenolic compositional analyses were performed in all sampling points during the wine-like extraction, and on skin and seed potential extraction. Total phenolic index (TPI), total flavonoid index (TFI), and non-anthocyanin flavonoid index (NFI) were determined through spectrophotometric analysis (UV-1800, Shimadzu Corporation, Kyoto, Japan), following the method proposed by Di Stefano and Cravero (1991) and reported by Torchio et al. (2010). TPI was determined by dilution of the sample in deionized water and absorbance measurement at 280 nm, and then expressed as mg (–)-epicatechin/kg of fresh berries. TFI was determined, after interference correction, with the absorbance value at 280 nm obtained through dilution of the sample in ethanol: water:37% hydrochloric acid (70:30:1, v/v). NFI was calculated by removing the estimated anthocyanin contribution to the TFI value. TFI and NFI were expressed as mg (+)-catechin/kg of fresh berries.

The proanthocyanidins (PAs) content was determined at the end of the model wine extraction and potential extraction through the methyl cellulose precipitation assay (Sarneckis et al., 2006), while their composition was evaluated following the method proposed by Kennedy and Jones (2001). Briefly, 5 mL of extract were diluted in 20 mL of water and then subjected to solid phase extraction using a previously-activated Sep-Pak tC18 cartridge (Waters Corporation, Milford, MA, USA). The eluted methanolic fraction was evaporated to dryness (Laborota 4011, Heidolph Instruments, Kelheim, Germany) and the residue was resuspended in 1 mL of methanol. Then, 200 µL of this solution were added to 200 µL of a methanolic solution of 0.1 mol/L HCl containing 50 g/L phloroglucinol and 10 g/L ascorbic acid. The phloroglucinolysis reaction occurred for 20 min at 50 °C, and then it was stopped by adding 1 mL of 40 mmol/L aqueous sodium acetate. After filtration using a 0.45 µm PTFE membrane filter, the sample was injected into the Agilent 1260 HPLC system equipped with a LiChroCART RP-18 analytical column (250 × 4 mm, 5 µm sorbent size; Merck KGaA, Darmstadt, Germany). The analysis was performed according to Kennedy and Jones (2001), in binary gradient mode, which consisted of two mobile phases containing 1% v/v aqueous acetic acid (A) and methanol (B). The elution conditions were 1.0 mL/min, 5% B for 10 min, a linear gradient from 5 to 20% B in 20 min, and a linear gradient from 20 to 40% B in 25 min. Between two injections, the column was washed with 90% B for 10 min and 5% B for 5 min. Eluting compounds were monitored at 280 nm using a diode array detector (DAD; Agilent 1260, Agilent Technologies). Acquisition and processing were performed using the Agilent ChemStation software (Agilent Technologies) based on external calibration curves of (+)-catechin, (–)-epicatechin, (–)-epicatechin gallate, and (–)-epigallocatechin (all supplied by Extrasynthese, Genay, France). The mean degree of polymerization (mDP), degree of galloylation (%G), percentage of prodelphinidins (%PD), and the percentage of the terminal and extension subunits were calculated according to Kennedy and Jones (2001).

The proportion of skin and seed tannins in the Sk + Se joint maceration treatment was evaluated according to Peyrot Des Gachons and Kennedy (2003) as follows: skin-derived PAs were calculated as the percentage ratio between the percentage of (–)-epigallocatechin extension units of PAs present in Sk + Se joint extraction and those in Sk; then, it was possible to obtain the percentage of seed-derived PAs by subtracting this value from 100%. These proportions were used to calculate the estimated content, expressed as mg (–)-epicatechin/kg berries, of seed and skin PAs in Sk + Se joint extraction. Phloroglucinolysis reaction yield was calculated as the ratio between the total detected terminal and extension subunits and the total tannin concentration, determined based on the sample total absorbing peak area at 280 nm, with both factors expressed as mg (+)-catechin equivalents/L (Downey et al., 2012).

Statistical Analysis

Statistical analysis was carried out using R software (R Foundation for Statistical Computing, Vienna, Austria). One-way analysis of variance (ANOVA) was performed for each variable. Differences among treatments were evaluated with the Bonferroni LSD post hoc test and considered statistically significant at p-value < 0.05. Shapiro–Wilk's and Levene's tests were used to assess normality and homoscedasticity of ANOVA assumptions, respectively. When assumptions were not met, ANOVA using Welch's correction with Games-Howell post hoc test was instead applied. Statistical correlations were evaluated as Pearson coefficient (r) and considered statistically significant at p-value < 0.05.

Results and Discussion

Skin and Seed Structural Changes During Maceration

Berry skin and seed mechanical and acoustic properties before and after maceration are reported in Fig. 1. Berry skin break force (F_{sk}) was evaluated on peeled fresh skins and after Sk and Sk + Se maceration (Fig. 1A). F_{sk} of fresh skins changes according to the variety: 'Aglianico' showed the highest F_{sk} values (0.71 ± 0.12 N) while 'Primitivo' the lowest ones (0.39 ± 0.12 N). In contrast to what was expected from previous winemaking experiments (Giacosa et al., 2015), two varieties ('Aglianico' and 'Primitivo') showed a significant F_{sk} increase after the maceration (although only after mixed maceration

