Research Article

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Combined effect of harvest time and postharvest dehydration length on the composition of withered grapes for *Sforzato di Valtellina* DOCG wine production

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Abstract

BACKGROUND: ***Sforzato di Valtellina*** **(*Sfursat*) is a PDO reinforced red wine produced in Valtellina (northern Italy) from partially withered red grapes (*Vitis vinifera* L.) cv. Nebbiolo. The present study aimed to evaluate the combined influence of different grape ripeness levels and withering length on the chemical composition, mechanical properties, and phenolic profile of Nebbiolo winegrapes from two Valtellina vineyards. During three consecutive vintages (2019, 2020, and 2021), three different technological binomials have been tested: early harvest/long withering (EL), medium-term harvest/medium-term withering (MM), and late harvest/short withering (LS).**

RESULTS: At the end of the withering process, EL thesis usually presented the highest values of sugars and acidity. Extractable seed polyphenols showed a decreasing trend by leaving the grapes on the plant longer, and this effect increased considerably after withering with respect to fresh samples. EL and MM evidenced the greater concentration of these compounds expressed on grape weight, particularly for tannins. Instead, skin-extracted total phenolics were less influenced by the harvest time, whereas their concentration increased after withering. The harvest time appears to have a higher impact than the withering length on the final extractable anthocyanin content, although the trend was no stable during the vintages or common for the two vineyards evaluated. EL and MM experienced the highest contents of grape skin tannins in most cases, suggesting that a longer withering increases their concentration.

CONCLUSION: Harvest time and withering length can be modulated according to the desired oenological objective, promoting the valorization of grape potentialities. The choice to harvest the grapes earlier and enhance the withering length should be preferred to obtain wines with higher acidity and phenolic content, more suitable for long-ageing period.

Supporting information may be found in the online version of this article.

Keywords: grape postharvest; phenolic compounds; withering process; red winegrapes; reinforced wines; *Sforzato di Valtellina* DOCG

INTRODUCTION

Territorial identity represents an added value for the winegrowing activities. It has a central role for the wine market for not only for economic aspects, but also cultural and social development reasons.[1-3] Therefore, it is essential to preserve the quality of unique and typical wines such as the *Sforzato di Valtellina* DOCG (Denominazione di Origine Controllata e Garantita or Protected Designation of Origin, PDO), one of the main identifying results of the so-called heroic steep slope viticulture and winemaking of Valtellina alpine valley (Northern Italy). This type of wine, which is also locally called '*Sfursat*', is a reinforced dry red wine produced with partially withered cv. Nebbiolo red winegrapes (*Vitis vinifera* L.). The Designation of Origin guidelines for

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this wine stipulates that the postharvest withering process must take place in uncontrolled conditions in fresh and dry dehydration rooms named *fruttai*. The grapes are usually placed in single-layer crates or on reed mats, and the withering starts immediately after the harvest and lasts until the grape crushing, which occurs no earlier than 1 December of the same year.[4]

In the production process of these special wines, there are two determining variables that can influence the chemical–physical features of the dehydrated grapes and, consequently, the quality of the wines: the ripeness degree at the harvest, and the withering process length and conditions such as temperature, relative humidity, and air flow speed.[5-9]

The importance of the ripeness degree and the withering conditions on grape phenolic composition have been separately studied in recent years,[10-13] but, to the best of our knowledge, there is little information available in the literature on the combined effect of these two variables on the grape quality features and phenolic profile. Grape skin and seeds contain several classes of phenolic compounds, which are significantly affected by these factors and strictly associated with red wines quality.[14-16]

The attempt to obtain a better understanding on this topic represents a considerable challenge because of several other factors requiring consideration, such as the different climatic conditions of the year or the vineyard location and management.[17-19] Accordingly, a 3-year experimental plan (vintages 2019, 2020, and 2021) was designed to answer the question: 'what is the best time to harvest red grapes destined for withering?'. The influence of three different binomials of grape ripeness degree and withering length have been studied, comparing their effects on grape must composition, grape skin and seed potential phenolic content, and grape skin mechanical properties of fresh and withered Nebbiolo grapes from vineyards with different locations in the Valtellina valley for the three vintages.

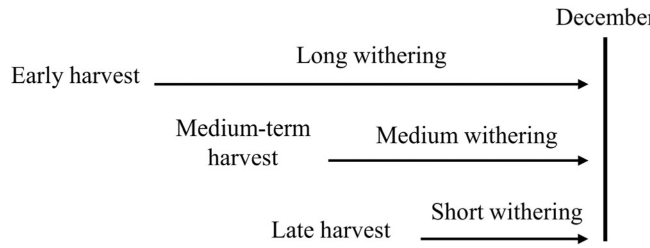

Figure 1. Experimental plan schematizing the length of the dehydration process for each binominal considered.

MATERIALS AND METHODS

Grape samples and the withering process

Grape samples of cv. Nebbiolo (*V. vinifera* L.) were harvested from two commercial vineyards located at the two opposite ends of the vine growing area in the Valtellina valley (northern Italy): (A) the upper-valley vineyard, set in the western part (Villa di Tirano, 46°12'N, 10°8'E, 400 m asl) and (B) the lower-valley vineyard, located in the eastern end of the valley (Berbenno di Valtellina, 46°10'N, 09°45'E, 370 m asl). For each vineyard, over the three consecutive years of experimentation (vintages 2019, 2020, and 2021), three different binomials have been tested: early harvest/long withering (EL), medium-term harvest/medium-term withering (MM), and late harvest/short withering (LS). To accomplish this task, the grapes were harvested every year for each vineyard at three different ripeness degrees according to the grape soluble solid content reached, with a target soluble solid contents of 21.5% (w/w) for EL thesis. Each year, MM target was 1% more than EL thesis, whereas LS was either targeted at 1% increment from MM or lower if the climate did not allow reaching this target. At each stage, approximately 300 kg of grapes were harvested. A sample of 10 kg of these grapes was randomly collected for the analysis on fresh material before withering, and the remaining grapes were placed in single-layer plastic crates in a typical *fruttaio* (uncontrolled dehydration room). For all the samples, as established by the DOCG product regulation guidelines,[4] the withering lasted until 1 December of the same harvest year. Consequently, the length of the dehydration process depended on the harvest date, as shown in Fig. 1.

Harvest date and total days of withering are shown in the Supporting information (Table S1). The long withering process lasted approximately 70 days in total and, among the three different withering periods, there were around 10 days of difference.

Eight randomized single-layer crates for each binomial have been weighted before and after the withering process to estimate the effective weight loss percentage (WL%), calculated as: [1 – (net weight of withered grapes in kg/net weight of fresh grapes in kg)]. A sample of withered grapes has been collected for each binomial/vineyard tested for the laboratory analyses.

Weather data

The meteorological data of temperature (°C) and precipitation (mm) were recorded at the weather station of Sondrio (SO, Italy) and provided by ARPA Lombardia[20] for the three entire consecutive harvest years of the study (2019, 2020, and 2021).

Chemical analysis

Chemical reagents and standards

Malvidin-3-*O*-glucoside chloride standard was provided by Extrasynthese (Genay, France). Methylcellulose, standards of (+)-catechin and (–)-epicatechin, and HPLC-gradient grade solvents were supplied by Sigma-Aldrich (St Louis, MO, USA). Deionized water used for preparing the solutions was produced by a Milli-Q system (Merck Millipore, Darmstadt, Germany).

Sample preparation and standard parameter determination

In the laboratory, for each sample of fresh or withered grapes, the berries were handpicked from the stalk without detaching the pedicel and visually inspected to eliminate the damaged ones. Three replicates of approximately 100 g of berries were collected and manually crushed for 2 min. The obtained grape must was centrifuged at 3000 × *g* for 15 min at 20 °C using a Hettich 32R centrifuge (Hettich, Tuttlingen, Germany) and the supernatant was analyzed. Total soluble solids were determined using a refractometer with automatic temperature compensation (Atago Palette 0-32; Atago Corporation, Tokyo, Japan). A pH meter (InoLab pH 730; WTW, Weilhelm, Germany) was used to measure pH by potentiometry, and total acidity (as g L^{-1} of tartaric acid) was determined by titration with sodium hydroxide 0.1 mol L^{-1} according to method OIV-MA-AS313-01.[21] Reducing sugars (as sum of glucose and fructose), glycerol, and organic acids (citric, tartaric, and malic acids) were determined using a HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a refractive index and a UV detector.[22]

Extraction and determination of phenolic compounds from grape skins and seeds

For each sample, three sets of 40 g of berries were randomly selected and weighted. The evaluation of extractable phenolic compounds was carried out separately for the different grape berry components. Grape skins and seeds were separated for each set, cleaned from the pulp with the aid of a laboratory spatula. Once cleaned, the flesh was discarded and each set of skins or seeds was immediately immersed in 50 mL of a wine-like solution (15% v/v ethanol, 5 g L^{-1} tartaric acid, and 100 mg L^{-1} $Na_2S_2O_5$, adjusted to pH 3.20 with NaOH 1 mol L^{-1}), following the proportions described by Mattivi *et al.*[23] to mimic a winemaking condition of a reinforced wine. The same weight of berries (40 g in 50 mL of wine-like solution) was maintained for both fresh and dehydrated grape samples in order to take into account the modifications of solid-to-liquid proportion due to weight loss. The flasks were placed at 25 °C controlled temperature and daily mixed for 5 min with the aid of internal magnetic stirring bars (20 × 6 mm). After 7 days of extraction, the liquid extracts were collected and used for the analyses.

