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Influence of Grape Density and Harvest Date on Changes in Phenolic Composition, Phenol Extractability Indices, and Instrumental Texture Properties during Ripening

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ABSTRACT: Changes in the phenolic composition, phenol extractability indices, and mechanical properties occur in grape berries during the ripening process, but the heterogeneity of the grapes harvested at different ripening stages affects the reliability of the results obtained. In this work, these changes were studied in Nebbiolo grapes harvested during five consecutive weeks and then separated according to three density classes. The changes observed in chemical and mechanical parameters through the ripening process are more related to berry density than harvest date. Therefore, the winemaker has to select the flotation density according to the objective quality properties of the wine to be elaborated. On the other hand, the stiffer grapes were associated with a higher accumulation of proanthocyanidins. The harder grapes provided the higher concentration and extractability of flavanols reactive to vanillin, whereas the thicker ones facilitated the extraction of proanthocyanidins.

KEYWORDS: phenolic composition, phenol extractability, anthocyanins, skin hardness, skin thickness, texture analysis, red grapes

INTRODUCTION

The phenolic composition of grapes is responsible for certain organoleptic properties intimately related to red wine quality, particularly color, astringency, and bitterness. Because color is one of the most important sensory characteristics in the initial valuation of red wine quality, anthocyanins play an important role in consumer acceptance of a wine as they are responsible for the color of red grapes and young wines.[1] On the other hand, proanthocyanidins strongly influence wine astringency, whereas the bitterness is restricted to small flavanol molecules.[2,3] Moreover, anthocyanins can react with other phenolic compounds to produce polymeric pigments resulting in the long-term color stability of aged red wines and in the decrease of the wine astringency.[3,4]

Anthocyanins are gradually accumulated in berry skins from veraison through grape ripening,[5,6] malvidin-3-glucoside being the most abundant anthocyanin in almost all red grape varieties.[6] However, the anthocyanin concentration may decline just before harvest and/or during over-ripening.[6] Instead, proanthocyanidins are mainly accumulated in berry skins before veraison.[7] The highest concentration of seed proanthocyanidins is achieved at veraison and, from this moment, they decline slowly until close to grape ripeness but thereafter remain relatively constant.[8]

Phenolic compounds are extracted from berry skins and seeds into the wine during the maceration/fermentation step and, therefore, assessment of the anthocyanin extractability through the winemaking process is required to predict the wine color from grape polyphenols.[9,10] Furthermore, the anthocyanin extractability varies throughout grape ripening,[6] as a consequence of the compositional changes occurring in the skin cell wall during its degradation by pectolytic enzymes.[11] In seeds, the histological and histochemical modifications that occur during the fruit development also affect the ability to release phenols.[8,12]

Many studies have been performed to define the best indices to evaluate phenol extractability from berry skins and seeds. Because the assessment of the extractability of phenolic compounds is strongly dependent on the extraction method used, the cellular maturity index (EA) and seed maturity index (MP) seem to provide an adequate robustness to predict those in the resulting wines.[13,14] Instrumental texture analysis parameters also permit the estimation of the anthocyanin extractability because the structural and chemical properties of the skin cell walls may determine the mechanical resistance, texture, and ease of processing berries.[11] In particular, the berry skin break force can be considered the best mechanical parameter to estimate anthocyanin extraction kinetics with adequate reliability.[15] Recently, Río Segade et al.[16] have proposed the use of the berry skin thickness to predict anthocyanin extractability. Furthermore, mechanical methods are inexpensive, which represents an additional advantage because it allows their application as a routine monitoring tool for the grape quality.

Most studies on the influence of the harvest date on the grape phenolic composition and phenol extractability have been carried out without considering the physiological homogeneity of samples. To reduce the heterogeneity of the physiological characteristics corresponding to the different ripening stages, Fournand et al.[6] calibrated berries according to their density. For the first weeks after veraison, the less dense classes were selected, and for the last weeks, the denser classes were selected, so that the physiological differences between the first and last sampling dates were emphasized.

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As the combined effect of both the harvest date and grape densimetric sorting was not previously studied, the aim of this work was first to investigate the changes in berry skin phenolic composition, phenol extractability indices, and skin mechanical properties through the last five ripening weeks at three different grape densities and then to establish a relationship that permits the harvest date to be related to certain chemical and physical parameters for each grape density. This knowledge could be interesting because a new technology, particularly automatic winery equipment that would perform berry densimetric sorting, has been recently developed and proposed for the enological industry. Because the effectiveness of grape selection is strongly dependent on the density of the floating solution, it should be previously optimized on the basis of the chemical−physical characteristics of the grape. Finally, this approach aimed to determine whether the skin mechanical attributes may influence the phenol composition and/or extractability, irrespective of the effect of the harvest date and/or sugar content. The study was carried out on *Vitis vinifera* L. cv. Nebbiolo because it is one of the most important and well-known Italian varieties, the grapes of which are usually used for the production of renowned red wines such as Barolo and Barbaresco DOCG, which are commercialized worldwide.

MATERIALS AND METHODS

Grape Samples. Grape samples of Nebbiolo red cultivar (*V. vinifera* L.) were collected at different physiological stages from a vineyard of 0.5 ha located in La Morra within the Cuneo province of Piedmont (northwestern Italy) during five consecutive weeks in 2009. About 12 kg of grape berries for each sampling date were randomly picked with attached pedicels from 500 vines by picking the berries one by one and/or in bunches (three or four berries) from each cluster. The berries were separated according to their density, which was estimated by flotation in different saline solutions (from 100 to 190 g/L sodium chloride).[6,17,18] These solutions had densities between 1069 and 1125 kg/m^3. The berries were introduced into the less dense solution and "floating" berries were considered to have the same density as the solution. These berries were separated from those that sank and were counted. Berries that sank were removed and introduced into the next denser solution. The same process was applied for all saline solutions. For each harvest date, the following density classes were studied: A = 1088 kg/m^3, B = 1094 kg/m^3, C = 1100 kg/m^3 (these are the densities to which most berries belong). The floating berries were washed with water and visually inspected before analysis; those with damaged skins were discarded.

For both density class and harvest date, a subsample of 30 sorted berries was used for the determination of the physical and mechanical properties. Three subsamples of 20 sorted berries were used for the determination of the skin phenolic composition and relative extractability. Another two subsamples of 200 berries were used for the determination of cellular maturity and seed maturity indices. The remaining berries, subdivided in three replicates, were used for determining standard physicochemical parameters in the grape must obtained by manual crushing and filtration.

Reagents and Standards. Solvents of HPLC gradient grade and all other chemicals of analytical reagent grade were purchased from Sigma (Milan, Italy). The solutions were prepared in deionized water produced by a Purelab Classic system (Elga Labwater, Marlow, U.K.). Anthocyanin standards (delphinidin-3-*O*-glucoside chloride, malvidin-3-*O*-glucoside chloride, petunidin chloride, peonidin-3-*O*-glucoside chloride, cyanidin-3-*O*-glucoside chloride) were supplied by Extrasynthèse (Genay, France). All of the standards were stored at −20 °C away from light before use.

Physical and Technological Maturity Parameters. Reducing sugars, pH, and total acidity were determined according to International Organization of Vine and Wine (OIV) methods. The distance between top and bottom sides (L) and the distance between both lateral sides at the middle of berry height (l) were measured using a caliper, which had an accuracy of 0.1 mm. The volume was then calculated, comparing the berry form to an ellipsoid, following eq 1[16]

$$volume\ (cm^3) = 4\pi abc/3 \quad (1)$$

where $a = b = l/2$ and $c = L/2$.