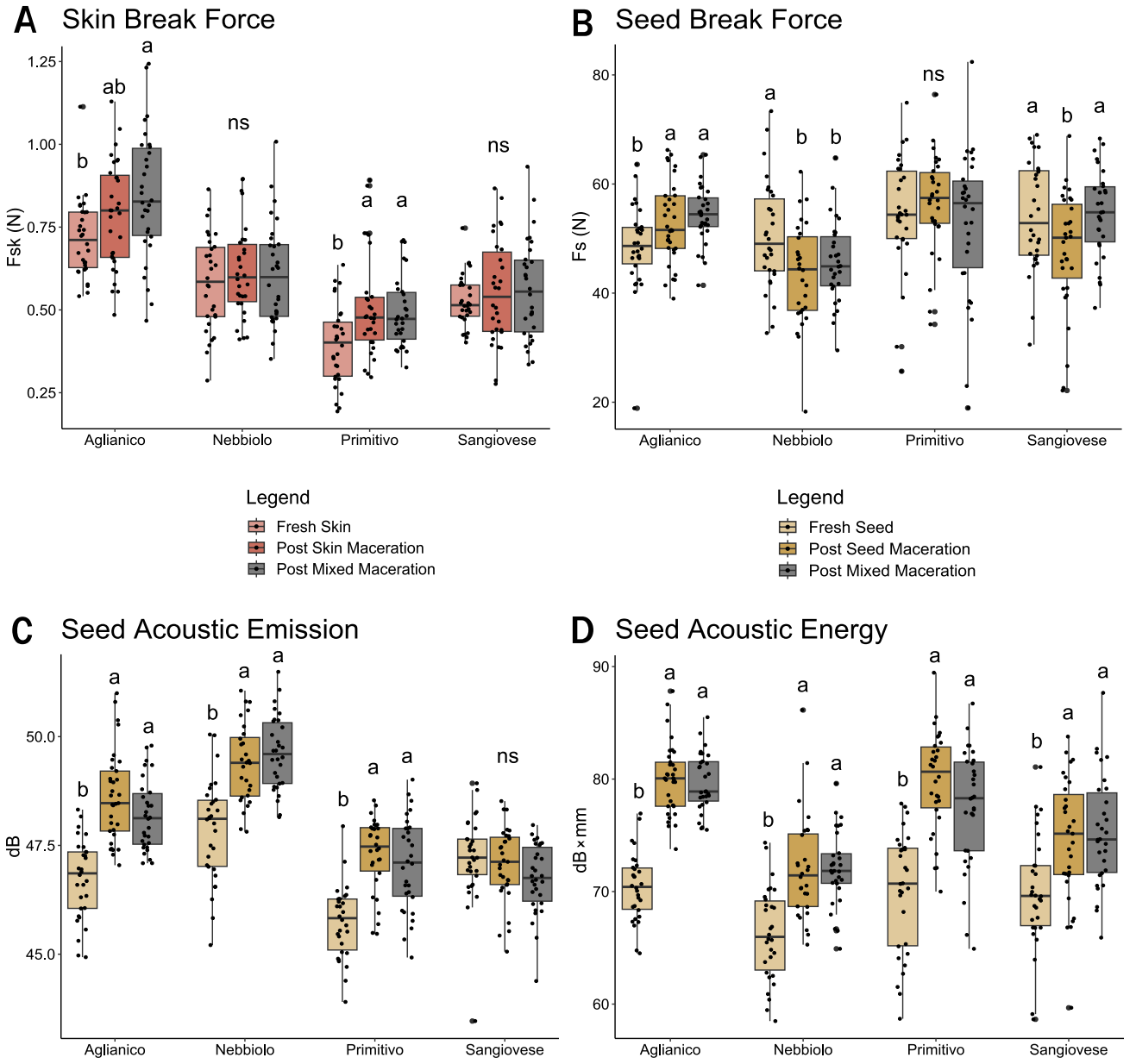

Fig. 1 Mechanical properties of grape skins, and mechanical-acoustic properties at breakage of seeds from four Italian red grape varieties. Skin break force was detected on fresh berry and after skin or jointly macerated with seeds in model wine solutions (**A**). Seed break force (**B**), acoustic emission (**C**), and acoustic energy (**D**) during compression were detected on fresh seeds and after seed-only or jointly macerated with skins. Skin and seed break force (F_{sk} and F_s, respectively) were expressed in N; acoustic emission from seed was expressed in dB, while acoustic energy as dB x mm. Data are reported as boxplots ($n = 30$). Significant differences within each variety are indicated by different letters ($p < 0.05$, one-way ANOVA with Bonferroni LSD post hoc test)

for 'Aglianico'), while 'Nebbiolo' and 'Sangiovese' did not show any difference. It should be noted that this experiment was carried out without yeast fermentation (and subsequent cap formation and management) and assesses the effect caused by the maceration; in regular winemaking, the yeast enzymatic activities present during fermentation could have an effect in damaging the skin cell walls, thus leading to a reduction of skin break force values (Giacosa et al., 2015). Concerning the variable effect among samples, this phenomenon may be due to several factors: (i) a higher hydration level that the skins get during 10 days of simulated maceration and (ii) a varietal effect induced by the different skin structural characteristics, as previously found among other red grape varieties (Ortega-Regules, et al., 2008). These different observations may lead to the conclusion that skin mechanical properties mainly depend on the variety, as previously reported by Giacosa et al. (2013).

Seed break force (F_s, Fig. 1B) changed during the maceration for 'Aglianico', 'Nebbiolo', and 'Sangiovese' without a clear trend, while no significant differences were detected in 'Primitivo' seeds. In particular, for 'Sangiovese' a difference between the two extraction trials (Sk + Se *vs* Se) was found, with the latter evidencing a significant seed break force decrease during the maceration. In general, similar to what happened for skins, it is reasonable to infer that seed mechanical properties also are a variety-related factor, as previously reported (Torchio et al., 2014).

To further examine changes during seed maceration, the acoustic emission released by seed compression, an indicator of the internal structure change with hydration, is reported in Fig. 1C. After the maceration in the model wine solution, 'Aglianico', 'Nebbiolo', and 'Primitivo' seeds generally increased their acoustic emission when compressed, although no differences were found for 'Sangiovese'. Similarly, the acoustic energy (AE, Fig. 1D) released by seeds during compression at the end of the maceration in model wine solutions was found significantly higher when compared with fresh seed measurements (i.e., higher sound emission with the compression). The observed increase in the acoustic emission and energy happened after the extraction in a hydro-alcoholic solution: this factor alone could hypothesize an increased level of hydration reached by seeds. Although specific experiences on seeds are not available in literature, on crisp food products, a higher water activity is considered to reduce significantly the acoustic emission at breakage (Lewicki et al., 2009); however, in our conditions, the seed acoustic emission after maceration increased in three cases out of four, and the acoustic energy released during compression increased in all cases (Fig. 1).