Phenolic compounds were determined with a UV-1800 spectrophotometer (Shimazdu Corp., Kyoto, Japan) by spectrophotometric methods.[24,25] Total phenolic index (TPI, expressed as mg (−)-epicatechin/kg berries) was obtained measuring the absorbance at 280 nm of the sample diluted 100 times in water and quantified using a (−)-epicatechin calibration curve ($y = 82.158x$, $r^2 = 0.999$). A dilution with ethanol:water:37% hydrochloric acid (70:30:1 v/v) solution was performed to determine total anthocyanins (TA, expressed as mg malvidin-3-glucoside chloride/kg berries) and total flavonoids (TF, mg (+)-catechin/kg berries), measuring the maximum absorbance at 536–540 nm for the former, and applying a graphical correction to the absorbance at 280 nm for the latter.[24] Condensed tannins (MCP, mg (−)-epicatechin/kg of grapes) were quantified by precipitation with methyl cellulose, using a 0.04% methyl cellulose solution and a sample dilution factor of 20.[26]

Grape skin mechanical properties

Grape skin mechanical properties were evaluated using a TA. XTplus Universal Testing Machine (Stable Micro Systems, Godalming, UK). The Texture Analyzer was equipped with an HDP/90 platform, a SMS P/2N needle probe used for skin hardness evaluation (berry skin break force, F_{sk}, N; berry skin break energy, W_{sk}, mJ; berry skin resistance against deformation, E_{sk}, N/mm) or a flat cylindrical probe (SMS P/2, diameter 2 mm) used for skin thickness evaluation (Sp_{sk}, μm), and a 5 kg load cell.[27] For each binomial/vineyard studied, 30 fresh or withered berries were randomly selected and individually subjected to the compression and penetration/puncture tests. The data were acquired using the Texture Exponent software (Stable Micro Systems).

Statistical analysis

Statistical analysis was executed using R, version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria). The Tukey *b* post-hoc test at $P < 0.05$ was used to define significant differences among the three binomials tested by one-way analysis of variance. A *t*-test was used to discriminate significant differences among fresh and withered grapes.

Multivariate analysis was performed through principal component analysis (PCA) to explore the association between the variables (grape chemical composition parameters) and groups (vineyards and treatment). Before conducting the PCA, data was normalized inside each year by using the *Z*-score transformation to exclude any variability caused by the vintage, as previously reported by Škrab *et al.*[28] The PCA was performed using R software and the package FactoMineR, and its results were extracted and visualized using R packages factoextra and ggplot2, respectively.

RESULTS AND DISCUSSION

Weather conditions

The climate of the east–west oriented alpine valley of Valtellina (46°10'N, Lombardy, northern Italy) is classified as endo-alpine, with an average of 800–1200 mm of yearly rainfall mainly distributed in the western part of the valley, and a windy regime characterized by breeze and *Föhn* phenomena.[29] As shown in Fig. 2, the weather conditions of the three vintages were very different from each other. The year 2019 was characterized by a dry and warm summer (with a maximum of 39.5 °C reached at the end of June) and a rainy autumn (466 mm), being the hottest of the 3 years in the period close to the harvest. The first half of 2020 was cooler than the previous year, whereas the summer was slightly hotter, and, in the second half of the year, rainfalls were significantly above average (314 mm in summer and 446 mm in autumn), especially over harvest time. In 2021, the beginning of the year was dry, the cool spring was followed by a very hot summer with a rainy July and a warm autumn. In general, the data recorded in the period close to the harvest time (from August to October, Fig. 2b) show that 2019 was the hottest of the 3 years considered (18.4 *versus* 17.6 *versus* 17.6 °C of average yearly temperature for 2019, 2020, and 2021, respectively), 2020 was the wettest vintage (497 mm from August to October *versus* 339 and 333 mm of vintages 2019 and 2021, respectively), and 2021 resulted the driest harvest year (with a decrease of 200 mm with respect to the total amount of rainfall of the previous years).

Grape must chemical composition

Technological parameters of fresh grapes

The standard parameters of fresh grapes for the years 2019, 2020, and 2021 are shown in Table 1. As provided by the research plan, higher sugars levels were found in late harvested grapes (224–258 g L^{-1}) with respect to the earliest ones (208–230 g L^{-1}). The glucose/fructose ratio in fresh Nebbiolo grapes was almost 1, which is the typical ratio for ripe grapes,[30] and experienced the tendency to decrease or remained almost constant leaving the grapes on the plant longer (0.96–1.00 for MM; 0.94–0.98 for LS), in accordance with the literature.[31]

Total acidity (expressed as g L^{-1} of tartaric acid) tended to decrease in fresh grapes from early to late harvest by an average of 1.9 and 0.9 g L^{-1} for vineyard A and B, respectively. Malic and tartaric acids tend to decrease progressively with the ripening process in fresh grapes as a result of respiratory metabolism and dilution, respectively.[32,33] In this case, this behavior was observed mainly in 2019–2020, with the exception of malic acid content in 2019 vintage for vineyard A and tartaric acid in 2019 for vineyard B. In the acidic composition described above, late harvested grapes of vintage 2021 were not in line, presenting a higher tartaric acid content in fresh grapes from vineyard A with respect to the previous harvest points of the same year. This behavior is probably influenced by the drought of the year, which could have induced a situation of slight dehydration of the grapes on the plant.[34,35]

The general effect on the ratio between juice sugar contents and total acidity values according to the harvest time is clearly visible: this ratio increases significantly from early to late harvest, reaching an average of approximately 30 points in the final harvest date (see Supporting information, Table S2).