Cellular Maturity and Seed Maturity Indices (Glories' Indices). The phenol extractability indices were assessed in accordance with the procedure proposed by Glories and Saint-Criq,[19] which was slightly modified for Nebbiolo grapes.[13] Two replicates of 200 grape berries were used. The following parameters were determined in both pH 1 and 3.2 solutions: total phenolic content (A_{280}), total anthocyanins (A_1 and $A_{3.2}$), total flavonoids (TF_1 and $TF_{3.2}$), and nonanthocyanin flavonoids (FNA_1 and $FNA_{3.2}$).[13,20] The cellular maturity index (EA) and the seed maturity index (MP) were calculated following eqs 2 and 3, respectively:[9,13,14]

$$EA\ (\%) = (A_1 - A_{3.2})/A_1 \times 100 \quad (2)$$

$$MP\ (\%) = (A_{280} - ((A_{3.2}/1000) \times TAR))/A_{280} \times 100 \quad (3)$$

The average ratio (TAR) between total phenols (A_{280}) and total anthocyanins in grape skins was 70 for Nebbiolo grapes when $A_{3.2}$ was expressed as g/L.[13]

Skin Phenolic Composition and Extractability. *Extraction.* Three replicates of 20 berries for each density class and harvest date were weighed before phenolic extraction. The berry skins were manually removed from the pulp. Afterward, they were quickly immersed in 75 mL of a buffer solution containing 12% (v/v) ethanol to simulate the extraction conditions during industrial production, 100 mg/L sodium metabisulfite to limit the oxidation of phenolic compounds,[6] 50 mg/L sodium azide, and 5 g/L tartaric acid. The pH value was adjusted to 3.20 by the addition of 1 mol/L sodium hydroxide.[20] The berries were then introduced in a controlled-temperature room at 25 °C for 48 h, and the supernatant was used for determining easily extracted phenols (solution A).[21] Residual berry skins were rinsed with the hydroalcoholic solution and quickly immersed in 75 mL of a new hydroalcoholic buffer containing a higher sodium metabisulfite concentration (600 mg/L). After homogenization at 8000 rpm for 1 min with an Ultraturrax T25 high-speed homogenizer (IKA Labortechnik, Staufen, Germany), the extract was centrifuged in a PK 131 centrifuge (ALC International, Milan, Italy) for 10 min at 3000g at 20 °C. The supernatant was then used for determining noneasily extracted phenols (solution B). The total extractable phenol content in berry skins (for each parameter evaluated) was calculated as $A + B$ and expressed as mg/kg grapes, whereas the extractability yield was calculated as $A/(A + B)$ and expressed as %.[21]

Spectrophotometric Methods. Phenolic compounds in the berry skin extracts were determined by spectrophotometric methods[20] using a UV-1601PC spectrophotometer (Shimazdu Scientific Instruments Inc., Columbia, MD). Total anthocyanins (TA_{sk}) were expressed as malvidin-3-glucoside chloride, whereas flavanols reactive to vanillin (flavanol vanillin assay, FVA_{sk}) and total flavonoids (TF_{sk}) were expressed as (+)-catechin. Proanthocyanidins (PRO_{sk}) were determined after acid hydrolysis with warming (Bate−Smith reaction) using a ferrous salt ($FeSO_4$) as catalyst. They were expressed as cyanidin chloride.

HPLC Method. An anthocyanin profile was performed after the berry skin extract had been submitted to solid phase extraction using a SEP-PAK C_{18} cartridge (Waters Corp., Milford, MA), methanol being the eluent. The chromatography system employed was a P100 pump equipped with an AS3000 autosampler (Spectra Physics Analytical, Inc., San Jose, CA), a 20 μL Reodyne sample loop, a LiChroCART analytical column

Table 1. Operating Conditions for the Measurement of Berry Mechanical Parameters

test	probe	test speed (mm/s)	compression-puncture (mm)	mechanical property
skin hardness	P/2N, 2 mm needle	1	3	F_{sk} = berry skin break force (N) W_{sk} = berry skin break energy (mJ) E_{sk} = berry skin Young's modulus (N/mm)
skin thickness	P/2, ∅ 2 mm	0.2		Sp_{sk} = berry skin thickness (μm)

(25 cm × 0.4 cm i.d.) purchased from Merck (Darmstadt, Germany), which was packed with LiChrosphere 100 RP-18 (5 μm) particles supplied by Alltech (Deerfield, IL), and a Spectra Focus diode array detector (DAD, Spectra Physics Analytical, Inc.) operating at 520 nm. The following mobile phases were used: A, 10% (v/v) formic acid in water; B, 10% (v/v) formic acid and 50% (v/v) methanol in water. All of the solvents were filtered through a 0.20 μm filter. The mobile phase flow rate was 1 mL/min. The following solvent A proportions were used: from 72 to 55%, 15 min; to 30%, 20 min; to 10%, 10 min; to 1%, 5 min; to 72%, 3 min. An equilibrium time of 10 min was selected.[20] Data treatment was carried out using the ChromQuest chromatography data system (ThermoQuest, Inc., San Jose, CA). Identification of the free forms of anthocyanins in berry skin extracts was performed by comparison with external standards. The acylated forms of anthocyanins were identified by matching the DAD spectrum and retention time of each chromatographic peak and by comparing these with data available in the literature.[22] Individual anthocyanins were expressed in percentages.

Skin Mechanical Properties. A Universal Testing Machine (UTM) TAxT2i Texture Analyzer (Stable Micro System, Godalming, Surrey, U.K.) equipped with an HDP/90 platform and a 5 kg load cell was used. The operating conditions applied and the mechanical properties measured in skins (sk) are shown in Table 1. All of the data acquisitions were made at 400 Hz, and data were evaluated using Texture Expert Exceed software version 2.54 for Windows 2000. For each berry weighed and measured, the skin hardness was assessed by a puncture test.[23] Thirty berries were placed on the metal plate of the UTM with the pedicel in a horizontal plane in order to be consistently punctured in the lateral face. The measurement of the skin thickness required the manual separation of a piece of skin (ca. 0.25 cm^2) from the lateral side of each berry with a razor blade and its subsequent drying with adsorbent paper. Care was taken in removing the pulp from the skin and in positioning the skin sample on the UTM platform to prevent folds in the skin.[16] Furthermore, it was convenient to insert an instrumental trigger threshold equal to 0.05 N that enabled the plane surface of the probe to adhere completely to the skin sample before the acquisition started. This allowed a reduction or elimination of the "tail" effect due to the postponement of the contact point.[23] Before each test, the instrument was calibrated for force and distance.

The hardness of the berry skin is assessed by the maximum break force (F_{sk}), by the break energy (W_{sk}), or by the material resistance to the axial deformation (E_{sk}). The first variable corresponds to the resistance to the needle probe penetration, whereas the second variable is represented by the area under the force/time curve, which is limited to between 0 and F_{sk}.[23] The third variable is defined as the slope of the stress−strain curve in the linear section and measures the stiffness of the skin to a load applied.[23,24] The berry skin thickness (Sp_{sk}) is given by the distance between the point corresponding to the probe contact with the berry skin (trigger) and the platform base during the compression test.[16]

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

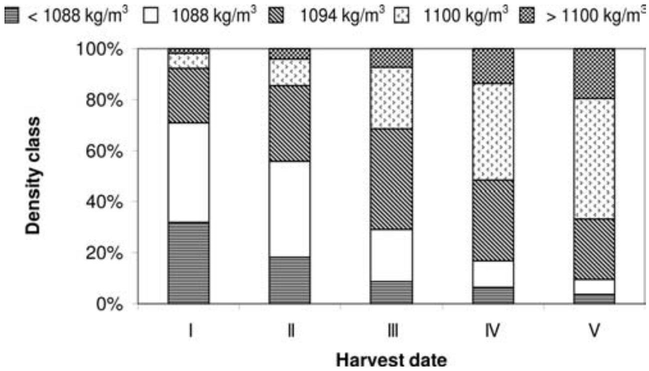

Figure 1. Percentage of Nebbiolo grape berries in different density classes, as a function of ripeness stage.