Phenolic Characterization of Skin and Seed Potential Extract

The potential contents of phenolic compounds are reported in Table 1. The use of an SO_2-rich media allowed to quantify the amount of skin and seed phenolics that may be potentially transferred from grape to wine. 'Aglianico' showed the highest TPI, TFI, and NFI values in both skin and seed extracts. Similarly, it resulted also in high values of PAs detected in skins and seeds. PAs were differently distributed between seeds and skins, and this variety had the lowest relative contribution of potential skin tannins (51.3%). 'Aglianico' skin PAs showed the lowest mDP and %PD and a high %G value. Similarly, 'Aglianico' seed PAs showed a high %G value and the lowest mDP. These results confirmed the high phenolic content of this variety reported by previous studies (Mattivi et al., 2002; Rinaldi et al., 2017) and the high astringency of its tannins due to the high galloylation rate (Rinaldi et al., 2015).

'Nebbiolo' showed a high contribution of skin PAs (59.8%). Furthermore, it had the highest mDP values in both skin and seed extracts. Skin PA concentration and mDP values are very similar to what was previously reported by Guaita and Bosso (2019). PAs extracted from 'Nebbiolo' showed the lowest G% in both skin and seed extracts, confirming Bordiga et al.'s (2011) findings, and also the highest values of %PD in skins.

'Primitivo' skin PAs did not show significant differences with those extracted from 'Nebbiolo' in terms of concentration, mDP, and %G. Additionally, 'Primitivo' seeds exhibited a concentration of PAs not significantly different to 'Nebbiolo' and 'Sangiovese'. However, 'Primitivo' seed PAs were distinguished from 'Nebbiolo' by having a lower mDP and a highest %G value.

Finally, 'Sangiovese' showed a low skin NFI value (on par with 'Primitivo'), resulting indeed in the lowest amount of skin PAs. Nevertheless, this variety reported the highest percentage of PAs in skins (66.1%), while seeds had the poorest potential phenolic content among those evaluated in terms of TPI, TFI, and PAs. However, 'Sangiovese' skin tannins showed the highest G%. Our results reflect those found in 'Sangiovese' wines as reported by Rinaldi et al. (2020).

As expected, seeds from all evaluated varieties showed lower mDP (average 20.1 in skins vs 2.5 in seeds) and higher G% values (average 2.6% in skins vs 12.6% in seeds) compared to those obtained in skin extraction, confirming general trends available in the literature for other varieties (Rousserie et al., 2019). The data on tannin composition, including terminal and extension units, and the phloroglucinolysis reaction yield can be found in Table S2. The latter showed non-significant differences for seeds among the varieties studied, whereas for skins, 'Aglianico' showed the

Table 1 Phenolic characterization of skins and seeds extracts to evaluate their potential content

		Grape skins					Grape seeds				
Parameter	Units	Aglianico	Nebbiolo	Primitivo	Sangiovese	Sign	Aglianico	Nebbiolo	Primitivo	Sangiovese	Sign
TPI	mg/kg berries	3753 ± 24 a	2219 ± 349 c	3237 ± 56 b	2871 ± 122 b	***	2546 ± 627 a	1465 ± 108 b	1150 ± 169 b	890 ± 234 b	*#
TFI	mg/kg berries	4099 ± 103 a	2958 ± 181 b	2904 ± 89 b	2987 ± 59 b	***	3801 ± 521 a	2729 ± 136 ab	2176 ± 128 b	2118 ± 233 b	*#
NFI	mg/kg berries	2865 ± 57 a	2364 ± 139 b	1985 ± 93 c	2055 ± 102 c	***	-	-	-	-	
mDP	-	15.7 ± 0.9 b	24.1 ± 3.4 a	20.7 ± 1.7 a	19.8 ± 2.5 ab	*#	2.1 ± 0.1 c	2.8 ± 0.1 a	2.5 ± 0.1 b	2.6 ± 0.1 b	***
%G	%	3.1 ± 0.2 a	1.6 ± 0.3 b	2.0 ± 0.1 b	3.6 ± 0.5 a	**	12.9 ± 0.9 ab	11.0 ± 0.3 b	13.9 ± 0.8 a	12.7 ± 1.9 ab	*#
%PD	%	19.3 ± 0.8 c	49.3 ± 3.7 a	23.2 ± 2.4 c	37.0 ± 3.5 b	***	-	-	-	-	
PAs	mg/kg berries	1189 ± 26 a	1002 ± 79 ab	1015 ± 108 ab	932 ± 122 b	*	1128 ± 154 a	677 ± 14 b	550 ± 81 b	477 ± 228 b	**

Values are presented as average ± standard deviation ($n = 3$). Sign.: *, **, and *** indicate significant differences at $p < 0.05$, 0.01, and 0.001, respectively, among varieties according to ANOVA or Welch's ANOVA (#). Values followed by different letters within the same row are significantly different (according to Bonferroni LSD or Games-Howell post hoc tests for ANOVA and Welch's ANOVA, respectively). *TPI* total phenolic index, *TFI* total flavonoids index, *NFI* non-anthocyanin flavonoids index, *mDP* mean degree of polymerization, *%PD* percentage of prodelphinidins, *%G* percentage of galloylation, *PAs* total proanthocyanidins. TPI and PAs are expressed as mg (−)-epicatechin/kg berries, while TFI and NFI are expressed as mg (+)-catechin/kg berries

highest (82.0%) and 'Sangiovese' the lowest (69.8%) reaction yield. These values agreed with the ranges previously reported in literature for *Vitis vinifera* L. grapevine varieties (Downey et al., 2012), as they can be influenced by the subunits composition as well as the ripeness level. A discrepancy between the PA and TPI values was observed in both skin and seed extracts across all grape varieties, primarily due to anthocyanins in the skins and monomeric flavan-3-ols in the seeds, which contribute to TPI but not to PA.

Polyphenolic Extraction Curve During Simulated Maceration

To understand the extraction curves of skins, seeds, and joint skins and seeds maceration, phenolic compounds were monitored through the TPI parameter during a 10-day maceration of these berry components in model wine solutions. An increasing ethanol concentration was used to simulate the increased extraction by this solvent in a regular fermentation-maceration in terms of diffusion, improvement on hydrophobic molecules release, and cuticle disgregation.