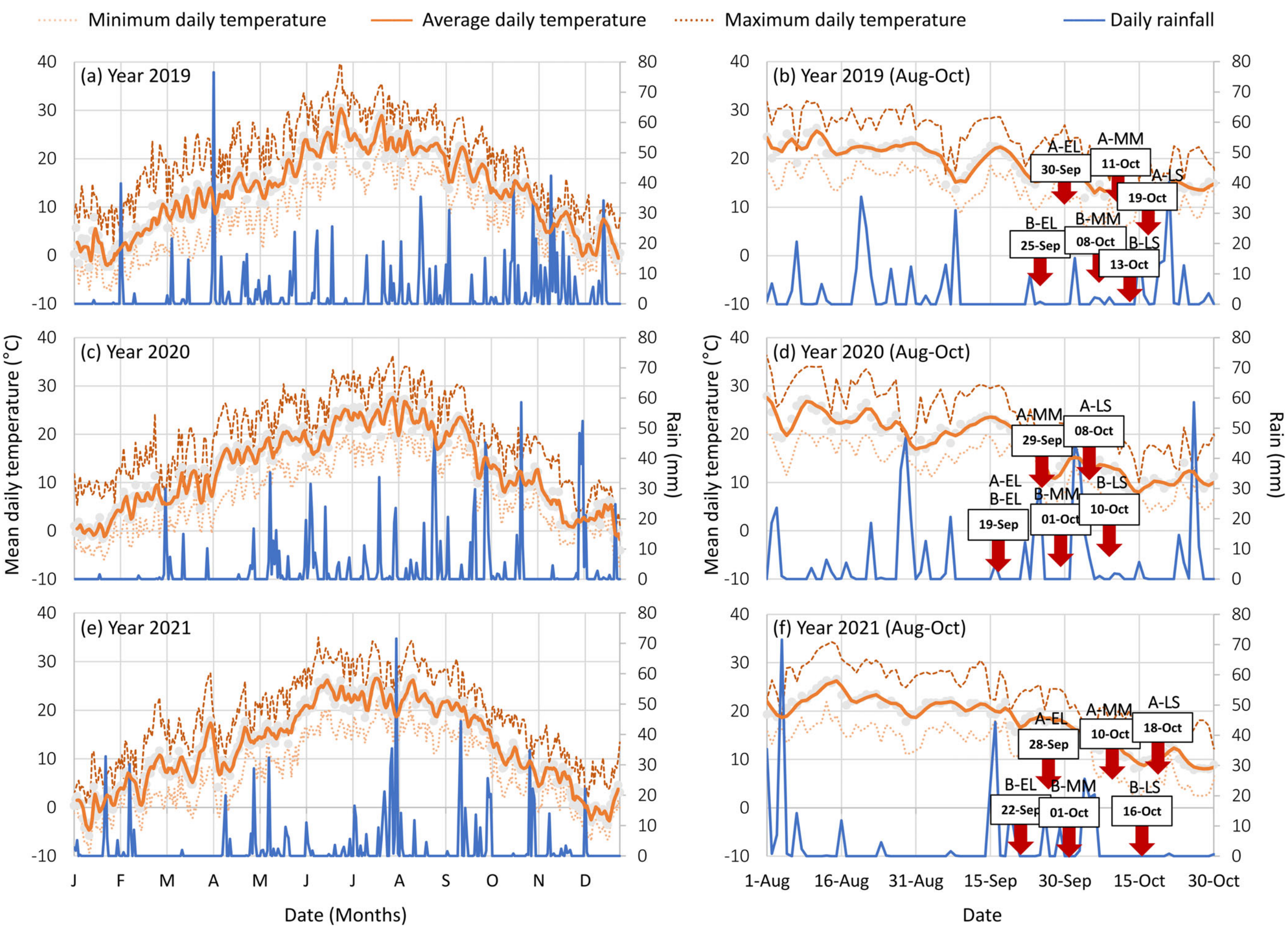

Figure 2. Minimum (dotted orange), maximum (dashed orange), and average (solid orange) daily temperature and rainfall (blue lines) of the three consecutive harvest years studied (a, c, e), harvest times and weather conditions of the months near the harvest (b, d, f) from the weather station located in Sondrio. Data from ARPA Lombardia.[18]

Technological parameters of withered grapes

The average percentages of grape weight loss (WL%) for the 3 years and two vineyards at the end of the withering process were 19 ± 5, 16 ± 4, and 12 ± 3% for EL, MM, and LS, respectively. These differences detected in WL% are consistent with the expectations. Indeed, the harvest time of each binomial resulted in a step decrease of approximately 10 days in terms of withering length between EL and LS trials.

Technological parameters of withered grapes for the years 2019, 2020, and 2021 are shown in Table 2. As regards sugar content in withered grapes, the longer the withering period, the greater the percentage increase of sugars in withered grapes with respect to fresh ones as a result of a concentration effect, leading the EL thesis to be the richest in sugar content at the end of the process (247–292 g L^{-1}, with a mean difference of +9.8 g L^{-1} with respect to LS samples). For each binomial studied, dehydrated grapes showed a decreased glucose/fructose proportion with respect to fresh ones from a range 0.94–1.02 (fresh) to 0.91–0.96 (dehydrated grapes), coherently with previous studies.[36-38] The observed movement of the ratio in favor of fructose suggested that, during the withering, glucose may have been used for the respiration or to feed other metabolic pathways.[39,40]

The combined effect of sugars accumulation and weight loss during withering was also studied through the SIR-to-WLR (sugar increase rate as °Brix/day-to-weight loss percentage rate/day) parameter[6]: The general average of this parameter was found 0.26 °Brix/%, with a non-significant increase in EL samples with respect to MM and LS (see Supporting information, Table S2), indicating a possible dominance of the concentration effect with relation to other metabolic processes such as sugars respiration.[6,41] The vintage effect was not significant, but a growing tendency in 2020 data can be seen compared to 2019 and 2021 data (see Supporting information, Table S2).

As regards total acidity, the concentration effect because of dehydration opposed the metabolic losses of acidity detected in withered grapes compared to the fresh ones. Consequently, at the end of the process, the EL thesis showed higher total acidity values (+1.21 g L^{-1} and + 0.85 g L^{-1}, respectively, on average with respect to LS and MM) and the lower pH values, confirming that the management of harvest time plays a central role in the achievement of a balanced sugar-to-acid ratio in withered grapes (see Supporting information, Table S2), as previously hypothesized by Failla *et al.*[42] Furthermore, this ratio was also found to be significantly influenced by the vintage (see Supporting information, Table S2), as previously demonstrated.[43]

The content of individual organic acids in withered grape juice also changed. Indeed, at the end of the withering process, the EL thesis showed the highest concentrations of malic and citric

Table 1. Standard parameters of fresh grapes

Harvest year	Ripeness parameter	Vineyard A				Vineyard B			
		EL	MM	LS	Significance	EL	MM	LS	Significance
2019	Reducing sugars (g L^{-1})	230 ± 1 c	245 ± 2 b	258 ± 8 a	**	230 ± 3	240 ± 3	239 ± 5	ns
	pH	3.04 ± 0.01 c	3.12 ± 0.01 b	3.23 ± 0.01 a	***	3.09 ± 0.05	3.07 ± 0.02	3.09 ± 0.02	ns
	TA (g L^{-1} tartaric acid)	10.20 ± 0.00 a	8.27 ± 0.21 b	8.23 ± 0.12 b	***	9.67 ± 0.50	9.23 ± 0.15	8.93 ± 0.31	ns
	Citric acid (g L^{-1})	0.22 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	ns	0.23 ± 0.01 a	0.19 ± 0.01 b	0.23 ± 0.01 a	**
	Tartaric acid (g L^{-1})	8.34 ± 0.16 a	7.83 ± 0.09 b	7.11 ± 0.20 c	***	8.08 ± 0.01	8.26 ± 0.20	8.07 ± 0.03	ns
	Malic acid (g L^{-1})	3.79 ± 0.04 a	2.94 ± 0.26 b	3.99 ± 0.26 a	**	3.54 ± 0.22	3.59 ± 0.26	3.25 ± 0.29	ns
	G/F ratio	1.00 ± 0.00 a	0.98 ± 0.00 b	0.98 ± 0.00 ab	*	0.97 ± 0.00	0.97 ± 0.00	0.98 ± 0.01	ns
2020	Reducing sugars (g L^{-1})	216 ± 2 b	234 ± 5 a	224 ± 1 b	**	220 ± 4	226 ± 7	227 ± 3	ns
	pH	3.16 ± 0.02 b	3.18 ± 0.01 b	3.32 ± 0.02 a	***	3.19 ± 0.01	3.18 ± 0.02	3.17 ± 0.04	ns
	TA (g L^{-1} tartaric acid)	8.76 ± 0.23 a	7.81 ± 0.08 b	6.44 ± 0.02 c	***	7.51 ± 0.13 a	7.64 ± 0.10 a	7.05 ± 0.26 b	*
	Citric acid (g L^{-1})	0.21 ± 0.01 a	0.19 ± 0.01 b	0.14 ± 0.00 c	***	0.16 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	ns
	Tartaric acid (g L^{-1})	7.29 ± 0.14 a	7.59 ± 0.09 a	7.05 ± 0.20 b	**	7.79 ± 0.09	7.69 ± 0.09	7.42 ± 0.28	ns
	Malic acid (g L^{-1})	2.76 ± 0.08 a	2.47 ± 0.30 a	1.46 ± 0.08 b	***	1.79 ± 0.13	1.79 ± 0.09	1.67 ± 0.08	ns
	G/F ratio	1.00 ± 0.00 a	0.99 ± 0.01 a	0.94 ± 0.00 b	***	0.98 ± 0.01 a	0.96 ± 0.00 ab	0.95 ± 0.01 b	**
2021	Reducing sugars (g L^{-1})	208 ± 2 c	226 ± 2 b	237 ± 6 a	***	217 ± 3 b	227 ± 5 a	231 ± 2 a	**
	pH	3.11 ± 0.02 b	3.06 ± 0.01 b	3.18 ± 0.02 a	**	3.10 ± 0.02 b	3.15 ± 0.02 ab	3.16 ± 0.03 a	*
	TA (g L^{-1} tartaric acid)	9.95 ± 0.34 a	9.09 ± 0.34 b	8.55 ± 0.20 b	**	10.18 ± 0.04 a	8.78 ± 0.44 b	8.54 ± 0.06 b	***
	Citric acid (g L^{-1})	0.21 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	ns	0.21 ± 0.00 a	0.17 ± 0.01 c	0.19 ± 0.01 b	***
	Tartaric acid (g L^{-1})	7.20 ± 0.08 b	7.12 ± 0.13 b	7.45 ± 0.07 a	*	7.97 ± 0.23 a	7.53 ± 0.12 b	7.91 ± 0.09 ab	*
	Malic acid (g L^{-1})	4.11 ± 0.20 a	3.60 ± 0.21 b	3.45 ± 0.03 b	**	3.78 ± 0.08 a	3.00 ± 0.29 b	3.01 ± 0.09 b	**
	G/F ratio	1.02 ± 0.00 a	1.00 ± 0.00 b	0.98 ± 0.00 c	***	1.00 ± 0.00 a	0.98 ± 0.00 b	0.96 ± 0.00 b	***