RESULTS AND DISCUSSION

The distribution percentage of Nebbiolo grape berries in different density classes at five harvest dates is reported in Figure 1. It is important to note that not all of the density classes had the same contribution, depending on the grape ripeness stage. As expected, the lower berries density (A) made up the majority in the less ripe grapes (harvest date I), whereas the contribution of the higher density berries (C) increased with grape ripeness and, therefore, with harvest date. The contribution of grapes belonging to density classes A, B, and C ranged as follows: 6.1−38.7, 21.4−39.2, and 6.2−47.0%, respectively, depending on the harvest date.

The distribution of the berries in the vineyard based on the density is already present at the beginning of the ripening process, and it changes during its advancement. Therefore, a non-negligible heterogeneity occurs through the ripening process. Consequently, this heterogeneity implies that a considerable percentage of unripe grapes are harvested and used to elaborate wine. Because unripe grapes provide a lower sugar content, higher acidity, fewer anthocyanins, and, in particular, more seed tannins, their presence can increase bitterness and astringency, affecting the final wine quality adversely.[18]

Physical and Technological Maturity Parameters. Table 2 shows the physical and technological maturity parameters for the three density classes at five grape ripeness stages of the Nebbiolo cultivar. Many differences were present in the physical characteristics among both the density classes and the harvest dates. This effect was particularly significant for the first harvest date, the grapes richer in sugars being the smaller and lighter ones. Smaller berries have a relatively higher solute to solvent ratio than larger berries and, therefore, it is widely known that berry size is a determining factor in wine grape quality.

The values of the technological maturity parameters obtained are those usually found for the Nebbiolo cultivar in the Piedmont

Table 2. Physical and Technological Maturity Parameters for Nebbiolo Grapes Harvested at Different Ripening Stages and Sorted According to Density[a]

density class[b]	harvest date	berry weight (g)	volume (cm^3)	reducing sugars (g/L)	pH	total acidity (g/L)
A	I	2.04 ± 0.06a,β	1.87 ± 0.06a,β	212 ± 2a,α	2.96 ± 0.06a,α	7.2 ± 0.1e,γ
	II	2.05 ± 0.07a,α	1.88 ± 0.08a,α	207 ± 5a,α	2.95 ± 0.02a,α	7.0 ± 0.2d,β
	III	2.00 ± 0.04a,α	1.84 ± 0.09a,α	212 ± 2a,α	2.95 ± 0.05a,α	6.4 ± 0.3c,β
	IV	1.96 ± 0.02a,α	1.80 ± 0.07a,α	211 ± 8a, α	3.06 ± 0.04a,α	6.0 ± 0.6b,β
	V	2.05 ± 0.04a,α	1.88 ± 0.08a,α	212 ± 7a,α	3.30 ± 0.04b,β	5.8 ± 0.2a,γ
signif (1)		ns	ns	ns	**	***
B	I	2.04 ± 0.06ab,β	1.86 ± 0.07a,β	224 ± 3a,β	3.00 ± 0.07a,αβ	6.8 ± 0.3d,β
	II	2.15 ± 0.04bc,α	1.96 ± 0.05ab,α	224 ± 6a,β	3.01 ± 0.06a,α	6.7 ± 0.1d,α
	III	2.03 ± 0.07ab,α	1.85 ± 0.05a,α	227 ± 4a,β	3.01 ± 0.04a,α	6.2 ± 0.4c,α
	IV	1.97 ± 0.08a,α	1.80 ± 0.02a,α	224 ± 4a,β	3.07 ± 0.04a,α	5.7 ± 0.3b,α
	V	2.28 ± 0.05c,β	2.07 ± 0.09b,β	230 ± 8a,β	3.10 ± 0.08a,α	5.4 ± 0.1a,β
signif (1)		**	**	ns	ns	***
C	I	1.72 ± 0.05a,α	1.56 ± 0.04a,α	241 ± 5a,γ	3.13 ± 0.05a,β	6.3 ± 0.3d,α
	II	2.16 ± 0.02c,α	1.96 ± 0.01c,α	235 ± 8a,γ	3.03 ± 0.03a,α	6.7 ± 0.6e,α
	III	1.96 ± 0.05b,α	1.78 ± 0.03b,α	235 ± 7a,γ	3.02 ± 0.03a,α	6.1 ± 0.2c,α
	IV	1.91 ± 0.04b,α	1.74 ± 0.07b,α	241 ± 8a,γ	3.07 ± 0.06a,α	5.7 ± 0.1b,α
	V	2.05 ± 0.09bc,α	1.86 ± 0.05b,α	241 ± 6a,γ	3.14 ± 0.05a,α	5.1 ± 0.3a,α
signif (1)		***	***	ns	ns	***
signif (2)		***,ns,ns,ns,**	**,ns,ns,ns,*	***,***,***,***,***	*,ns,ns,ns,*	***,**,**,***,***

[a] Data are expressed as the average value ± standard deviation: $n = 30$ for berry weight and volume, $n = 3$ for technological maturity parameters. Different Latin letters within the same column indicate significant differences (1) among harvest dates at the same berry density (Tukey b test; $p < 0.05$). Different Greek letters within the same column indicate significant differences (2) among the three density classes at the same harvest date (Tukey b test; $p < 0.05$). *, **, ***, and ns indicate significance at $p < 0.05$, 0.01, and 0.001 and not significant, respectively. [b] A = 1088 kg/m^3; B = 1094 kg/m^3; C = 1100 kg/m^3.

region. At the same density class, the sugar content agreed among the five harvest dates. Fournand et al.[6] reported that the difference in the total sugar content of the berries belonging to two consecutive density classes was ~17 g/L (i.e., 1% v/v potential alcohol). For Nebbiolo grapes, these differences ranged from 8 to 18 g/L. Although the grape berries showed a similar floating behavior among the harvest dates, a significant decrease was found in the total acidity when both the berry density increased and the ripening stage advanced (Table 2). On the contrary, the pH values of the floated grapes showed little or no differences among both density classes and harvest dates.

Total acidity was the only technological maturity parameter that is statistically correlated with the percentage of grape berries belonging to each density class in the different harvest dates. For density class A, a correlation coefficient of 0.997 ($p = 0.0002$, $n = 5$) was obtained, whereas this was −0.930 ($p = 0.022$, $n = 5$) for berry density C. This implies that total acidity is the technological maturity parameter more dependent on the harvest date. Although total acidity diminished through the ripening period, the correlation coefficient was positive for density class A but negative for density class C. This is due to the fact that the proportion of grapes with lower pulp sugar content diminishes in the most advanced ripening stages, whereas that of grapes richer in sugars increases.