The TPI extraction curves are reported in Fig. 2. Given that the TPI index represents a general assessment of total phenolics extracted, this value is also influenced by phenolics other than PAs, such as anthocyanins extracted from skins. For all varieties, the TPI extraction from skins (Sk) reached the plateau on the 2nd day, except for 'Aglianico' whose curve slowly increased until the end of the maceration. Our results are consistent with those reported by Canals et al. (2005) who demonstrated that the extraction of total phenolics from the berry skin tends to stabilize after an initial increase, while seed phenolic compounds are continuously extracted during simulated maceration. Our results generally agreed with other studies which showed that skin PAs reached a maximum after a few days of maceration (Bautista-Ortín et al., 2016a; Mattivi et al., 2002) given that skin tannins fastly diffuse in wine thanks to their grape tissue location (Miller et al., 2019; Rousserie et al., 2020). Indeed, Busse-Valverde et al. (2012) demonstrated that skin tannins are present in higher proportion in shorter maceration as they are readily extracted. One possible explanation for the phenolic extraction slowdown in the Sk curve is the continuous tannin and polymeric pigment re-adsorption on cell wall materials (Beaver et al., 2020).

TPI constantly increased during seed maceration for all the varieties assessed. Despite the constant increase, the seed (Se) extract showed the lowest concentration of total phenolic compounds at every sampling point. As reported in the literature, grape seeds need to be hydrated to release phenolics (Hernández-Jiménez et al., 2012). Several authors reported a slow and constant phenolic extraction from seeds, which initially showed a slower diffusion rate that increased only after about halftime of

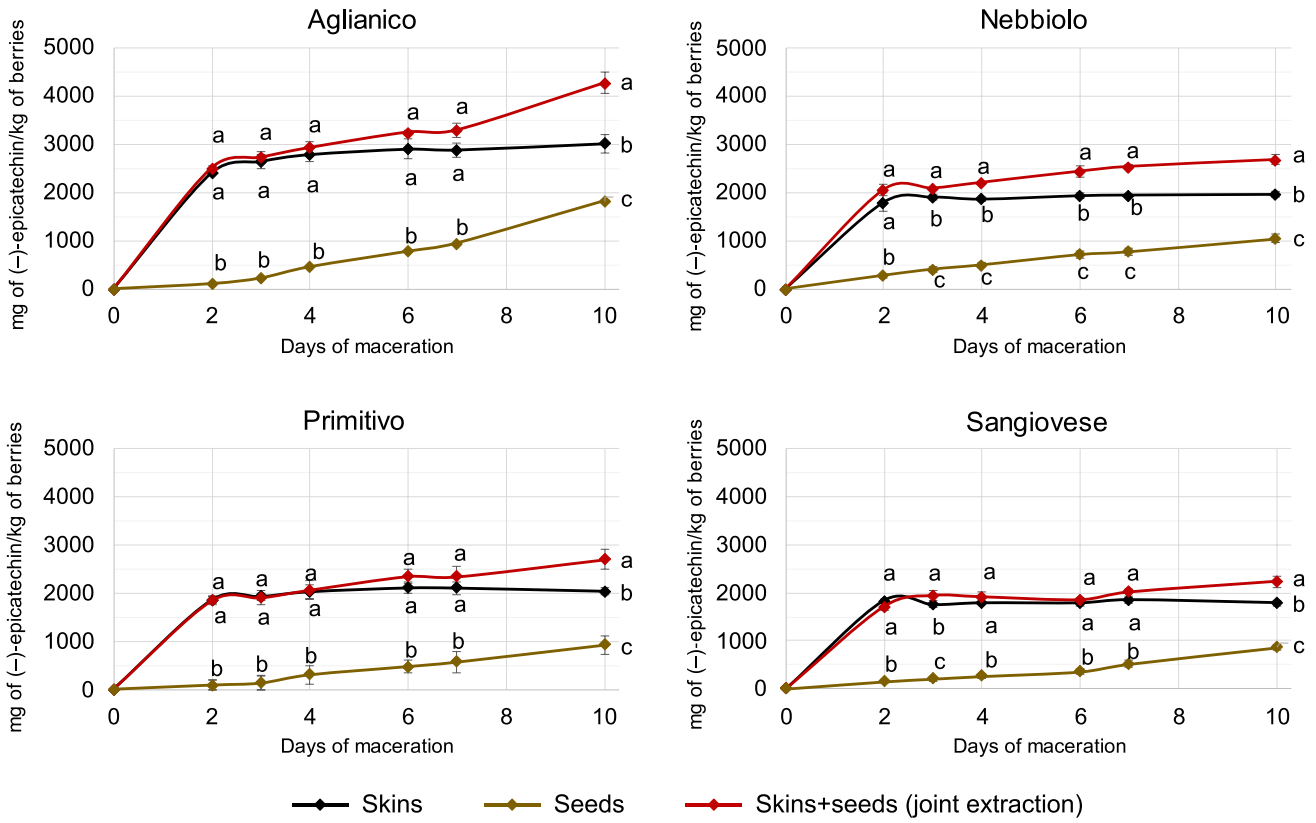

Fig. 2 Total phenolic extraction curves from skins (black), seeds (brown), and skins + seeds (red) of four Italian red grapevine varieties during 10 days of maceration in model wine solution with increasing ethanol content. Data are expressed as average values in mg (−)-epicatechin/kg berries, with bars showing the standard deviation ($n = 3$). Different letters within the same sampling point indicate significant differences among treatments ($p < 0.05$, one-way ANOVA with Bonferroni LSD post hoc test)

