Research Article

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Impact of berry size at harvest on red wine composition: a winemaker's approach

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Abstract

BACKGROUND: A classical postulate of viticulture declares that small grape berries produce the best red wines. The popularity of this postulate among winemakers leads them to consider berry size at harvest as a tool to measure the grape's potential to obtain great red wines. To address this issue, two vineyards from the same cultivar and subjected to the same physiological conditions during growing were selected for their difference in average grape berry size at harvest. Grapes from both origins were characterized and used for red winemaking by the same way. Release of volatile compounds and phenolic compounds during the alcoholic fermentation was monitored, and the finished wines were chemically characterized.

RESULTS: Larger grapes have a lower theoretical surface-to-volume ratio but have thicker skins and a greater proportion of skins (m/m). Wines made from grapes with a greater proportion of skins contain higher amounts of phenolic compounds, terpenes, volatile acids, acetate esters and polysaccharides.

CONCLUSION: According to the results, it seems that grape skin extraction is more related to skin proportion than to berry size. Thus not always smaller grapes produce darker red wines.

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

Keywords: berry size; wine phenolic composition; wine aroma; wine polysaccharides; Carménère cv.

INTRODUCTION

Red wine production is an ancient labor, and as such, it entails traditional postulates or paradigms that are not fully supported by experimental data. One of those traditional postulates is that small berries produce the best red wines.[1,2] This classical postulate seems quite logical for winemakers (for several reasons mentioned below), and often it conditions the winemaking techniques applied for each grape batch when the fruit is received in the cellar. Hence, when winemakers receives small berries, they tend to perform harder maceration processes to enhance grape extraction, leading to more concentrated wines.

The reasons argued traditionally to justify the best quality of smaller grapes are related, firstly, with the smaller and more spherical shape of wine grapes when compared with table grapes, besides that some of the most appreciated red wines on the market use to come from grape cultivars with a spherical shape and small berry size (e.g. Cabernet Sauvignon cv.). Secondly, it has been demonstrated that most secondary metabolites that confer appreciable sensory attributes to wine come from grape skin tissues, while some cannot be found in the flesh (mesocarp) of grape berries.[3,4] Hence, if we regard grape berry shape as a perfect sphere, it can be asserted that berry surface (corresponding to the formula $4\pi r^2$, where r corresponds to berry radius) is related to skin amount, while berry volume (corresponding to the formula $4\pi r^3/3$) is related to flesh amount. Thus, following this elegant model, the surface-to-volume ratio (corresponding to the formula $3/r$) is inversely correlated with berry size. This could suggest that smaller grapes have a higher skin-to-flesh ratio and, accordingly, a higher extraction of secondary metabolites during winemaking could take place. However, such an elegant argument does not consider differences in grape skin thickness or differences in the contribution of the seeds to berry fresh weight on the final wine composition.

These concerns regarding the berry size paradigm were reviewed by Matthews and Nuzzo[5] and Poni and Libelli,[6] highlighting that is very difficult to evaluate the effect of berry size *per se* on skin potential extractability and/or final wine composition, since grape berry size at harvest is strongly related to the physiological status of vines during the season. Indeed, as reported