Cellular Maturity Index and Seed Maturity Index (Glories' Indices). The modifications found in the phenol extractability indices for the Nebbiolo cultivar through the grape ripening at three different berries densities can be seen in Table 3. EA and MP are considered maturity indices. Although anthocyanins are located in the vacuoles in a free form, the skin cell wall constitutes a barrier for these compounds. Skin cell walls undergo compositional and structural changes during grape ripening that modify the capability to diffuse anthocyanins.[11] In seeds, the histological and histochemical modifications that occur during grape development also affect the ability to release phenols because the solidification of the cells rich in tannins, before harvest, can negatively affect the aptitude for extraction of these compounds.[8]

In the same density class, the results obtained for A_1 and $A_{3.2}$ indicate an increasing tendency to accumulate more anthocyanins in the berry skin when the harvest date is later, except the first one. Furthermore, the percentage variation of total anthocyanin concentration ranged from 37.2 to 40.7% for both density classes A and B; it varied between 19.7 and 21.0% for density class C. When the differences in the anthocyanin accumulated in the same harvest date for the three berry densities were studied, an increase in both A_1 and $A_{3.2}$ with the increase in density was observed. This agrees with the increase previously reported for anthocyanin accumulation in berry skin from 235 to 269 g/L sugars for sorted Barbera grapes that were harvested on the same date.[17] Nevertheless, the differences among total anthocyanin concentrations corresponding to density classes A and C diminished as the ripening stage advanced. In a previous study performed on sorted berries (by selecting only one class of berries for each harvest date), the total red pigments increased rapidly until 170 g/L sugars in the pulp.[6] Afterward, the amount of total red pigments remained nearly unchanged.

In general, FNA_1, $FNA_{3.2}$, and MP showed a decreasing tendency with harvest date for each density class defined by flotation,

Table 3. Phenol Extractability Indices for Nebbiolo Grapes Harvested at Different Ripening Stages and Sorted According to Density[a]

density class[b]	harvest date	$A_{3.2}$ (mg/L malvidin-3-glucoside chloride)	$TF_{3.2}$ (mg/L (+)- catechin)	$FNA_{3.2}$ (mg/L (+)-catechin)	A_{280}	A_1 (mg/L malvidin-3-glucoside chloride)	TF_1 (mg/L (+)-catechin)	FNA_1 (mg/L (+)-catechin)	EA (%)	MP (%)
A	I	245 ± 7b,α	2028 ± 60c,α	1672 ± 50b,α	50.0 ± 1.5b,α	388 ± 11b,α	3482 ± 104c,α	2917 ± 96b,α	36.9 ± 1.1b,α	65.7 ± 2.1c,α
	II	195 ± 5a,α	1859 ± 55ab,α	1576 ± 47b,β	67.0 ± 2.2c,α	304 ± 14a,α	2662 ± 85a,α	2219 ± 62a,α	36.0 ± 1.2ab,α	79.6 ± 2.7d,α
	III	210 ± 9a,α	1722 ± 48a,α	1416 ± 58a,α	45.2 ± 1.8a,α	387 ± 15b,α	3397 ± 98c,αβ	2834 ± 85b,β	45.6 ± 1.6c,γ	67.4 ± 2.2c,β
	IV	267 ± 10c,α	1851 ± 78ab,α	1462 ± 66a,α	47.8 ± 2.2ab,α	436 ± 21c,α	3032 ± 102b,α	2396 ± 99a,α	38.8 ± 1.0b,α	60.9 ± 2.3b,α
	V	329 ± 6d,α	1927 ± 84bc,α	1447 ± 44a,α	50.2 ± 1.9b,α	495 ± 12d,α	3064 ± 115b,α	2344 ± 89a,α	33.5 ± 1.8a,α	54.1 ± 2.7a,α
signif (1)		***	**	*	**	**	***	***	**	**
B	I	291 ± 9b,β	2307 ± 69c,β	1883 ± 56c,β	59.6 ± 1.8c,β	487 ± 17c,β	3917 ± 117d,β	3209 ± 87c,β	40.2 ± 1.6a,β	65.8 ± 1.7c,α
	II	226 ± 8a,β	1775 ± 75a,α	1445 ± 38a,α	71.8 ± 0.9d,α	375 ± 9a,β	2829 ± 81a,α	2283 ± 69a,α	39.7 ± 1.5a,β	77.9 ± 1.8d,α
	III	278 ± 11b,β	2066 ± 85b,β	1661 ± 47b,β	51.2 ± 2.8a,β	444 ± 16b,β	3211 ± 105b,α	2565 ± 56b,α	37.3 ± 1.2a,β	62.0 ± 2.0bc,α
	IV	313 ± 15c,β	2104 ± 63b,β	1648 ± 74b,β	54.4 ± 1.7ab,β	504 ± 13c,β	3313 ± 83bc,β	2579 ± 88b,β	37.8 ± 1.8a,α	59.7 ± 1.5ab,α
	V	364 ± 11d,β	2112 ± 68b,β	1583 ± 56b,β	57.4 ± 2.0bc,β	597 ± 11d,γ	3482 ± 101c,β	2612 ± 76b,β	39.1 ± 2.0a,β	55.7 ± 1.5a,α
signif (1)		***	***	***	***	***	***	***	ns	***
C	I	303 ± 9a,β	2475 ± 70d,γ	1930 ± 45d,β	62.1 ± 0.7b,β	495 ± 13ab,β	4031 ± 119d,β	3350 ± 90d,β	42.0 ± 1.5b,β	66.0 ± 2.3b,α
	II	282 ± 10a,γ	1876 ± 127a,α	1466 ± 52a,α	83.8 ± 1.7c,β	478 ± 12a,γ	3169 ± 95a,β	2474 ± 76a,β	41.0 ± 0.9b,β	76.5 ± 3.2c,α
	III	353 ± 11c,γ	2233 ± 68c,γ	1720 ± 56c,β	62.8 ± 3.2b,γ	528 ± 19bc,γ	3550 ± 111bc,β	2781 ± 89bc,β	33.2 ± 1.3a,α	60.7 ± 2.8a,α
	IV	327 ± 8b,β	2079 ± 95b,β	1602 ± 60b,β	55.2 ± 1.3a,β	595 ± 17d,γ	3708 ± 107c,γ	2842 ± 97c,γ	44.9 ± 0.8c,β	58.5 ± 3.6a,α
	V	357 ± 6c,β	2248 ± 64c,β	1728 ± 44c,γ	63.8 ± 1.5b,γ	550 ± 9c,β	3412 ± 96b,β	2611 ± 82ab,β	35.1 ± 0.6a,α	60.8 ± 2.2a,β
signif (1)		***	***	***	***	***	***	***	***	***
signif (2)		***,***,***,***,*	***,ns,***,**,**	**,*,***,**,**	***,***,***,**,***	***,***,***,***,***	**,**,*,***,**	**,*,*,**,**	**,**,***,**,**	ns,ns,*,ns,**

[a] All data are expressed as the average value ± standard deviation ($n = 2$). Different Latin letters within the same column indicate significant differences (1) among harvest dates at the same berries density (Tukey *b* test; $p < 0.05$). Different Greek letters within the same column indicate significant differences (2) among the three density classes at the same harvest date (Tukey *b* test; $p < 0.05$). *, **, ***, and ns indicate significance at $p < 0.05$, 0.01, and 0.001 and not significant, respectively. $A_{3.2}$ = total anthocyanins extracted at pH 3.2; $TF_{3.2}$ = total flavonoids extracted at pH 3.2; $FNA_{3.2}$ = nonanthocyanin flavonoids extracted at pH 3.2; A_{280} = total phenolic content; A_1 = total anthocyanins extracted at pH 1; TF_1 = total flavonoids extracted at pH 1; FNA_1 = nonanthocyanin flavonoids extracted at pH 1; EA = cellular maturity index; MP = seed maturity index. [b] A = 1088 kg/m^3; B = 1094 kg/m^3; C = 1100 kg/m^3.