the alcoholic fermentation (Bautista-Ortín et al., 2016a; Rousserie et al., 2020). Seed hydration, together with the effect of the increasing alcohol content in the media that facilitates the dissolution of the cuticle, could have led to the fast increase in phenolic extraction at the last stages of seed maceration. Our observation agrees with the literature, which reported the increasing proportion of seed tannins after longer maceration due to the increased seed hydration (Busse-Valverde et al., 2012) and which showed that the tannin extraction from seeds follows that from skins, requiring typically 4–5 days to start, while the amount extracted from the seeds depends on the variety and winemaking process (Mattivi et al., 2002). Similarly, other studies (Busse-Valverde et al., 2011; Panprivech et al., 2015) demonstrated that long macerations allowed important hydration of seed cells, which led to higher extraction of tannins and resulted in more astringent wines. These observations confirmed that phenolic extraction from seeds is strongly affected by the maceration time. Our results concerning the mechanical properties and acoustic emission at seed breakage were unable to provide additional information on the possible effect of seed hydration. At the end of the maceration, skins + seeds (Sk + Se) joint maceration treatment always showed significantly higher TPI values when compared to individual extraction from skins and seeds ($p < 0.05$). Sk + Se joint maceration presented an extraction curve more similar to the one obtained in the Sk extraction, also considering the delayed seed extraction (Fig. 2). This result is in agreement with the common belief that skins mainly contribute to the general phenolic composition of wine thanks to the rapid extraction of anthocyanins and skin tannins (Bautista-Ortín et al., 2016a). Furthermore, Sk + Se treatment resulted in higher TPI values than Sk at different times during the maceration, depending on the variety. 'Nebbiolo' showed a significant TPI increase in Sk + Se from the third day. Although 'Sangiovese' Sk + Se TPI was briefly found significantly higher than Sk on the third day, this behavior was not statistically confirmed until the final point. In 'Primitivo' and 'Aglianico', significant differences between Sk + Se and Sk curves were found only at the end of the maceration. Observing the extraction curve of Sk + Se treatments, it seems clear that the increases during the last days of maceration are directly influenced by the extraction from grape seeds.

Based on findings from a previous study demonstrating that anthocyanin extraction and retention in the medium do not change whether skins are extracted alone or alongside seeds (Giacosa et al., 2023), we may hypothesize that differences observed between Sk and Sk + Se treatments in 'Aglianico' and 'Nebbiolo' are only due to the late phenol extraction from seeds. Moreover, the lack of differences between Sk and Sk + Se treatments in 'Primitivo' and 'Sangiovese' from the fourth to the tenth day of maceration could be attributed to the comparatively lower phenolic content of seeds in these varieties (Table 1).

After 240 h of simulated maceration, 'Aglianico' showed the highest TPI extracted from skins or seeds (3023 ± 184 and 1825 ± 33 mg (–)-epicatechin/kg of berries, respectively; Table 2). Accordingly, this variety also resulted in the highest TPI values in the Sk + Se treatment (4276 ± 219 mg (–)-epicatechin/kg of berries). Moreover, 'Aglianico' showed the highest values of TFI in all treatments, and NFI in Sk and Sk + Se treatment, confirming the high phenolic and specifically tannin content of this variety. On the other hand, 'Sangiovese' showed the lowest phenolic concentration in Se, Sk, and Sk + Se, confirming the result obtained in the potential seed extraction.

Regarding the phenolic extraction yield according to TPI values of Sk + Se treatment when compared to the sum of skins and seeds separate extractions, 'Sangiovese' evidenced the lowest yield (84.3%), followed by 'Aglianico' and 'Nebbiolo' (88.2% and 88.7%, respectively), and 'Primitivo' (91.5%). Therefore, in terms of total phenolics, the joint extraction from skins and seeds evidenced decreases from about 9 to 16%, without clear, definite behaviors according to phenolic richness.

Proanthocyanidin Profile of Skin and Seed Extracts

At the end of each grape component maceration in model wine solutions with increasing ethanol content, several flavan-3-ol parameters were assessed (Table 2). Regarding the methyl cellulose precipitation assay (PAs), 'Aglianico' showed the highest extracted amount of PAs from skins and seeds, 'Primitivo' and 'Nebbiolo' resulted in a similar extractable skin PA concentration, while 'Sangiovese' was the variety that showed the poorest skin PA concentration (528 ± 30 mg (–)-epicatechin/kg of berries). No significant differences were found in PA extraction from 'Nebbiolo', 'Primitivo', and 'Sangiovese' seeds ($p > 0.05$).

Structural characteristics of PAs extracted during the simulated macerations are also reported in Table 2. The values of mDP in skin extracts (Sk) were higher in all cases compared to those obtained in the other treatments (Se; Sk + Se), with 'Nebbiolo' showing the highest mDP (15.5 ± 0.9), followed by 'Primitivo', 'Sangiovese', and 'Aglianico'. Differences in %G values were found among varieties, but in this case, the lowest value belonged to 'Nebbiolo' extractions both from skins and seeds (1.4 ± 0.1 and 8.5 ± 0.3, respectively), while 'Sangiovese' showed the highest values (2.9 ± 0.1 for skins and 11.9 ± 0.4 for seeds extraction). The composition of PA terminal and extension subunits was also characterized. Generally, catechin terminal subunit percentage (%C-term) was higher than that of epicatechin (%EC-term) in both Sk and Se extracts. 'Aglianico' skins showed the highest %C-term and the lowest %EC-term. 'Sangiovese' was the only variety that evidenced a higher %EC-term in seed extract. Regarding the extension subunits, epicatechin subunits were the most abundant in both Sk and Se extracts. Notably, a sustained variability among varieties can be found for %EC-ext in skin extraction with the highest values corresponding to 'Aglianico' and 'Primitivo', but not in seeds.

The extraction yield of skin PAs was calculated by comparing skin PA concentration at the end of the simulated maceration (Table 2) with the skin potential extraction (Table 1). The extent of skin PA extraction was found different among varieties and accounted for 89% in 'Aglianico', 67% in 'Nebbiolo', 62% in 'Primitivo', and 56% in 'Sangiovese'. These results confirm that the extractable phenolic content does not only depend on their concentration in grapes but also their extractability. For tannins, varietal effects on extractability were previously found on multiple grape batches presenting different concentration (Bindon et al., 2014), allowing to decouple concentration and extractability factors. However, the difference in extraction methods makes it difficult to compare directly the findings across the available literature. It is also to be noted that the result in the present study was obtained on berries selected according to their density, a factor that allows to mitigate berry heterogeneity also in terms of phenolic traits (Rolle et al., 2012). A significant positive correlation was found between tannins in skin potential extraction and skin simulated maceration ($r = 0.9899$, $p < 0.05$). Moreover, also skin mDP, %G, and %PD for the potential extraction correlated with the values obtained after the skin maceration in model wine solutions with the following Pearson coefficients: $r = 0.9553$ ($p < 0.05$), 0.9946 ($p < 0.01$), and 0.9941 ($p < 0.01$), respectively.