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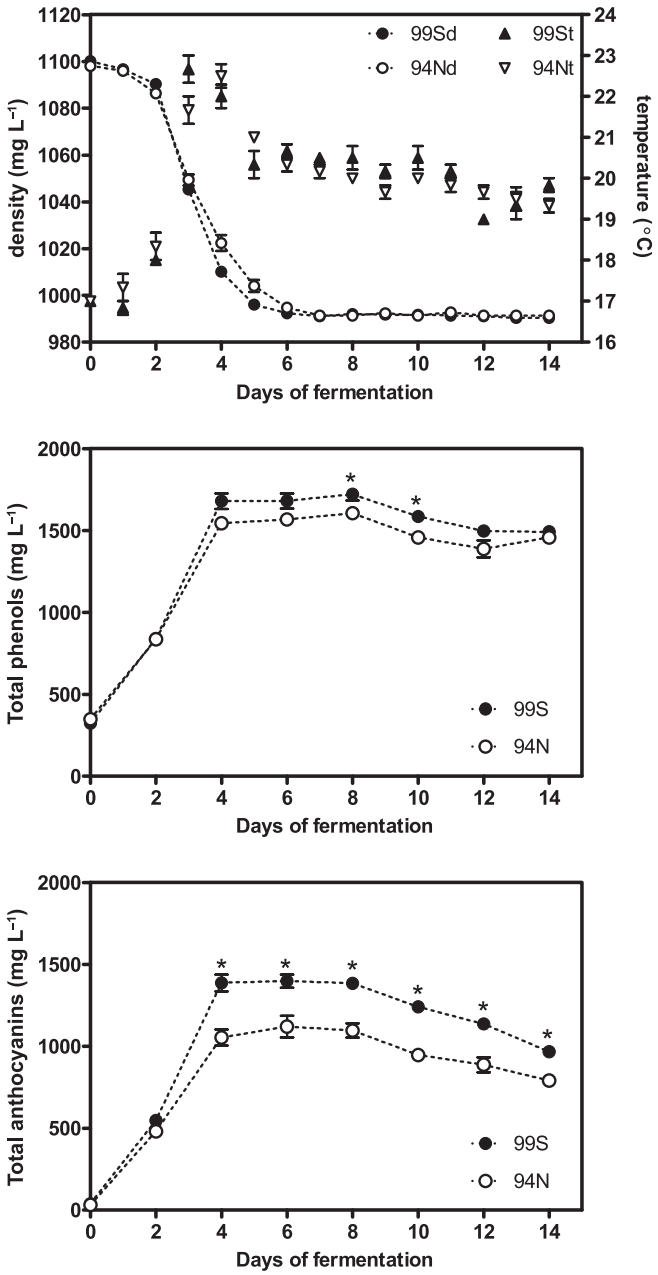

Figure 1. Extraction of total phenols and total anthocyanins during maceration-fermentation of grapes from 94N and 99S plot-fields. Asterisks (*) indicate significant differences ($P < 0.05$) between plot-fields for each chemical family at each fermentation stage. Total phenol content is expressed as gallic acid equivalent. Total anthocyanin content is expressed as malvidin-3-*O*-glucoside equivalent.

in the aforementioned reviews, several published works have demonstrated the impact of pruning treatments,[7] crop yield,[8] cluster light exposure,[9] partial defoliation[10] and water deficits[11–16] on grape berry size at harvest. Moreover, it has been demonstrated that all of these factors alter grape composition (by modifying the vine's physiological status and, in turn, the biosynthesis of secondary metabolites) in addition to berry size at harvest. In consequence, it is very difficult to distinguish whether the impact on wine composition is due to differences just in grape berry size or due to differences in grape composition. All these scientific data could be very useful for vine growers or boutique wineries that elaborate their own field-grown wines, which try to apply viticultural practices in order to obtain smaller grapes. However, it is not a useful point of view for those winemakers that receive grapes (differing in berry size) from different geographical origins or cultivated by different growers.

To evaluate the berry size influence on red wine composition while avoiding other growing physiological factors, Gil *et al.*[17] performed an assay in which grapes from a single harvest from the same vineyard were screened by size and separately used for red winemaking. This experiment was used to assess the impact of berry size from grapes from the same vines (obviously subject to the same physiological conditions during the season) on the final wine composition. This publication demonstrates that screening berries by size could be a good tool for red wine quality management, since wines made from smaller berries have deeper color and a higher amount of phenolic compounds. Moreover, the screening of grape berries by size before winemaking takes advantage of the natural heterogeneity of grape berries at harvest. Nonetheless, such a study does not indicate if berry size *per se* could be used as a grape quality parameter for winemakers when they receive different batches of grapes. One strategy to find the answer to this question is to search for two vineyards planted with the same cultivar, subjected to comparable viticultural practices, shown similar edaphoclimatic conditions and the same physiological state during the season, but which differ in average grape berry size at harvest. These grapes from both batches can be used for red winemaking in the same conditions, in order to evaluate if smaller grapes really lead to more concentrated wines.