Note: All data are expressed as the mean ± SD ($n = 3$). ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$; ns, not significant. Different lowercase letters indicate significant differences among the three binomials tested for each vineyard studied according to the Tukey *b* test ($P < 0.05$). A, upper-valley vineyard; B, lower-valley vineyard; EL, early harvest/long withering; MM, medium-term harvest/medium withering; LS, late harvest/short withering; TA, total acidity; G/F ratio, glucose/fructose ratio.

acids with respect to the other binomials studied for each year (except for 2020 vineyard B). Interestingly, the concentrations of citric and malic acids progressively increased from fresh to withered grapes, presumably because of a positive balance between catabolism and concentration effect (*t*-test, $P < 0.01$ with respect to fresh and withered citric acid values, whereas the difference was not statistically significant for malic acid). By contrast, a decreasing trend in the concentration of tartaric acid was observed from fresh to withered grapes. Rösti *et al.*[44] explained the drop in tartaric acid observed during Merlot and Syrah winegrapes dehydration as consequence of precipitations occurred already inside the berries, probably because of a loss of compartmentation over the process. Nevertheless, the ratio between juice malic and tartaric acid seemed more influenced by the vintage rather than the harvest date, both on fresh and withered grapes (see Supporting information, Table S2), especially for year 2020 fresh grapes that reported the lowest values.

A small amount of glycerol has been detected only in withered grapes (from 0.05 to 2.14 g L^{-1}), more prominently in the grapes from 2019 vintage. The increase of the glycerol content as a result of the withering process has often been observed in the literature.[45,46] Indeed, during dehydration, grape cells under hyperosmotic stress for the increasing sugar concentration appear to react to stress by increasing the intracellular glycerol.[47] However, the differences in glycerol contents from EL to LS observed after withering were not statistically significant.

Grape skin mechanical properties

The instrumental texture parameters of fresh and withered grape skins determined in the three consecutive harvest years are shown in Table 3. Berry skin hardness (F_{sk}) and thickness (Sp_{sk}) are important qualitative indexes used in oenology as predictors of anthocyanin extractability.[48] The possibility to estimate the extractability of phenolic compounds during the maceration phase is particularly interesting for Nebbiolo winegrapes, which are rich in di-substituted anthocyanins, the easiest extractable and oxidable ones.[49,50] Moreover, it has been demonstrated that the berry skin hardness at harvest affects the dehydration kinetics.[51]

The F_{sk} values detected in fresh grapes in the present study were slightly higher (0.55–0.74 N) than the ranges present in the literature on Nebbiolo grapes from Piedmont region (around the range of 0.23–0.55 N), probably because of the influence of the Valtellina mountainous growing area, as previously found for Carema mountainous growing area compared to the La Morra and Barbaresco hill areas.[52] Indeed, several studies demonstrated that grape mechanical properties, particularly F_{sk}, are influenced by many variables, such as variety, clonal differences, grape-growing

Table 2. Standard parameters of withered grapes

Harvest year	Ripeness parameter	Vineyard A EL	Vineyard A MM	Vineyard A LS	Significance	Vineyard B EL	Vineyard B MM	Vineyard B LS	Significance
2019	Reducing sugars (g L^{-1})	290 ± 5 a	289 ± 1 a	277 ± 6b	*	292 ± 2 a	278 ± 7 b	279 ± 2 b	*
	pH	3.14 ± 0.02 c	3.22 ± 0.02b	3.29 ± 0.02a	***	3.14 ± 0.03 b	3.21 ± 0.03 a	3.23 ± 0.01 a	*
	TA (g L^{-1} tartaric acid)	9.50 ± 0.60 a	7.73 ± 0.15b	8.07 ± 0.15b	**	9.13 ± 0.25 a	8.93 ± 0.15 a	8.10 ± 0.36 b	**
	Citric acid (g L^{-1})	0.33 ± 0.03	0.26 ± 0.04	0.29 ± 0.06	ns	0.36 ± 0.04	0.29 ± 0.03	0.29 ± 0.06	ns
	Tartaric acid (g L^{-1})	7.75 ± 0.78	7.13 ± 0.43	7.05 ± 0.09	ns	6.75 ± 0.24 b	7.81 ± 0.14 a	7.43 ± 0.09 a	***
	Malic acid (g L^{-1})	4.07 ± 0.18 a	3.18 ± 0.19 b	3.84 ± 0.18 a	**	3.95 ± 0.11 a	3.33 ± 0.09 b	3.18 ± 0.21 b	**
	G/F	0.95 ± 0.00 a	0.94 ± 0.00 b	0.95 ± 0.00 a	***	0.95 ± 0.00 a	0.93 ± 0.00 b	0.94 ± 0.01 b	*
	Glycerol (g L^{-1})	1.98 ± 1.03	1.58 ± 0.63	1.12 ± 0.09	ns	2.14 ± 0.97	2.11 ± 1.10	1.36 ± 0.52	ns
2020	Reducing sugars (g L^{-1})	278 ± 4 a	280 ± 3 a	265 ± 3 b	**	275 ± 8	273 ± 1	267 ± 15	ns
	pH	3.25 ± 0.01 c	3.36 ± 0.03 b	3.58 ± 0.01 a	**	3.35 ± 0.05	3.35 ± 0.02	3.36 ± 0.02	ns
	TA (g L^{-1} tartaric acid)	8.43 ± 0.08 a	7.33 ± 0.50 b	5.81 ± 0.24 c	***	7.46 ± 0.23	7.49 ± 0.14	7.03 ± 0.24	ns
	Citric acid (g L^{-1})	0.32 ± 0.02	0.28 ± 0.00	0.31 ± 0.04	ns	0.26 ± 0.01 a	0.25 ± 0.02 a	0.20 ± 0.01 b	**
	Tartaric acid (g L^{-1})	6.94 ± 0.29 a	5.91 ± 0.14 ab	5.00 ± 0.56 b	**	6.67 ± 0.12	6.72 ± 0.17	6.72 ± 0.49	ns
	Malic acid (g L^{-1})	2.95 ± 0.06 a	2.70 ± 0.04 b	2.01 ± 0.05 c	***	2.20 ± 0.07	2.31 ± 0.06	2.15 ± 0.11	ns
	G/F	0.94 ± 0.00 a	0.94 ± 0.00 a	0.92 ± 0.00 b	***	0.92 ± 0.00 a	0.92 ± 0.00 ab	0.91 ± 0.00 b	*
	Glycerol (g L^{-1})	0.37 ± 0.13	0.76 ± 0.11	1.00 ± 0.34	ns	0.36 ± 0.09	0.45 ± 0.30	0.20 ± 0.16	ns
2021	Reducing sugars (g L^{-1})	247 ± 2 b	257 ± 2 a	250 ± 6 ab	*	264 ± 3 a	270 ± 5 a	249 ± 2 b	***
	pH	3.19 ± 0.01 a	3.13 ± 0.02 b	3.21 ± 0.01 a	***	3.24 ± 0.02 b	3.30 ± 0.02 a	3.26 ± 0.00 b	**
	TA (g L^{-1} tartaric acid)	10.20 ± 0.15 a	9.44 ± 0.24 b	9.62 ± 0.04 b	**	9.49 ± 0.07 a	8.22 ± 0.12 b	8.28 ± 0.11 b	***
	Citric acid (g L^{-1})	0.28 ± 0.00 a	0.23 ± 0.01 b	0.24 ± 0.00 b	*	0.27 ± 0.01 a	0.21 ± 0.03 b	0.18 ± 0.02 b	**
	Tartaric acid (g L^{-1})	6.84 ± 0.07	7.00 ± 0.16	6.70 ± 0.27	ns	7.36 ± 0.13	7.27 ± 0.10	7.37 ± 0.19	ns
	Malic acid (g L^{-1})	4.31 ± 0.12 a	3.77 ± 0.08 b	4.26 ± 0.06 a	***	4.15 ± 0.08 a	3.25 ± 0.02 b	2.96 ± 0.08 c	***
	G/F	0.96 ± 0.00	0.96 ± 0.00	0.96 ± 0.01	ns	0.94 ± 0.01	0.94 ± 0.01	0.93 ± 0.00	ns
	Glycerol (g L^{-1})	0.14 ± 0.03	0.14 ± 0.07	0.05 ± 0.02	ns	0.47 ± 0.15	0.61 ± 0.37	0.27 ± 0.23	ns