Some differences in the results obtained after the potential extraction (SO_2-rich media) and the simulated maceration are worth to be noted. During the maceration, high molecular weight tannins were less extracted from skins as the mDP values for the potential extraction were higher compared to values obtained at the end of the simulated maceration. Similarly, galloylated skin PAs were less extracted during simulated macerations compared to the potential extraction; indeed, %G values were higher in potential extracts for all varieties. These observations agreed with those of Rinaldi et al. (2015), who reported that the release of galloylated skin tannins is minimally or not at all promoted under real

Table 2 Proanthocyanidin composition of skin-only (Sk), seed-only (Se), and joint skins + seeds (Sk + Se) extracts at the end of the simulated maceration in model wine solution

		Aglianico				Nebbiolo				Primitivo				Sangiovese			
Parameter	Units	Sk	Sk + Se	Se	Sign	Sk	Sk + Se	Se	Sign	Sk	Sk + Se	Se	Sign	Sk	Sk + Se	Se	Sign
TPI	mg/kg berries	3023 ± 184 b	4276 ± 219 a	1825 ± 33 c	***	1972 ± 58 b	2688 ± 103 a	1059 ± 93 c	***	2036 ± 87 b	2699 ± 205 a	915 ± 92 c	***	1784 ± 53 b	2233 ± 117 a	865 ± 71 c	***
TFI	mg/kg berries	1731 ± 136 b	2817 ± 106 a	1573 ± 21 b	***	970 ± 77 b	1585 ± 82 a	843 ± 87 b	**	1155 ± 84 b	1673 ± 165 a	887 ± 150 c	**	754 ± 30 b	1055 ± 119 a	689 ± 58 b	**
NFI^	mg/kg berries	971 ± 76 c	2031 ± 54 a	-	***	649 ± 84 b	1289 ± 127 a	-	**	637 ± 59 c	1190 ± 124 a	-	**	426 ± 27 c	699 ± 79 b	-	**
PAs	mg/kg berries	1070 ± 102 b	1457 ± 42 a	817 ± 80 c	***	674 ± 30 b	856 ± 49 a	503 ± 53 c	***	636 ± 73 b	858 ± 62 a	464 ± 48 c	**	528 ± 30 b	677 ± 58 a	446 ± 45 b	**
mDP	-	11.7 ± 0.8 a	3.5 ± 0.1 b	2.0 ± 0.1 c	***#	15.5 ± 0.9 a	5.1 ± 0.2 b	2.6 ± 0.1 c	***	13.5 ± 0.6 a	4.2 ± 0.1 b	2.2 ± 0.1 c	***	12.5 ± 1.1 a	5.2 ± 0.3 b	2.8 ± 0.1 c	***#
%PD	%	17.6 ± 0.4 a	10.6 ± 0.5 b	-	***	44.9 ± 1.5 a	29.3 ± 0.8 b	-	***	24.1 ± 0.7 a	13.3 ± 0.4 b	-	**#	34.4 ± 0.6 a	24.5 ± 1.5 b	-	***
%G	%	2.7 ± 0.4 c	5.8 ± 0.2 b	10.3 ± 0.4 a	***	1.4 ± 0.1 c	3.7 ± 0.1 b	8.5 ± 0.3 a	***	1.8 ± 0.1 c	5.6 ± 0.4 b	10.3 ± 1.5 a	***	2.9 ± 0.1 c	4.2 ± 0.7 b	11.9 ± 0.4 a	***
%C-term	% w/w	91.0 ± 2.3 a	63.5 ± 0.6 b	56.4 ± 0.4 c	**	78.9 ± 2.7 a	59.2 ± 0.8 b	53.7 ± 0.9 c	***	61.3 ± 2.7 a	56.2 ± 0.7 b	53.9 ± 0.5 b	**	71.9 ± 5.2 a	44.7 ± 1.8 b	36.2 ± 0.8 c	***
%EC-term	% w/w	9.0 ± 2.3 c	30.2 ± 0.7 b	34.4 ± 0.6 a	***	21.1 ± 2.7 c	35.9 ± 0.4 b	39.5 ± 0.8 a	***	38.7 ± 2.7	36.4 ± 0.9	37.6 ± 0.7	ns	28.1 ± 5.2 b	51.5 ± 1.3 a	53.8 ± 1.5 a	***
%ECG-term	% w/w	-	6.3 ± 0.2 b	9.3 ± 0.3 a	***	-	4.9 ± 0.4 b	6.8 ± 0.4 a	***	-	7.4 ± 0.3 b	8.5 ± 1.0 a	***	-	3.8 ± 0.5 b	10.0 ± 0.7 a	***
%C-ext	% w/w	1.1 ± 0.1 c	1.3 ± 0.1 b	2.5 ± 0.1 a	***	1.4 ± 0.2 c	2.1 ± 0.1 b	3.6 ± 0.2 a	***	1.0 ± 0.1 b	1.1 ± 0.1 b	1.5 ± 0.1 a	***	2.7 ± 0.2 a	2.3 ± 0.1 ab	1.9 ± 0.1 b	*
%EC-ext	% w/w	76.8 ± 0.2 c	78.2 ± 0.6 b	86.1 ± 0.5 a	***	49.1 ± 1.3 c	58.0 ± 0.3 b	86.7 ± 0.6 a	***	71.0 ± 0.8 c	76.4 ± 0.3 b	86.7 ± 1.8 a	***	56.8 ± 0.1 c	63.0 ± 1.2 b	85.0 ± 0.1 a	***
%ECG-ext	% w/w	3.0 ± 0.5 c	5.6 ± 0.2 b	11.4 ± 0.5 a	***	1.5 ± 0.1 c	3.4 ± 0.1 b	9.6 ± 0.3 a	***	1.9 ± 0.1 c	5.0 ± 0.4 b	11.8 ± 1.7 a	***	3.2 ± 0.1 c	4.3 ± 0.7 b	13.1 ± 0.1 a	***
%EGC-ext	% w/w	19.2 ± 0.3 a	14.6 ± 0.8 b	-	***	48.0 ± 1.4 a	36.5 ± 0.6 b	-	***	26.0 ± 0.8 a	17.5 ± 0.3 b	-	***	37.4 ± 0.5 a	30.3 ± 1.9 b	-	**