The aim of this study was to select two different plot-fields of the same vine cultivar, subjected to the same edaphoclimatic conditions during the season and reaching the same maturity degree, but showing different averaged berry size at harvest. Afterwards, grapes from both plot-fields were used for red winemaking in triplicate, in order to evaluate the impact of berry size on the aromatic, phenolic and polysaccharidic composition of red wines.

MATERIALS AND METHODS

Vineyard, grapes and wines

The grapes for this study were from own-rooted *Vitis vinifera* cv. Carménère (from the same massal selection) obtained from plots 94N (33° 40′ 12.0″ S and 70° 34′ 56.6″ W) and 99S (33° 40′ 22.4″ S and 70° 34′ 15.1″ W) of the William Fevre vineyards in the Maipo Valley (Chile). The vines were trained on a vertical trellis system and managed by the same vine grower according to standard viticultural practices for the cultivar and region. The vineyard was equipped with a drip irrigation system, and both plot 94N and plot 99S were irrigated in the same way. The 94N plot corresponds to a 1.5 ha field of sand-slime soil type, with vines planted in 1994 and arranged in rows spaced 2.8 m apart, with 1.2 m spacing within each row. The 99S plot corresponds to a 6 ha field of sand-slime soil type (slightly stonier and less deep than the 94N one), with vines planted in 1999 and arranged in rows spaced 2.3 m apart, with 0.9 m spacing within each row. The crop yields for the 2015 vintage correspond to 2.56 and 2.73 kg per vine for 94N and 99S respectively. No evidence of biotic or abiotic stress was observed for vines in the 94N plot or the 99S plot. The grapes from each plot were harvested during the 2015 vintage (8 May) from approximately 30 vines selected from three consecutive rows from a homogeneous soil zone. Grapes were manually harvested when reaching approximately 24 °Brix. The grapes were placed in 20 kg plastic boxes and

transported to the Department of Agro-industry and Enology of the University of Chile. Grapes from each plot were split into three different batches. A 1 kg quantity of grapes from each batch was reserved for berry characterization. The remaining grapes of each batch (~20 kg) were de-stemmed, crushed (Delta E2, Bucher Vaslin, South America, Santiago de Chile, Chile), sulfited (100 mg $K_2S_2O_5$ kg^{-1}) and placed in 25 L polyethylene tanks. The tanks were immediately inoculated with 200 mg kg^{-1} of the selected *Saccharomyces cerevisiae* yeast (Lalvin® EC1118, Lallemand Inc.) and placed in a room with controlled temperature (20 °C). Alcoholic fermentation was controlled daily by measuring the temperature and density of the juice (Fig. 1). Every 2 days, the cap was gently punched down and 100 mL of sample (containing both liquid and solid to avoid a bleeding effect) was taken to perform phenolic and aromatic analyses. After 15 days of maceration, after completion of alcoholic fermentation, the wines from each tank were racked, sulfited (200 mg $K_2S_2O_5$ L^{-1}) and kept at 4 °C for 15 days for tartaric stabilization. After tartaric stabilization, the wines were placed in 750 mL green glass bottles, capped with natural cork and stored in a dark cellar at 16 °C until analysis. The analyses were performed 3 months after bottling.

Grape characterization

Grape berries were characterized as previously described[17] using 100 berries for each replicate. Skin thickness was calculated as the quotient between the average mass of berry skin and its theoretical surface. The analytical methods recommended by the International Organisation of Vine and Wine (OIV)[18] were used to determine the sugar concentration, pH and titratable acidity. The phenolic index (I_{280}) and anthocyanin content were determined as previously described.[19]