whereas a clear tendency was not observed for TF_1, $TF_{3.2}$, A_{280}, and EA, independent of the berry density measured. For each harvest date, TF_1, $TF_{3.2}$, FNA_1, $FNA_{3.2}$, and A_{280} increased with density class in most of cases, but this increase was not always significant. In a previous work, sorted Barbera grapes harvested on the same date also showed an increasing tendency of the above-mentioned phenol extractability indices when the sugar content increased from 235 to 269 g/L in three levels.[17]

For the last harvest date, the values of the phenol extractability indices (A_1, $A_{3.2}$, TF_1, $TF_{3.2}$, A_{280}, EA, and MP) agreed with those previously published for Nebbiolo grapes in the same production area.[13] On the other hand, the results obtained for A_1, EA, and MP fell within the range reported by Ribéreau-Gayon et al.,[25] who considered values of 500−2000 mg/L, 70−20%, and 60−0%, respectively, as the normal variation range. The values of A_1 and EA depend on the ripeness degree and variety, whereas those of MP depend also on the number of seeds per berry. Furthermore, values lower than 30% for both EA and MP are recommended to indicate a good phenolic maturity. Therefore, the values of EA obtained indicate a good skin cell wall fragility that facilitates anthocyanin extractability, whereas the high values of MP associated with this wine grape variety involve a high contribution of seed tannins.

The phenol extractability indices are determining factors in wine grape quality and have a great impact on the selection of the most suitable winemaking methodology. The elaboration of high-quality red wines requires not only a sufficient accumulation of anthocyanins in berry skins through the ripening period but also the assessment of anthocyanin extractability.[14] Therefore, high color intensity requires management of the winemaking process based on the tendency of the berry skin to yield up anthocyanins.[9,11] It is well-known that the anthocyanin extractability varies through the grape ripening as a consequence of the compositional changes occurring in the skin cell wall during its degradation by pectolytic enzymes.[9,11] In fact, no clear influence of the density class on the anthocyanin extractability in the same harvest date, or with the ripening stage at the same density class, was found. This agrees with two previously published studies in which no significant difference was found in the anthocyanin extractability for sorted Mencía grapes containing 176, 193, and 210 g/L sugars[16] or for sorted Barbera grapes containing 235, 252, and 269 g/L sugars.[17]

A correlation study was performed in an attempt to find a relationship between the percentage of grape berries belonging to each density class in the different ripening stages and the phenol extractability indices. This correlation study permits a better understanding of the effect of the ripening stage on the phenol extractability indices. For density class A, correlation coefficients of −0.983 ($p = 0.017$, $n = 4$) and 0.992 ($p = 0.008$, $n = 4$) were obtained for A_1 and MP, respectively, when the values corresponding to the first harvest date were not considered. This signifies that anthocyanin accumulation in berry skins increased and the contribution of seed tannins diminished with harvest date for those grapes with lower density.

Skin Phenolic Composition and Extractability. The total extractable phenolic composition of the berry skin and the extractability yield in a model hydroalcoholic solution for the five grape ripening stages studied at three different density classes are shown in Table 4. At the same harvest date, total extractable concentrations of TA_{sk}, TF_{sk}, PRO_{sk}, and FVA_{sk} increased with berry density in most cases. However, the variations were only significant for TA_{sk} concentration at harvest dates I−IV and for TF_{sk} content for the first three dates. In particular, an important increase in TA_{sk} with density class was observed, and the difference between classes A and C was 154−204 mg/kg of grapes for harvest dates I, II, and III. Furthermore, TF_{sk} experienced a relevant increase from density class A to C of 371−522 mg/kg of grapes. Therefore, a useful grape densimetric separation could be performed in the winery, by densimetric sorting equipments, at 1094 kg/m^3 to obtain wines with differences in the last two parameters. At the same density class, an irregular tendency was generally found for the total extractable concentration of TA_{sk}, TF_{sk}, PRO_{sk}, and FVA_{sk} through the ripening process. In this case, the more significant differences among harvest dates were associated with density class C, except for FVA_{sk}. The extraction yield ranged as 91.1−94.2, 53.0−63.3, 73.8−80.7, and 77.5−89.7% for TA_{sk}, TF_{sk}, PRO_{sk}, and FVA_{sk}, respectively, but very few significant changes were found for each phenol with the harvest date or the density of berries. In the last stages of grape ripening, the decline rates for all flavanols slowed and, therefore, the composition changed very little.[26]

Other work previously published on Barbera grapes, harvested on the same date and classified into three soluble solid classes defined by flotation, reported significant increases in the concentrations of TA_{sk} and TF_{sk}, but those of PRO_{sk} and FVA_{sk} appeared to be independent of the sugar accumulation in the berry pulp.[17] This also agrees with the increase previously reported in the anthocyanin accumulation in the berry skin of sorted Mencía grapes.[16]

With regard to the anthocyanin profile, Table 5 shows few significant differences among harvest dates or density classes because the anthocyanin profile can be considered as a chemical−taxonomic marker of a certain variety. Nevertheless, some authors showed that environmental factors influence the anthocyanin synthesis.[27,28] The Nebbiolo variety is characterized by a higher percentage of simple glucosides (85.0−88.9%), malvidin and peonidin derivative forms being the majority anthocyanin compounds (26.7−39.9 and 32.8−36.9%, respectively). The results obtained agreed with others previously reported in the literature.[28,29]

In the same ripening stage, it is important to bear in mind that the higher berry density involved a lower percentage of malvidin derivatives and higher ones of petunidin, cyanidin, and delphinidin derivative forms. Hence, the higher proportions of disubstituted anthocyanins and nonacylated anthocyanins contribute to a greater sensitivity to the oxidation reactions and to the color degradation; the color of the denser Nebbiolo grapes may be more easily degraded. The scarcity of significant differences also suggests that the variations observed in the anthocyanin profile are not related to a different sugar accumulations.[17,22]

At the same density class C, significant differences in the anthocyanin profile were obtained for the first harvest date. Higher percentages of simple glucosides and cyanidin derivative and peonidin derivative forms were found in favor of lower percentages of acylated glucosides and malvidin derivative forms. Therefore, the later ripening stages may also involve red wine production with less sensitivity to color degradation.