Values are presented as average ± standard deviation ($n = 3$). Sign.: *, **, ***, and "ns" indicate significant differences at $p < 0.05$, 0.01, 0.001, and not significant, respectively, among values within the same variety according to ANOVA or Welch's ANOVA (#). Values followed by different letters within a variety are significantly different (according to Bonferroni LSD or Games-Howell post hoc tests for ANOVA and Welch's ANOVA, respectively). *TPI* total phenolic index, *TFI* total flavonoids index, *NFI* non-anthocyanin flavonoids index, *PAs* total proanthocyanidins, *mDP* mean degree of polymerization, *%PD* percentage of prodelphinidins, *%G* percentage of galloylation, *%C-term* percentage of catechin terminal subunits, *%EC-term* percentage of epicatechin terminal subunits, *%ECG-term* percentage of epicatechin gallate terminal subunits, *%EGC-ext* percentage of epigallocatechin extension subunits, *%C-ext* percentage of catechin extension subunits, *%EC-ext* percentage of epicatechin extension subunits, *%ECG-ext* percentage of epicatechin gallate extension subunits. TPI and PA are expressed as mg (−)-epicatechin/kg berries, while TFI and NFI are expressed as mg (+)-catechin/kg berries. ^ NFI statistical grouping includes the respective seeds (Se) TFI value

maceration conditions. Consequently, in wine production, the contribution of galloylation is made predominantly by seeds.

The extraction rates found in seeds were 72% in 'Aglianico', 74% in 'Nebbiolo', 84% in 'Primitivo', and 93% in 'Sangiovese'. A significant positive correlation was also found between PAs detected in seed potential extraction and seeds simulated maceration ($r = 0.9598$, $p > 0.05$). Contrary to what was observed for the skins, no significant correlation was found in mDP and %G values between seed potential extraction and seed simulated maceration in model wine solution. %G values in seeds were always lower in simulated maceration than potential extraction, confirming that under softer extraction conditions, smaller and less galloylated tannins are extracted.

Overall, these results indicate that seed and skin tannins are differently extracted from grape tissues, and the potential tannin content of grapes partially affects the amount of tannins that can be extracted. Lastly, it is worth noting that, under real winemaking conditions, the presence of fermenting yeasts and enzyme activity could further influence the extraction and re-adsorption of phenolic compounds extracted during maceration, leading to different results.

Skin and Seed Tannin Extraction Repartition in Joint Maceration

As expected, the joint Sk + Se maceration always resulted in a significantly higher amount of PAs compared to the individual extraction of seeds or skins (Table 2). 'Aglianico' showed the highest PA value obtained after the Sk + Se treatment, while 'Sangiovese' in the lowest, and minor differences were found in 'Nebbiolo' and 'Primitivo'. Interestingly, the PA content detected in Sk + Se consistently exhibited abundantly lower levels compared to the sum of PAs from the Sk and Se treatments. Despite utilizing equal quantities of seeds and skins in the Sk + Se treatment as in the Se or Sk treatments individually, the final extracted PA content was notably lower. Specifically, it was 23% lower for 'Aglianico', 27% for 'Nebbiolo', 22% for 'Primitivo', and 31% for 'Sangiovese'. Given that phenolics are extracted according to a concentration-driven diffusion (Boulton, 1995), it is logical to believe that the increasing concentration reported in the joint Sk + Se treatment has affected the extraction, lowering the amount of PA extracted from both skins and seeds. Moreover, in Sk and Se separate treatments, there was a higher volume to contact surface ratio that could have enhanced the extraction of tannins from grape tissues. In light of this data, it is possible to hypothesize that tannins are extracted differently if skins and seeds are extracted alone or together, but it is not demonstrated if this reduction is equally distributed on skin and seed fractions. The PA content in joint Sk + Se was found to be significantly correlated with those found in Sk and Se with the following Pearson coefficients: $r = 0.9976$ ($p < 0.01$) and $r = 0.9843$ ($p < 0.05$), respectively. Given that tannins extracted in Sk and Se treatments are both correlated with those extracted in joint Sk + Se, the reduction may occur to a similar extent from skins and seeds when extracted together despite the later PA extraction from seeds.

To better understand the extraction repartition between seeds and skins, the possible contribution of these components on the total content of PAs in the joint Sk + Se extraction was calculated by applying the method proposed by Peyrot Des Gachons & Kennedy (2003) based on (–)-epigallocatechin extension unit content. The distribution of PAs in the combined treatment of skins and seeds (Sk + Se) was contrasted with the tannin ratio derived from the PA content corresponding to separate skin and seed extractions (Sk|Se) on their sum. This comparison is reported in Fig. 3.

The estimated content of skin tannins (skin PAs) in joint Sk + Se treatment was always significantly higher compared to one of the seeds (seed PAs) for all varieties. Similar results were found in wine in previous studies (Busse-Valverde et al., 2012). 'Sangiovese' showed the highest percentage of skin PAs in Sk + Se treatment (81.2%), followed by 'Aglianico' (77.6%), 'Nebbiolo' (76.1%), and 'Primitivo' (67.2%).

It is possible to notice a discrepancy between the values of skin and seed PAs in the estimation from Sk + Se treatment compared to the PA content obtained from separate extractions (Sk|Se). For all varieties, the content of seed PAs in Sk + Se was always lower compared to seed PAs in Sk|Se. 'Sangiovese', which exhibited the lowest estimated seed contribution in the joint extraction (Sk + Se), did not show any differences in the ratio of PAs in Sk|Se. On the other hand, the estimation of skin PAs in Sk + Se treatment seemed to be similar to what was observed in Sk|Se for all varieties. Therefore, it may be hypothesized that the reduction of the PA content observed in Sk + Se when compared to the sum of Sk and Se contributions may be due to a lower extraction from seeds. In general, the ratio of skin and seed tannins obtained from the separate extraction was not kept in Sk + Se, and therefore it can be confirmed that grape phenolics are extracted with different extents and proportions when seeds and skins undergo maceration together, the seed contribution being particularly affected.

Prodelphinidins are exclusively found as extension subunits in skin tannins. Therefore, the %PD was significantly higher in Sk treatment compared to Sk + Se (Table 2). Likewise, the %EGC-ext subunit on the total extension subunits was higher in Sk. The structural characteristics of PAs extracted in Se and Sk treatments separately were poorly correlated with those of tannins extracted in joint Sk + Se. Only %G in Se was significantly correlated with Sk + Se ($r = 0.984$, $p < 0.05$).