Note: All data are expressed as mean ± SD ($n = 3$). ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$; ns, not significant. Different lowercase letters indicate significant differences among the three binomials tested for each vineyard studied according to the Tukey *b* test ($P < 0.05$). A, upper-valley vineyard; B, lower-valley vineyard; EL, early harvest/long withering; MM, medium-term harvest/medium withering; LS, late harvest/short withering; TA, total acidity; G/F ratio, glucose/fructose ratio.

location, and environmental conditions.[53,54] However, among the three harvest points (early, medium, and late), no significant differences were found in fresh grapes F_{sk} for vintages 2019 and 2020, confirming that high variability in the skin break force is found in grapes regardless of the changes in soluble solids happening in the advanced phases of grape.[25,55] However, in 2021, the late harvest points presented a higher F_{sk} value for both the vineyards studied (0.74 and 0.67 N for vineyard A and B, respectively). This trend could be imputable to the dry season, which characterized the year 2021 until the heavy rain event that occurred in the first days of October, therefore before the A-MM, A-LS, and B-LS sampling points (Fig. 2). Indeed, water availability appears to influence the grape skin physical features, especially in the period before the harvest.[56] After the withering process, F_{sk} tended to increase, even if the differences among treatments resulted statistically significant only in a few cases. However, this phenomenon was more evident in berry skin break energy (W_{sk}), confirming that previously reported in the literature.[57]

The berry skin resistance against deformation (Young's modulus; E_{sk}) decreased significantly from fresh to withered grapes in all tested cases (vintage, vineyard, binomials assessed combinations) (−24%). However, the different level of WL reached appeared to influence this parameter more than harvest date: at the end of the whole process, EL samples showed generally lower values of E_{sk} than MM and LS berries, resulting in lower skin stiffness.[57] This information could be useful in programming the manipulation activities of grapes especially during the first wine maceration phase, such as the frequency of pumping-over, punching down, and *délestage* pomace cap management operations.

As regards berry skin thickness (Sp_{sk}), as already observed for F_{sk}, the values detected on fresh skins in the 3 years of experiments (197–262 μm) were generally higher than those present in literature for Nebbiolo grapes from other regions.[52,58] As expected, Sp_{sk} had an increasing trend from early to late-harvested samples (+10%) and increased (significantly in 12 cases out of 18) from fresh to withered grapes (+17%), as already demonstrated by Rolle *et al.*[36] However, the different lengths of the withering process and the high variability of this parameter balanced these differences, often resulting in no significant differences among the binomials at the end of the process.

Table 3. Fresh and withered grape skins mechanical properties

Harvest year		2019						2020						2021					
Vineyard		A			B			A			B			A			B		
		Fresh	Withered	Significance[‡]	Fresh	Withered	Significance[‡]	Fresh	Withered	Significance[‡]	Fresh	Withered	Significance[‡]	Fresh	Withered	Significance[‡]	Fresh	Withered	Significance[‡]
Fsk (N)	**EL**	0.69 ± 0.19	0.70 ± 0.13	ns	0.63 ± 0.13	0.69 ± 0.17	ns	0.68 ± 0.17	0.65 ± 0.14	ns	0.60 ± 0.14	0.61 ± 0.15	ns	0.64 ± 0.10 b	0.68 ± 0.17 b	ns	0.72 ± 0.15 a	0.71 ± 0.17	ns
	MM	0.69 ± 0.13	0.75 ± 0.13	ns	0.62 ± 0.11	0.61 ± 0.16	ns	0.62 ± 0.15	0.68 ± 0.12	ns	0.60 ± 0.14	0.60 ± 0.13	ns	0.68 ± 0.13 ab	0.83 ± 0.13 a	***	0.55 ± 0.11 b	0.71 ± 0.20	***
	LS	0.66 ± 0.18	0.67 ± 0.14	ns	0.61 ± 0.13	0.61 ± 0.14	ns	0.60 ± 0.09	0.67 ± 0.10	ns	0.59 ± 0.14	0.54 ± 0.11	ns	0.74 ± 0.16 a	0.75 ± 0.26 ab	ns	0.67 ± 0.15 a	0.70 ± 0.17	ns
	Significance[†]	ns	ns		ns	ns		ns	ns		ns	ns		*	*		***	ns	
Wsk (mJ)	**EL**	0.71 ± 0.19	0.85 ± 0.18 a	**	0.64 ± 0.19	0.95 ± 0.31 a	ns	0.70 ± 0.23 a	0.86 ± 0.26	*	0.62 ± 0.20	0.77 ± 0.21 a	**	0.60 ± 0.16 b	0.79 ± 0.28 b	**	0.70 ± 0.21 a	0.97 ± 0.31	***
	MM	0.74 ± 0.21	0.88 ± 0.25 a	*	0.67 ± 0.18	0.66 ± 0.23 b	ns	0.59 ± 0.20 a	0.82 ± 0.23	***	0.59 ± 0.21	0.74 ± 0.20 a	**	0.68 ± 0.19 ab	1.00 ± 0.26 a	***	0.52 ± 0.16 b	0.87 ± 0.30	***
	LS	0.64 ± 0.24	0.73 ± 0.17 b	ns	0.65 ± 0.21	0.73 ± 0.24 b	ns	0.59 ± 0.13 a	0.79 ± 0.17	***	0.58 ± 0.20	0.59 ± 0.21 b	ns	0.80 ± 0.25 a	0.88 ± 0.40 ab	ns	0.70 ± 0.24 a	0.81 ± 0.29	ns
	Significance[†]	ns	*		ns	***		*	ns		ns	**		*	*		***	ns	
Esk (N/mm)	**EL**	0.30 ± 0.04 ab	0.21 ± 0.05 b	***	0.27 ± 0.05	0.19 ± 0.04 b	***	0.28 ± 0.06 a	0.18 ± 0.04 b	***	0.25 ± 0.04	0.18 ± 0.05 a	***	0.31 ± 0.05	0.22 ± 0.03 b	***	0.33 ± 0.05 a	0.20 ± 0.04 b	***
	MM	0.28 ± 0.05 b	0.24 ± 0.04 a	**	0.25 ± 0.05	0.22 ± 0.05 a	**	0.29 ± 0.05 a	0.21 ± 0.04 a	***	0.27 ± 0.03	0.19 ± 0.04 a	***	0.30 ± 0.04	0.26 ± 0.03 a	***	0.26 ± 0.03 b	0.22 ± 0.04 b	***
	LS	0.31 ± 0.06 a	0.24 ± 0.05 a	***	0.31 ± 0.06	0.20 ± 0.03 ab	***	0.26 ± 0.02 a	0.21 ± 0.02 a	***	0.26 ± 0.04	0.20 ± 0.03 a	***	0.29 ± 0.03	0.25 ± 0.05 a	***	0.28 ± 0.03 b	0.24 ± 0.04 a	***
	Significance[†]	*	*		ns	*		*	***		ns	*		ns	**		***	***	
Spsk (μm)	**EL**	197 ± 41 b	260 ± 42 ab	***	215 ± 45	289 ± 48	**	218 ± 41 b	261 ± 43 b	***	237 ± 39 b	285 ± 50	***	217 ± 39 b	236 ± 47 b	ns	215 ± 34 b	264 ± 41	***
	MM	244 ± 34 a	265 ± 47 a	ns	233 ± 40	279 ± 40	*	262 ± 43 a	271 ± 49 ab	ns	256 ± 42 a	294 ± 45	**	248 ± 49 a	269 ± 46 a	ns	235 ± 45 ab	266 ± 47	*
	LS	220 ± 37 b	235 ± 51 b	ns	221 ± 34	289 ± 50	***	237 ± 26 b	292 ± 48 a	***	261 ± 33 a	290 ± 34	***	232 ± 51 ab	274 ± 49 a	**	252 ± 35 a	270 ± 48	ns
	Significance[†]	***	*		ns	ns		***	*		**	ns		*	**		**	ns	

Note: All data are expressed as the mean ± SD ($n = 30$). ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$; ns, not significant with respect to the differences among the three binomials studied (†) and between fresh and withered grapes (‡) according to analysis of variance and a *t*-test. Different lowercase letters in the same column indicate significant differences (†) according to the Tukey *b* test ($P < 0.05$). A, upper-valley vineyard; B, lower-valley vineyard; EL, early harvest/long withering; MM, medium-term harvest/medium withering; LS, late harvest/short withering; F_{sk}, berry skins break force; W_{sk}, berry skins break energy; E_{sk}, berry skin resistance against deformation; Sp_{sk}, berry skin thickness.