A correlation study was performed to establish a relationship between the percentage of Nebbiolo grapes belonging to each density class at the different harvest dates and both total extractable phenolic composition and the easily extractable anthocyanin profile. With regard to total extractable phenolic composition, the TF_{sk} concentration was significantly correlated for density class B when the values corresponding to the last ripening

Table 4. Skin Total Extractable Phenolic Composition and Relative Extractability in Model Hydroalcoholic Solution for Nebbiolo Grapes Harvested at Different Ripening Stages and Sorted According to Density[a]

density class[b]	harvest date	TA_{sk} (mg/kg malvidin-3-glucoside chloride)	TA_{sk} (% extraction)	TF_{sk} (mg/kg (+)-catechin)	TF_{sk} (% extraction)	PRO_{sk} (mg/kg cyanidin chloride)	PRO_{sk} (% extraction)	FVA_{sk} (mg/kg (+)-catechin)	FVA_{sk} (% extraction)
A	I	415 ± 9a,α	94.1 ± 0.1a,α	2435 ± 12b,α	59.5 ± 1.8ab,α	2385 ± 258a,α	79.2 ± 0.9ab,α	828 ± 45ab,α	85.9 ± 0.6a,α
	II	328 ± 52a,α	92.3 ± 1.2a,α	2040 ± 61a,α	54.5 ± 2.2ab,α	2249 ± 63a,α	74.6 ± 0.6a,α	703 ± 142a,α	77.5 ± 2.2a,α
	III	332 ± 29a,α	91.9 ± 0.1a,α	2053 ± 28a,α	55.2 ± 0.3ab,α	2319 ± 104a,α	74.7 ± 1.8a,α	1334 ± 163b,α	88.3 ± 2.5a,α
	IV	420 ± 29a,α	93.8 ± 0.1a,α	2262 ± 134ab,α	61.4 ± 2.8b,α	2406 ± 397a,α	80.7 ± 1.8b,α	625 ± 278a,α	80.0 ± 9.4a,α
	V	418 ± 10a,α	91.1 ± 1.2a,α	2277 ± 22ab,α	53.0 ± 1.2a,α	2034 ± 164a,α	73.8 ± 1.6a,α	786 ± 32ab,α	83.7 ± 1.2a,α
signif (1)		ns	ns	**	*	ns	*	*	ns
B	I	511 ± 32a,β	94.2 ± 0.0b,α	2717 ± 60b,β	61.2 ± 1.6a,α	2595 ± 346ab,α	78.8 ± 4.1a,α	1075 ± 225a,α	88.0 ± 3.2a,α
	II	451 ± 19a,αβ	92.9 ± 0.1ab,α	2463 ± 154ab,β	63.3 ± 0.6a,β	2839 ± 36b,β	78.0 ± 2.7a,α	1296 ± 429a,α	86.5 ± 2.0a,β
	III	409 ± 12a,β	92.4 ± 0.6ab,α	2236 ± 22a,β	57.0 ± 1.1a,αβ	2397 ± 117ab,α	76.0 ± 0.8a,α	1407 ± 41a,α	88.8 ± 1.7a,α
	IV	482 ± 25a,αβ	92.7 ± 0.0ab,α	2396 ± 93ab,α	61.8 ± 0.4a,α	2640 ± 123ab,α	78.7 ± 0.4a,α	993 ± 103a,α	88.1 ± 1.8a,α
	V	457 ± 56a,α	91.5 ± 0.7a,α	2151 ± 198a,α	58.5 ± 4.0a,α	2076 ± 116a,α	77.5 ± 0.3a,α	845 ± 168a,α	86.5 ± 3.1a,α
signif (1)		ns	*	*	ns	*	ns	ns	ns
C	I	619 ± 16b,γ	94.1 ± 1.1a,α	2957 ± 13d,γ	63.0 ± 2.6a,α	3202 ± 152c,α	80.3 ± 4.7a,α	1346 ± 251a,α	89.5 ± 0.9a,α
	II	482 ± 19a,β	93.4 ± 0.5a,α	2411 ± 1b,β	62.9 ± 1.3a,β	2642 ± 217b,αβ	80.0 ± 2.2a,α	1616 ± 382a,α	88.7 ± 2.6a,β
	III	499 ± 3a,γ	92.0 ± 0.0a,α	2430 ± 24b,γ	59.5 ± 0.5a,β	2456 ± 51b,α	76.3 ± 1.8a,α	1527 ± 209a,α	89.7 ± 3.1a,α
	IV	533 ± 16a,β	93.5 ± 0.3a,α	2594 ± 37c,α	62.2 ± 0.3a,α	2540 ± 20b,α	78.2 ± 0.7a,α	1002 ± 22a,α	87.6 ± 0.0a,α
	V	483 ± 49a,α	92.2 ± 1.0a,α	2224 ± 98a,α	60.3 ± 2.6a,α	1905 ± 147a,α	78.3 ± 2.5a,α	713 ± 83a,α	86.9 ± 2.2a,α
signif (1)		*	ns	***	ns	**	ns	ns	ns
signif (2)		**,*,**,*, ns	ns,ns,ns,ns,ns	**,*,**, ns, ns	ns,*,*,ns,ns	ns,*, ns, ns, ns	ns,ns,ns,ns,ns	ns, ns, ns, ns, ns	ns,*,ns,ns,ns

[a] All data are expressed as the average value ± standard deviation ($n = 3$). Different Latin letters within the same column indicate significant differences (1) among harvest dates at the same berries density (Tukey *b* test; $p < 0.05$). Different Greek letters within the same column indicate significant differences (2) among the three density classes at the same harvest date (Tukey *b* test; $p < 0.05$). *, **, ***, and ns indicate significance at $p < 0.05$, 0.01, and 0.001 and not significant, respectively. TA_{sk} = skin total anthocyanins; TF_{sk} = skin total flavonoids; PRO_{sk} = skin proanthocyanidins; FVA_{sk} = skin flavanols vanillin assay. [b] A = 1088 kg/m^3; B = 1094 kg/m^3; C = 1100 kg/m^3.

stage were discarded (−0.995, $p = 0.005$, $n = 4$). On the other hand, the FVA_{sk} content showed a significant correlation for density class C when the values associated with the first harvest date were not considered (−0.959, $p = 0.041$, $n = 4$). For the higher berry density, a significant correlation was found for delphinidin derivatives (−0.978, $p = 0.004$, $n = 5$). When the results obtained for the first harvest date were discarded, this correlation factor increased to −1.000 ($p = 0.000$, $n = 4$), and a new statistical correlation was found for petunidin derivatives (−0.973, $p = 0.027$, $n = 4$). Both delphinidin and petunidin derivatives also showed a good correlation for density class B when the values associated with the last harvest date were not considered (−0.965, $p = 0.035$, $n = 4$; −0.976, $p = 0.024$, $n = 4$, respectively). This confirms that the anthocyanin compounds more prone to oxidation diminished with the increase in the proportion of berries belonging to density classes B and C through the grape ripening and that the skin flavanol reactivity decreased with the increase in the proportion of the denser berries. It agreed with the loss in the skin tannins reactivity during ripening.[25]

Skin Mechanical Properties. The mechanical properties of berry skins for Nebbiolo grapes harvested on five different dates and classified in three density classes are shown in Table 6. The puncture parameters of berry skins increased with berry density at each harvest date studied. However, this increment was not always significant. Although very few significant changes were reported in the parameters that characterize the berry skin hardness (F_{sk} and W_{sk}) and the tissue rigidity or stiffness (E_{sk}) of Barbera grapes containing different soluble solid contents, an increasing tendency of Sp_{sk} values with the sugar accumulation was observed.[17]

When the modification of the skin mechanical characteristics at a certain density class through the five ripening stages evaluated was studied, Table 6 shows values of the E_{sk} parameter significantly higher and those of Sp_{sk} significantly lower for the three density classes at harvest date III, particularly for berry density A. Furthermore, a correlation study was then carried out to establish a relationship among the percentage of grape berries belonging to each density class in the different ripening stages and the skin mechanical attributes. Nevertheless, no significant correlation was found.

Several studies suggested that the behavior of F_{sk} values close to the harvest time could limit the choice of this parameter as a maturity indicator in grape berries. In fact, from veraison to ripeness, an increase in the F_{sk} parameter is observed, particularly in the first ripening phases, with a steady value or a slight decrease close to technological maturity.[24] This same behavior was also observed in Nebbiolo grapes for density classes A and C, achieving the higher F_{sk} values at harvest dates II and III.