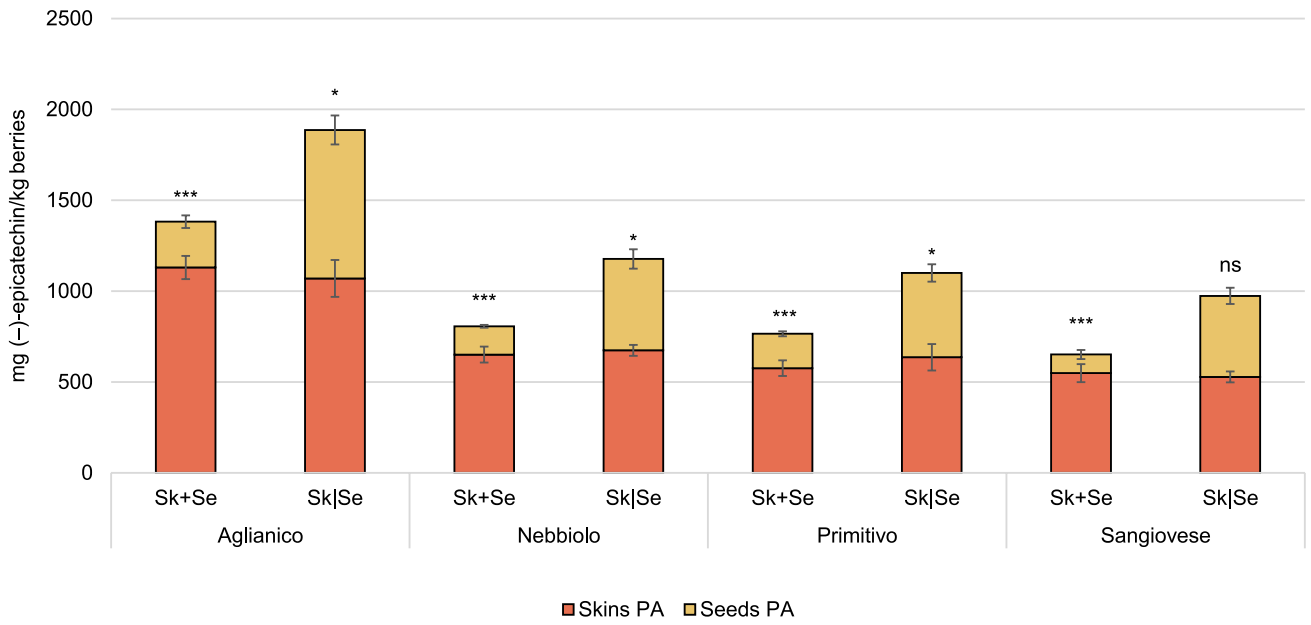

Fig. 3 Comparison between the estimated content of seed and skin PAs in Sk + Se joint extraction according to phloroglucinolysis (−)-epigallocatechin units (Peyrot Des Gachons & Kennedy, 2003) with the PAs detected in Sk and Se separate treatments (Sk|Se). Data are expressed as average values in mg (−)-epicatechin/kg berries, with bars showing the standard deviation ($n = 3$). Sign.: *, ***, and "ns" indicate significant differences at $p < 0.05$, 0.001, and not significant, respectively, among skins and seeds contributions according to *t*-test

Therefore, it is likely that the simultaneous maceration of seeds and skins in the Sk + Se treatment led to changes in both extracted tannin concentrations and their structural characteristics, differing from when seeds and skins were macerated alone.

Given that Sk treatment gave significantly lower %G and higher mDP values compared to Se in all tested varieties, the presence of seeds in the Sk + Se could have affected the extraction by significantly lowering the mDP and increasing %G. In previous studies, it was observed that the presence of seeds during long macerations led to an mDP decrease due to the increasing extraction of smaller tannins from seeds (Busse-Valverde et al., 2012). Therefore, an increase of skin PAs during red winemaking may not be always associated with an increase in mDP values due to the presence of seed tannins that contribute to lowering the average degree of polymerization (Bautista-Ortín et al., 2016a). In addition, cell wall material is reported to have a greater affinity with high molecular weight tannins (Bindon et al., 2010); therefore, the adsorption of skin PAs may have played a role in lowering the mDP in Sk + Se. These phenomena may have been enhanced by the presence of seeds, whose cell walls are reported to be able to adsorb phenolic compounds (Giacosa et al., 2023). Therefore, the simultaneous presence of skins and seeds in Sk + Se could have led to a higher presence of suspended cell wall materials that might have played a role in reducing the final PA concentration and lowering the mDP.

Conclusions

The present experiment provides novel information on how phenolic substances are extracted from grape components. The four Italian red grape varieties studied showed distinct differences in terms of potential phenolic content, extractable content, and extractability. Therefore, the variety must be considered one of the most influential factors affecting the wine phenolic content.

In literature, several authors reported the difficulty of establishing a correlation between the total phenolic content of grapes and the extractable phenolics under maceration conditions. In the present study, the potential tannin pool (estimated with a SO_2-rich extracting medium) in skins and seeds was instead positively correlated with the amount of tannins extracted during the maceration in a wine-like solution. Nevertheless, seed and skin potential extracts contained high molecular weight and highly galloylated tannins that were not extracted in model wine solutions.

Different tannin extraction patterns from skins and seeds have been observed when macerated together versus separately. This is supported by (i) the discrepancy

between the combined tannin content detected in skin and seed extractions versus the joint skins + seeds extraction, (ii) the non-conservation of tannin distribution between separate versus joint skins + seeds extractions, and (iii) the poor correlation in structural tannin characteristics (mDP, %G) between separate skins and seeds extractions compared to skins + seeds.

Therefore, the extraction of tannins depends not only on the variety, native tannin pool, and extractability, but also on the presence and proportion of seeds and skins during maceration. Overall, the novel insights provided by this study may find a useful application in some enological practices, such as seed removal, a widespread technique beneficial in winemaking involving grapes with high amounts of extractable tannins from seeds.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing Interests The authors declare no competing interests.

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