Considering the different locations, vineyard A presented slightly lower Sp_{sk} values than vineyard B; meanwhile, F_{sk} showed the opposite trend (*t*-test, $P < 0.001$ for both parameters) (Table 3). In 2021, this tendency has been less remarkable than the previous vintages probably for the higher variability of grape samples (*t*-test, $P = 0.09$ and 0.308 for F_{sk} and Sp_{sk}, respectively). These mechanical properties may have influenced the extractable phenolic profile, as they are related with the extractability of these compounds, particularly for anthocyanins.[48] Indeed, lower values of Sp_{sk} and higher values of F_{sk} are linked to an easier diffusion of anthocyanins in wine during the maceration phase.[48] The results of phenolic compounds presented in the next section confirm this hypothesis.

Extractable phenolic composition of fresh and withered grapes

Here, the results obtained from the analysis of seed and skin extracts using wine-like solution for both fresh and dehydrated samples are presented and discussed considering the concentration effect in withered grapes and simulating winemaking conditions.

Grape seed extractable phenolics

The content of extractable total polyphenols (TPI), total flavonoids (TF), and condensed tannins (MCP) in seeds appeared to show a decreasing trend by leaving the grapes on the plant longer by an averaged value of the 3 years, respectively, of −15/26%, −27/23%, and − 20/28% for vineyards A/B (Fig. 3), in accordance with previous studies about the evolution of phenolic profile along ripening.[59,60] In seeds, the main phenolic compounds are represented by flavanol monomers and their condensed forms; therefore, the trends emerging from the different analysis carried out (TPI, TF, MCP) are generally in accordance (correlation coefficients of 0.99, 0.88, and 0.89 for TPI-TF, TPI-MCP, and TF-MCP, respectively). The decrease in phenolic compounds observed in this study from early to late harvested samples was previously attributed to oxidation reactions,[61] and it is also probably strongly related to the conjugation with other molecules, which reduced their extractability such as proteins and grape cell wall polysaccharides.[62,63] This tendency has been observed in all the three vintages considered, although the

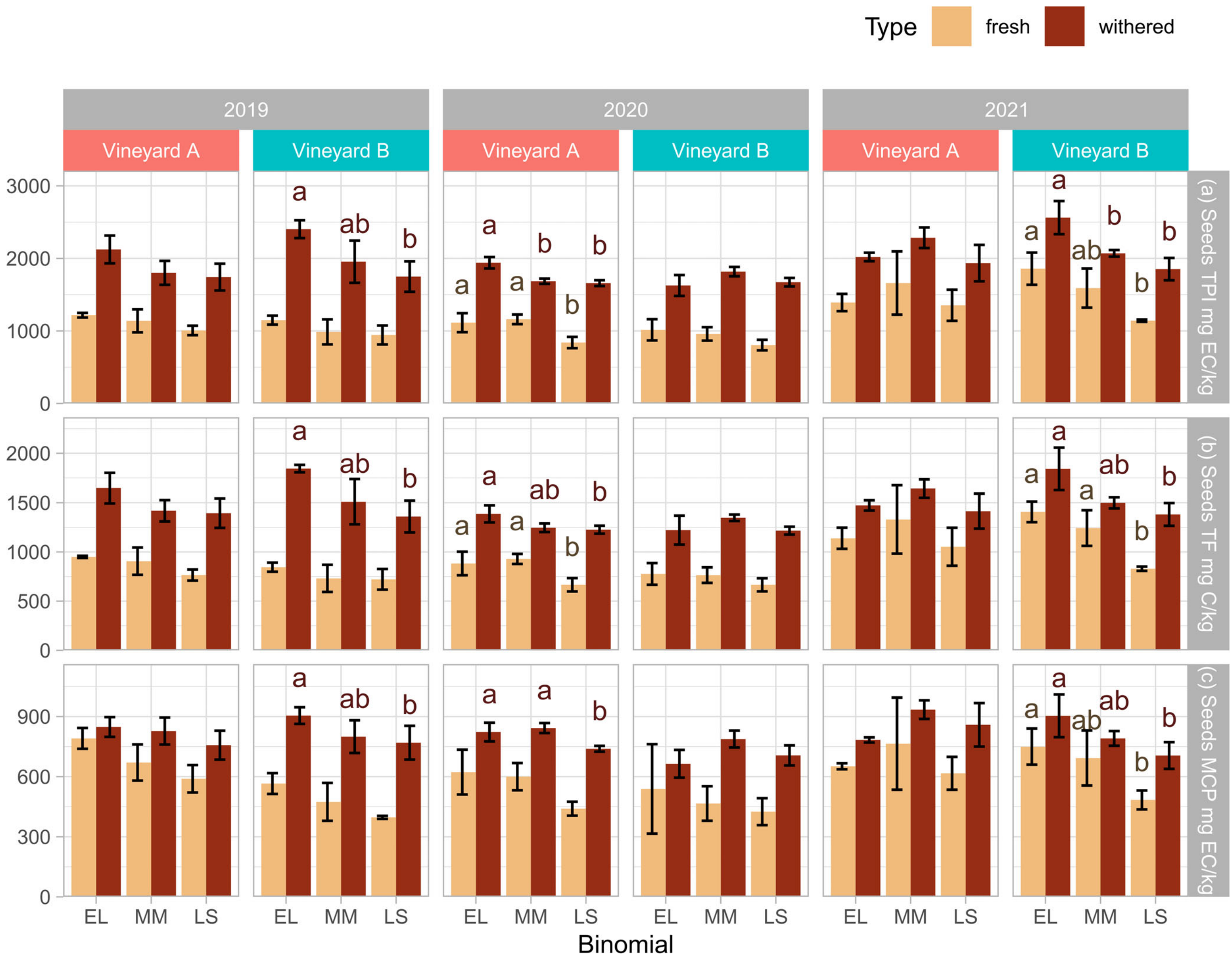

Figure 3. Phenolic profile of fresh and withered grape seeds. TPI, extractable total phenolic compounds (a); TF, extractable total flavonoid compounds (b); MCP, extractable condensed tannins determined by methyl cellulose assay (c). ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$; ns, not significant. Different lowercase letters indicate significant differences among the three binomials tested for each vineyard and year studied according to the Tukey *b*-test ($P < 0.05$). A, upper-valley vineyard; B, lower-valley vineyard; EL, early harvest/long withering; MM, medium-term harvest/medium withering; LS, late harvest/short withering.

differences were not always statistically significant because of sample variability.

The impact of the grape's ripeness degree observed in fresh grapes increased considerably after withering because of the concentration effect, with percentage amounts comprised from +30 to +109% for TPI, from +21 to +118% for FT, and from +7 to +94% for MCP. Therefore, at the end of the withering process, the greatest phenolic contents extracted from seeds were mainly found for EL and MM grape samples. The highest contents of seed polyphenols, particularly for condensed tannins (MCP), detected in EL and MM samples after 7 days of maceration make the earlier harvested/longer withered grapes more suitable than the other binomials with respect to producing wines destined for long ageing periods, as is the case for *Sforzato di Valtellina* DOCG wines.