Recently, instrumental texture analysis has also been used for a rapid estimation of anthocyanin extractability. In particular, berry skin break force can be considered the best mechanical parameter to estimate the kinetics of anthocyanin extraction with adequate reliability,[15] whereas berry skin thickness has been proposed to predict the percentage of extractable anthocyanins.[16] Mechanical

Table 5. Extractable Anthocyanin Profile for Nebbiolo Grapes Harvested at Different Ripening Stages and Sorted According to Density[a]

density class[b]	harvest date	simple glucosides (%)	acetyl-glucosides (%)	cinnamoyl-glucosides (%)	delphinidin derivatives (%)	cyanidin derivatives (%)	petunidin derivatives (%)	peonidin derivatives (%)	malvidin derivatives (%)
A	I	87.9 ± 0.0a,α	4.8 ± 0.1a,αβ	7.3 ± 0.1a,α	8.5 ± 0.8a,α	12.2 ± 1.0a,α	7.5 ± 0.5a,α	34.0 ± 2.7a,α	37.8 ± 2.4a,β
	II	86.3 ± 0.9a,α	5.1 ± 0.3a,α	8.5 ± 0.6a,α	7.9 ± 0.1a,α	11.9 ± 0.5a,α	7.0 ± 0.1a,α	34.4 ± 0.2a,α	38.9 ± 0.7a,α
	III	85.0 ± 1.9a,α	5.6 ± 0.6a,α	9.4 ± 1.3a,α	7.3 ± 0.6a,α	11.8 ± 2.9a,α	6.7 ± 0.1a,α	34.3 ± 2.2a,α	39.9 ± 5.6a,α
	IV	86.0 ± 0.5a,α	5.0 ± 0.3a,α	9.0 ± 0.1a,β	7.9 ± 0.2a,α	12.4 ± 0.2a,α	7.1 ± 0.1a,α	33.8 ± 0.4a,α	38.8 ± 0.8a,β
	V	86.1 ± 0.7a,α	5.0 ± 0.0a,α	8.9 ± 0.7a,α	7.7 ± 0.2a,α	13.4 ± 1.5a,α	6.8 ± 0.1a,α	34.5 ± 1.0a,α	37.6 ± 2.8a,α
signif (1)		ns	ns	ns	ns	ns	ns	ns	ns
B	I	87.5 ± 0.6a,α	5.1 ± 0.1a,β	7.4 ± 0.5a,α	10.1 ± 0.2a,α	14.6 ± 0.7a,α	8.2 ± 0.0a,α	32.8 ± 0.4a,α	34.3 ± 0.5a,β
	II	87.3 ± 0.7a,α	5.2 ± 0.2a,α	7.5 ± 0.5a,α	9.6 ± 1.1a,α	14.5 ± 1.0a,α	7.7 ± 0.6a,α	34.4 ± 3.0a,α	33.8 ± 2.3a,α
	III	85.3 ± 1.6a,α	5.9 ± 0.6a,α	8.8 ± 1.0a,α	8.5 ± 0.3a,αβ	12.8 ± 1.0a,α	7.3 ± 0.1a,β	33.0 ± 0.4a,α	38.4 ± 1.8a,α
	IV	86.5 ± 0.3a,α	5.2 ± 0.2a,α	8.3 ± 0.1a,αβ	9.0 ± 0.2a,α	14.3 ± 1.3a,α	7.5 ± 0.0a,α	34.7 ± 0.2a,α	34.6 ± 1.2a,α
	V	86.2 ± 0.5a,α	5.2 ± 0.4a,α	8.5 ± 0.2a,α	8.7 ± 0.5a,α	14.6 ± 0.2a,α	7.3 ± 0.2a,αβ	35.3 ± 1.5a,α	34.1 ± 1.0a,α
signif (1)		ns	ns	ns	ns	ns	ns	ns	ns
C	I	88.9 ± 0.5b,α	4.7 ± 0.1a,α	6.4 ± 0.4a,α	10.1 ± 0.7a,α	18.5 ± 0.5b,β	7.8 ± 0.2a,α	36.9 ± 0.8b,α	26.7 ± 0.6a,α
	II	86.7 ± 0.3a,α	5.4 ± 0.1b,α	7.9 ± 0.4b,α	10.3 ± 0.5a,α	15.0 ± 1.7a,α	8.1 ± 0.0a,α	33.8 ± 0.1a,α	32.7 ± 2.2b,α
	III	86.6 ± 0.2a,α	5.5 ± 0.2b,α	7.9 ± 0.0b,α	9.7 ± 0.4a,β	15.3 ± 0.6a,α	7.7 ± 0.0a,γ	34.6 ± 0.2ab,α	32.8 ± 1.2b,α
	IV	86.8 ± 0.6a,α	5.4 ± 0.3b,α	7.9 ± 0.3b,α	9.2 ± 0.8a,α	15.0 ± 0.5a,α	7.6 ± 0.4a,α	35.5 ± 0.8ab,α	32.7 ± 0.9b,α
	V	86.5 ± 0.2a,α	5.5 ± 0.1b,α	8.1 ± 0.1b,α	8.8 ± 0.1a,α	13.3 ± 0.4a,α	7.4 ± 0.1a,β	34.9 ± 0.7ab,α	35.7 ± 1.1b,α
signif (1)		**	*	**	ns	*	ns	*	**
signif (2)		ns,ns,ns,ns,ns	*,ns,ns,ns,ns	ns,ns,ns,*, ns	ns,ns,*,ns, ns	**,ns,ns,ns,ns	ns,ns,**,ns,*	ns,ns,ns,ns,ns	*,ns,ns,*,ns

[a] All data are expressed as the average value ± standard deviation ($n = 3$). Different Latin letters within the same column indicate significant differences (1) among harvest dates at the same berries density (Tukey b test; $p < 0.05$). Different Greek letters within the same column indicate significant differences (2) among the three density classes at the same harvest date (Tukey b test; $p < 0.05$). *,**, and ns indicate significance at $p < 0.05$ and 0.01 and not significant, respectively. [b] A = 1088 kg/m^3; B = 1094 kg/m^3; C = 1100 kg/m^3.

methods are inexpensive, allowing their application as a routine monitoring tool for grape quality. Thinner skins seem to be characterized by a greater cellular maturity index,[30] but this behavior was confirmed only when Sp_{sk} values among the three density classes were compared for harvest dates I, II, and III. Separation of the grape berries on the basis of the anthocyanin extractability estimated from Sp_{sk} could be possible using flotation with the density of 1094 kg/m^3, which was already proposed for the determination of the berry skin phenolic composition.

Relationship among Mechanical and Chemical Parameters of Berry Skins. To summarize, this study evaluated the possible dependence of phenol composition and/or extractability on skin mechanical attributes, irrespective of the effect of harvest date or density class (Table 7). The mechanical properties do not seem to be well related to the red pigments accumulated in berry skins. On the other hand, the puncture parameters have been also evaluated as potential estimators of the accumulation power of berry skins for total and easily extractable concentrations of flavonoids, proanthocyanidins, and flavanols reactive to vanillin, as well as of the facility of berry skins to yield them. Total and easily extractable concentrations of TF_{sk} and FVA_{sk} also showed a low correlation with the E_{sk} parameter, whereas the concentrations of PRO_{sk} achieved factors ranging 0.705 and 0.756 ($p < 0.004$, $n = 15$). Furthermore, better relationships for total and easily extractable concentrations of FVA_{sk} were obtained with the F_{sk} parameter with correlation factors varying between 0.766 and 0.774 ($p < 0.001$, $n = 15$). The extractability of FVA_{sk} is also little correlated with the F_{sk} parameter. On the other hand, the extraction yield for TF_{sk} and PRO_{sk} is correlated with the Sp_{sk} parameter, correlation factors being 0.567 ($p = 0.028$, $n = 15$) and 0.671 ($p = 0.006$, $n = 15$), respectively. The Sp_{sk} parameter facilitates the estimation of the skin cell wall degradability and, therefore, of the extractability of proanthocyanidins. According to Rolle et al.,[29] higher skin hardness probably involves greater cell wall fragility, which agrees with the tendency of the extraction yield of flavanols reactive to vanillin to increase when the F_{sk} parameter increases.

Although further studies are necessary with increasing grape varieties, growing areas, and vintages to achieve more robust conclusions, this first approach showed that, for a given harvest date, the denser grapes provide, in general, higher total and easily extractable concentrations of phenolic compounds. Because the heterogeneity of the grapes harvested determines the variability of the results obtained, the relevance of the information provided for the management of the winemaking process diminishes according to grape heterogeneity. The lack and low reliability of the statistical correlations found between the percentage of grape berries belonging to each density class, in the different ripening stages, and the mechanical/chemical parameters confirmed that the changes observed in the latter through the grape ripening process are more related to berry density than harvest date. This suggests the effectiveness of the automatic winery equipment of berry densimetric sorting recently developed whenever the flotation density is selected according to objective

Table 6. Berry Skin Mechanical Parameters for Nebbiolo Grapes Harvested at Different Ripening Stages and Sorted According to Density[a]

density class[b]	harvest date	F_{sk} (N)	W_{sk} (mJ)	E_{sk} (N/mm)	Sp_{sk} (μm)
A	I	0.652 ± 0.089a,α	0.483 ± 0.100a,α	0.402 ± 0.051a,α	205 ± 23b,β
	II	0.696 ± 0.092a,α	0.547 ± 0.161a,α	0.409 ± 0.054a,α	186 ± 23b,α
	III	0.744 ± 0.122a,α	0.564 ± 0.158a,α	0.457 ± 0.042b,α	145 ± 18a,α
	IV	0.643 ± 0.136a,α	0.511 ± 0.177a,α	0.383 ± 0.063a,α	202 ± 29b,α
	V	0.649 ± 0.145a,α	0.521 ± 0.169a,α	0.374 ± 0.051a,α	192 ± 33b,α
signif (1)		ns	ns	***	***
B	I	0.734 ± 0.111a,α	0.563 ± 0.146a,α	0.435 ± 0.048bc,α	185 ± 24b,α
	II	0.714 ± 0.146a,α	0.566 ± 0.183a,α	0.416 ± 0.066ab,α	206 ± 26b,β
	III	0.733 ± 0.109a,α	0.533 ± 0.119a,α	0.468 ± 0.061c,α	161 ± 21a,β
	IV	0.753 ± 0.164a,β	0.593 ± 0.232a,α	0.458 ± 0.064bc,β	200 ± 32b,α
	V	0.657 ± 0.186a,α	0.554 ± 0.266a,α	0.373 ± 0.054a,α	183 ± 36b,α
signif (1)		ns	ns	***	***
C	I	0.728 ± 0.130a,α	0.578 ± 0.162a,α	0.417 ± 0.076ab,α	206 ± 28bc,β
	II	0.800 ± 0.126ab,β	0.700 ± 0.173a,β	0.412 ± 0.047ab,α	223 ± 23c,γ
	III	0.861 ± 0.117b,β	0.714 ± 0.188a,β	0.481 ± 0.056c,α	164 ± 23a,β
	IV	0.740 ± 0.103a,β	0.570 ± 0.134a,α	0.446 ± 0.059bc,β	182 ± 21ab,α
	V	0.730 ± 0.125a,α	0.650 ± 0.195a,α	0.375 ± 0.055a,α	200 ± 49bc,α
signif (1)		**	ns	***	***
signif (2)		ns,*,**,*,ns	ns,*,**,ns,ns	ns,ns,ns,***,ns	*,***,*,ns,ns

[a] All data are expressed as the average value ± standard deviation ($n = 30$). Different Latin letters within the same column indicate significant differences (1) among harvest dates at the same berries density (Tukey b test; $p < 0.05$). Different Greek letters within the same column indicate significant differences (2) among the three density classes at the same harvest date (Tukey b test; $p < 0.05$). *, **, ***, and ns indicate significance at $p < 0.05$, 0.01, and 0.001 and not significant, respectively. F_{sk} = berry skin break force; W_{sk} = berry skin break energy; E_{sk} = berry skin Young's modulus; Sp_{sk} = berry skin thickness. [b] A = 1088 kg/m^3; B = 1094 kg/m^3; C = 1100 kg/m^3.

Table 7. Correlation Study among Mechanical and Chemical Parameters of Berry Skins for Nebbiolo Grapes[a]

phenolic composition	F_{sk} (N)	W_{sk} (mJ)	E_{sk} (N/mm)	Sp_{sk} (μm)
TA_{sk} total extractable (mg/kg malvidin-3-glucoside chloride)	ns[b]	ns	0.515*	ns
TA_{sk} easily extractable (mg/kg malvidin-3-glucoside chloride)	ns	ns	0.524*	ns
TF_{sk} total extractable (mg/kg (+)-catechin)	ns	ns	0.621*	ns
TF_{sk} easily extractable (mg/kg (+)-catechin)	ns	ns	0.560*	ns
PRO_{sk} total extractable (mg/kg cyanidin chloride)	ns	ns	0.756**	ns
PRO_{sk} easily extractable (mg/kg cyanidin chloride)	ns	ns	0.705**	ns
FVA_{sk} total extractable (mg/kg (+)-catechin)	0.766**	0.561*	0.615*	ns
FVA_{sk} easily extractable (mg/kg (+)-catechin)	0.774**	0.572*	0.624*	ns
TF_{sk} (% extraction)	ns	ns	ns	0.567*
PRO_{sk} (% extraction)	ns	ns	ns	0.671**
FVA_{sk} (% extraction)	0.618*	ns	0.538*	ns

[a] F_{sk} = berry skin break force; W_{sk} = berry skin break energy; E_{sk} = berry skin Young's modulus; Sp_{sk} = berry skin thickness; TA_{sk} = skin total anthocyanins; TF_{sk} = skin total flavonoids; PRO_{sk} = skin proanthocyanidins; FVA_{sk} = skin flavanols vanillin assay. [b] Significance: *, **, and ns indicate significance at $p < 0.05$, 0.01, and not significant, respectively.

quality properties of the grape such as TA_{sk}, TF_{sk}, and Sp_{sk}. Therefore, the winemaker has to select the flotation density on the basis of the wine type he wishes to elaborate. This work also highlights the importance of improving knowledge of the physical modifications of the cell tissues through grape ripening to assess the evolution of the phenol composition and extractability because the influence of skin mechanical properties on total flavonoids, proanthocyanidins, and flavanols reactive to vanillin has not been previously studied. Stiffer grapes allowed accumulation of more proanthocyanidins, whereas harder ones provided higher concentration and extractability of flavanols reactive to vanillin. On the other hand, the thicker grapes facilitated the extraction of proanthocyanidins. This first approach demands further research on histological and histochemical changes in berry skins during grape development.

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