Grape skin extractable phenolics

The grape skin extractable total phenolic compounds (TPI) and total flavonoids (TF) appeared to be less influenced by the harvest period compared to those of seeds, although their contents expressed on grape weight generally increased after withering, as a result of a balance between concentration and degradation effects (Fig. 4a,b). In general, the increase from fresh to withered for EL samples was less evident for grape skins than for seeds, probably because the skin phenolic compounds are more

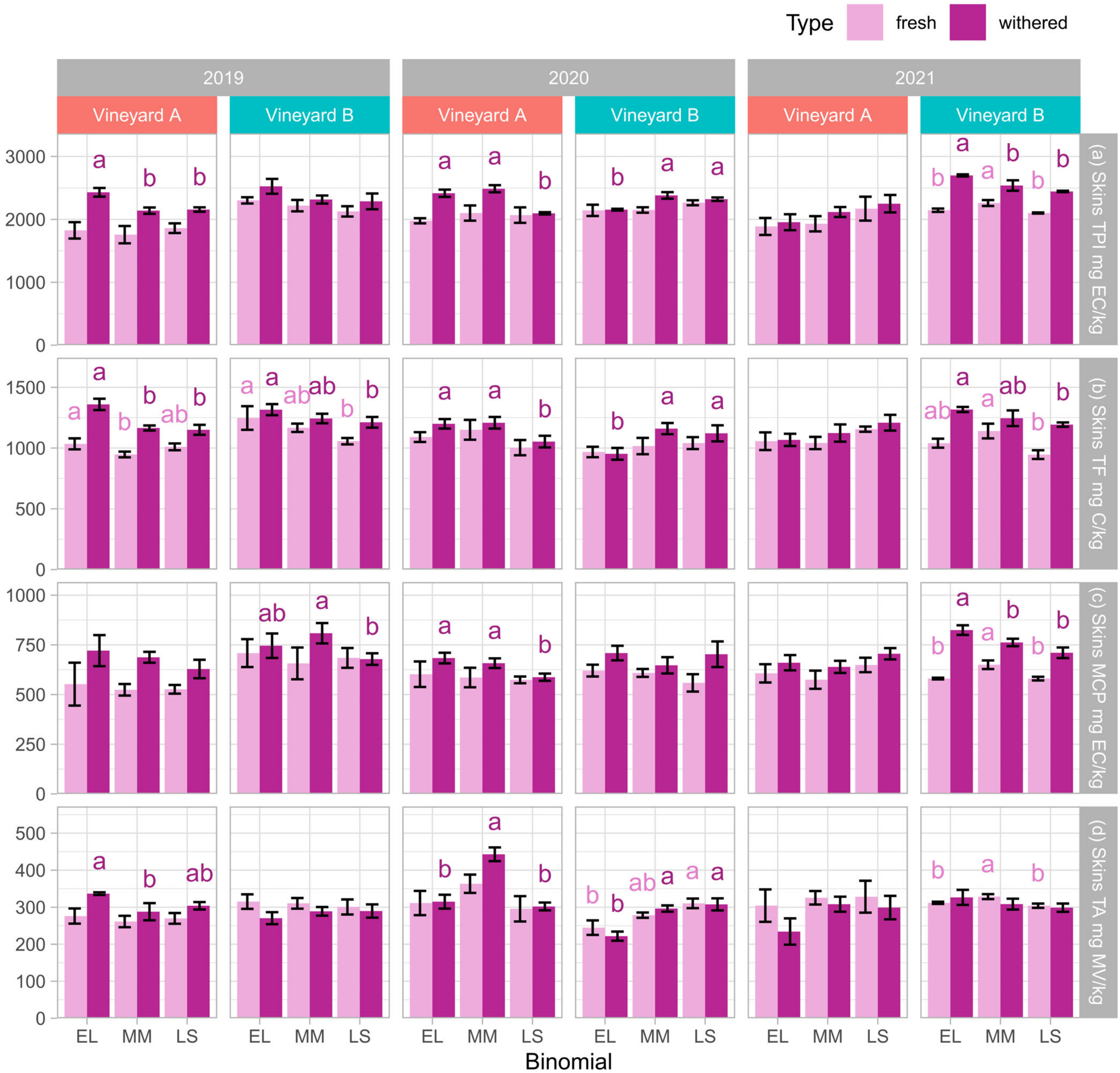

Figure 4. Phenolic profile of fresh and withered grape skins. TPI, extractable total phenolic compounds (a); TF, extractable total flavonoid compounds (b); MCP, extractable condensed tannins determined by methyl cellulose assay (c); TA, extractable total anthocyanins (d). ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$; ns, not significant. Different lowercase letters letters indicate significant differences among the three binomials tested for each vineyard and year studied according to the Tukey *b* test ($P < 0.05$). A, upper-valley vineyard; B, lower-valley vineyard; EL, early harvest/long withering; MM, medium-term harvest/medium withering; LS, late harvest/short withering.

subjected to biotic and abiotic stress, mitigating the concentration effect.[64,65] Previous studies[66-68] have reported that a long withering time and greater water loss could determine a significant loss of phenolic compounds as a result of oxidation and senescence metabolism. Nevertheless, the cool temperatures that occur during the period when the natural withering is performed in Valtellina probably delayed the water loss stress, as hypothesized by Bellincontro *et al.*[66] This results in a final increase in polyphenols, in accordance with the results of Panceri *et al.*[69] on Merlot and Cabernet sauvignon grapes under controlled withering conditions. Indeed, Nicoletti *et al.*,[70] when investigating Nebbiolo grapes destined for the production of *Sfursat* and subjected to different withering rate under controlled temperatures, observed an increase in skins polyphenols (at 10 and 20% WL) after dehydration at 10 °C.

Extractable anthocyanins (TA) expressed as the malvidin-3-*O*-glucoside equivalent on berry weight (mg kg^{-1} berries) (Fig. 4d) showed no consistent differences among the three harvest points in fresh grape skins of vineyard A in any year under evaluation; meanwhile, a significant increase from early to late harvest was observed in vineyard B during 2020 vintage (from 245 to 310 mg kg^{-1} berries), as well in 2021 from early to medium harvest (from 312 to 329 mg kg^{-1} berries). Their ratio with respect to juice sugars showed significant differences only among vintages (see Supporting information, Table S2). The withering process affected the final concentrations of skins TA, without changing the trends observed in the corresponding fresh grapes. Therefore, for these compounds, the harvest time appears to have a higher impact than the withering length on the final extractable content, although the trend was not stable during the vintages or common for the two vineyards evaluated. Hence, considering the risks involved (climate, loss of product, etc.,) leaving the grapes on the vine longer does not appear to be justified in terms of any real gain in anthocyanin compounds. Moreover, during the first 2 years of experiments, their content from fresh to withered grape skins experienced a distinct trend for the two vineyards assessed: their concentration increased in withered samples from the vineyard A (upper-valley; from +1% to +22%) and slightly decreased or remained almost constant in those from the vineyard B (lower-valley; from −14% to −1%) except for vintage 2020 B-MM (+6%). The grapes mechanical properties, particularly the higher F_{sk} and the lower Sp_{sk} values found after withering in the vineyard A compared to B, may have promoted enhanced extractable anthocyanin contents in withered grapes, as previously discussed above. Instead, in 2021, the high variability likely induced by the dry season of the vintage makes it difficult to highlight the trend observed in the previous years. This confirms the greater variability in the grape composition observed in dry years.[71] Therefore, among the compounds analyzed, anthocyanin was the compound most affected by the vineyard and by the vintage effect.

As regards the amounts of extractable condensed tannins determined by methyl cellulose precipitation assay (MCP) (Fig. 4c), the differences among the three harvest dates resulted statistically significant in fresh grapes only in the case of vineyard B in vintage 2021, presenting a slight increase from EL (580 mg kg^{-1} berries) to MM (650 mg kg^{-1} berries), as observed by Ó-Marques *et al.*[60] on Cabernet sauvignon and Tinta Roriz varieties with the progress of maturation, but followed by a decrease in LS point to 580 mg kg^{-1} berries. However, for MCP, the withering length appeared to have a greater influence on the extractable grape skin tannins than the harvest time. Indeed, at the end of the process, EL and MM binomials often showed the highest concentrations of condensed tannins, as already observed for seeds (with an enhancement among EL and LS comprised between +29 and +114 mg kg^{-1} grapes), although not always significant, indicating an important impact of the concentration effect over degradation during withering. Condensed tannin content has been previously reported to be less affected by dehydration than other phenolic compounds on a dry weight basis.[72] The main changes are connected with structural modification, as observed previously in dehydrated Nebbiolo grapes at 10% and 20% weight loss,[73] which is in agreement with the present study.

Multivariate evaluation of data

By performing a preliminary PCA on the original data, the effect of vintage was predominant over all other variables (data not shown); therefore, to assess the real impact of the effect of vineyard and thesis, the data were normalized inside each vintage. Because of the strong differences between fresh and dehydrated grapes, these two matrices were studied separately. The biplots demonstrating the characterization of our samples and the correlation among variables are shown in Fig. 5. In fresh grapes (Fig. 5a–c), the first two PCs explained 58.8% of the variance of the samples, with a PC1 contribution of 39.4%. Concerning withered grapes (Fig. 5b–d), the first two PCs accounted for 35.9% and 21.5%, respectively, explaining 57.4% of the total variance.

As expected, in fresh grapes, the juice pH correlated positively with sugars and negatively with the total acidity parameter. On the other hand, in withered grapes, pH was no longer correlated with sugars level. The phenolic parameters of the skins (Skins TA and Skins MCP) were close and positively correlated in both fresh and withered grapes, whereas Seeds MCP was found to be poorly correlated with these parameters in both cases. These results show that the extraction of phenolic substances from the different berry components did not appear to influence each other.

Interestingly, malic acid and Seeds MCP were found to be correlated, especially in fresh grapes: one hypothesis concerning this behaviour may involve ripeness evolution through sampling points, with a decreasing tendency of malic acid content in juice (Table 1) and the extractable condensed tannins from seeds (Fig. 3c).

In Fig. 5(a) (fresh grapes) and Fig. 5(b) (dehydrated grapes), it is possible to observe that vineyards A and B are well separated on each PC2. In general, grapes from vineyard A were characterized by higher values of malic acid and Seeds MCP, whereas grapes from vineyard B were more associated with Skins TA, Skins MCP, and tartaric acid. It is worth noting that differences between vineyards were kept also at the end of the withering process (Fig. 5b), with sugars and glycerol contents also contributing to the differentiation.

The biplots in Fig. 5(c) (fresh grapes) and Fig. 5(d) (dehydrated grapes) reported the binomial harvest date/length of withering effect. In fresh grapes (Fig. 5c), the three binomials show differences mostly related to the acid–sugar composition of the grapes. It is interesting to note that the groups were separated on each PC1, following the harvest date order on which they were picked. In particular, LS is characterized by high pH and sugar values, EL by high total acidity, malic acid and also Seeds MCP values, and the MM sample group stands in the middle. Therefore, the late harvest date resulted in grapes with a higher sugar content and higher pH value.

In withered grapes (Fig. 5d), it is possible to observe that treatments (binomials) are still well separated. As previously noted, EL showed a good correlation with high sugar content, confirming that long withering periods give a higher sugar increase. On the other hand, high pH values characterize the LS thesis, showing

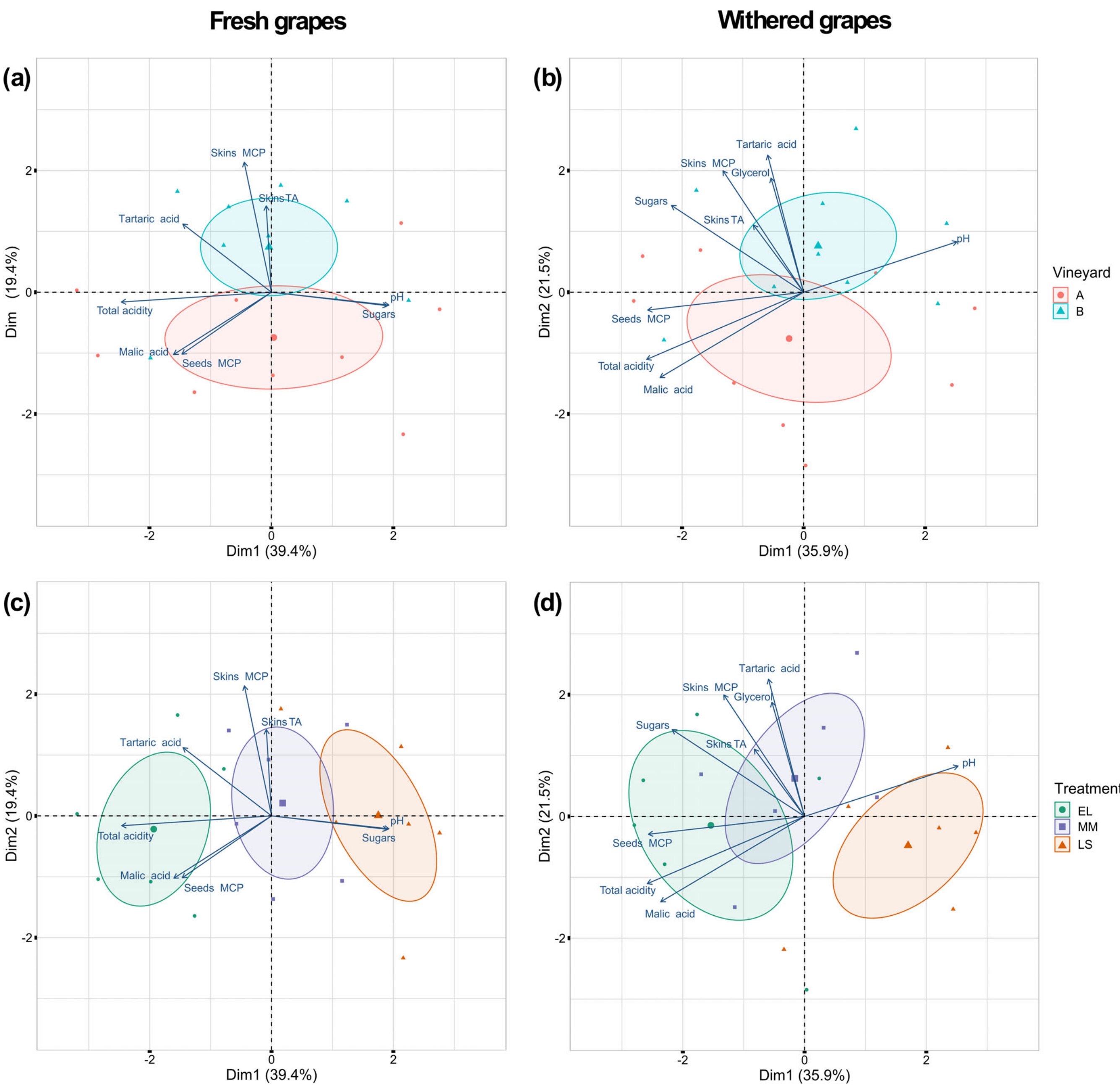

Figure 5. Biplots of the Principal component analyses (PCAs) performed on fresh (a, c) and withered grapes (b, d) examining the effect of vineyards (a, b) and treatments (c, d). The observations shown in the biplots represent the mean of three replicates of grapes from the same vineyard and vintages and subjected to the same treatment. The arrows indicate the strength and direction of the relationship between the variables and the principal components.

that the combination of late harvest date and short withering period contributes to lower the juice total acidity and to increase the pH values.

Considering these results, we may confirm that different combinations of harvest date and withering length result in different grape composition and that the effect of the treatment can be observed across different vintages.

CONCLUSIONS

During the three consecutive vintages, all the analyzed parameters were affected by the close interaction between the harvest time and the withering length. The most evident changes were observed in technological parameters. The grapes harvested earlier and subjected to long-withering process were found not only to be richer in sugars, but also showed higher total acidity and lower pH values, presenting a balanced sugar-to-acid ratio. By contrast, the combination of late harvest date and short withering period contributes to lowering the juice total acidity and increasing the pH. These observations confirm not only the great importance of ripeness degree for grapes destined for dehydration, but also the importance of considering the withering process.

Mechanical properties were affected by the combined effect of the studied variables: the skin stiffness (E_{sk}) was generally lower in

EL than in MM and LS withered grapes. Skin break force (F_{sk}) and thickness (Sp_{sk}) increased in withered grapes with respect to their fresh counterpart, but the different withering rates tended to compensate the effect of grape ripeness degree. Nevertheless, the same parameters showed some different trends between the two vineyards tested.

Seed extractable polyphenols showed a decreasing trend when leaving the grapes on the plant longer, whereas the concentration effect considerably enhanced this impact after withering. The extracted skin phenolic compounds were less influenced by harvest period, but their potential impact increased after dehydration. In most cases, EL and MM trials resulted in withered grapes characterized by higher amounts of extractable phenolic compounds, particularly for seeds and skins condensed tannins. For anthocyanins, harvest time appeared to be more of an influence than withering length regarding final extractable content, even if a sustained variability among vintages and vineyards was present.

The great weather differences among the three vintages allowed a common trend to be highlighted in very different situations, although further studies are needed to better clarify the impact of the climate conditions. Our results suggest that the choice with respect to anticipating harvest time for Nebbiolo grapes destined for withering should be preferred in view of the production of reinforced wines destined for long wine ageing, as a result of higher phenolic compounds contents, higher acidity, and lower pH, as well as for grape health reasons, aiming to avoid possible adverse climate and pests.

AUTHOR CONTRIBUTIONS

GS, MAP, CDP, DŠ, AZ, GM, LF and SBDA were responsible for formal analysis. GS, SG, MAP and SRS were responsible for investigations. GS, CDP, DŠ, AZ, GM, LF and SBDA were responsible for data curation. GS and LF were responsible for visualization. GS was responsible for writing the original draft. SG, MAP, SRS, VG and LR were responsible for conceptualization. SG and MAP were responsible for methodology. SG and VG were responsible for project administration. SG, MAP, SRS, CDP, DŠ, AZ, GM, LF, SBDA, VG and LR were responsible for reviewing and editing. SRS, VG and LR were responsible for supervision. VG and LR were responsible for funding acquisition.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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