Spectrophotometric analyses of wines

All wine samples were centrifuged (4500 × *g* for 20 min) before analysis. A 20 µL aliquot of an aqueous solution of acetaldehyde (100 mL L^{-1}) was added to 2 mL of wine 45 min before the color measurements to avoid sulfite effects. Color intensity (CI) and hue were determined as described in the literature.[19] CIELab coordinates for the wines were determined as previously described.[17] The phenolic index (I_{280}) and total anthocyanin content were determined according to Ribéreau-Gayon *et al.*[19]

Flavanol and proanthocyanidin analysis

Wine proanthocyanidins were analyzed using various approaches, including the methylcellulose method described by Sarneckis *et al.*,[20] the LA (LA) method described by Ribéreau-Gayon *et al.*,[19] a gel permeation chromatography (GPC) method[21] for proanthocyanidin extracts obtained by solid phase extraction (SPE), as described by Pastor del Rio and Kennedy,[22] and phloroglucinolysis of the same extracts.[23] The signal at 280 nm (recorded with a diode array detector) was used to monitor the GPC profile of proanthocyanidins, and results were expressed as gallic acid (used as external standard) equivalent. Moreover, wine proanthocyanidins were also fractionated according to molecular mass by SPE as described by Sun *et al.*[24] Fractions I and II (monomers and oligomers of flavanols) were combined, concentrated to dryness with a Labconco CentriVap concentrator (Merck), re-suspended in 1 mL of methanol/water (1:1 v/v) and analyzed by reverse phase high-performance liquid chromatography (RP-HPLC) as previously described.[25] Finally, fraction III (polymeric flavanols) was analyzed by GPC and phloroglucinolysis using the method previously described for whole proanthocyanidin extracts.

Polysaccharide analysis

Grape skins' soluble polysaccharides were extracted from skin tissues and analyzed by High Resolution Size Exclusion Cromathography coupled to a Refraction Index Detector (HRSEC-RID) as previously described,[26] with some modifications. Briefly, 5 g of grape skins were mixed with 100 mL of solvent and crushed in an Ultra-Turrax T25 Basic (IKA, Staufen, Germany). The homogenized mixture was then placed on an orbital shaking platform for 1 h. Two different extractions were performed using an ammonium oxalate buffer (7.5 g L^{-1}, adjusted to pH 7.5) and a wine-like solution (a hydroalcoholic solution of 12 vol% acidified with tartaric acid (5 g L^{-1}) and adjusted to pH 3.5) as extraction solvents. Polysaccharides from grape skin extracts and wine polysaccharides were precipitated with cold acidified ethanol as described by Ayestarán *et al.*[27] and analyzed by HRSEC-RID as previously reported.[28]

Volatile compounds

To determine the volatile compounds, the method employed by Úbeda *et al.*[29] was followed, with some modifications. Headspace solid phase microextraction (SPME) was employed by putting 7.5 mL of sample, 1.5 g of sodium chloride and 10 µL of internal standard 4-methyl-2-pentanol (0.75 g L^{-1}) into a 20 mL glass vial that was placed on an autosampler tray. First, the vial with the sample was incubated for 20 min at 45 °C and 500 rpm, and volatiles from the headspace of the wine were extracted using a 2 cm 50/30 µm Carboxen/DVB/PDMS SPME fiber (Agilent Technologies, Santa Clara, CA, USA) for 40 min. Afterwards, the fiber was desorbed for 180 s and the sample was injected in splitless mode with a transfer line temperature of 280 °C. Gas chromatography analysis was then performed using an Agilent 7890B gas chromatograph coupled to an Agilent 5977 Inert quadrupole mass spectrometer (Agilent Technologies). A DB-WAX capillary column (60 mm × 0.25 mm, 0.25 µm film thickness; Agilent Technologies) was used with helium carrier gas at a flow rate of 1 mL min^{-1}. The oven temperature was programmed to start at 35 °C for 10 min, then rise to 100 °C at 5 °C min^{-1} and finally to 210 °C at 3 °C min^{-1} (holding for 40 min). Electron ionization mass spectra in scan mode were recorded at 70 eV in the range *m/z* 40–300. All data were recorded using MS ChemStation (Agilent Technologies). Biological triplicates were analyzed. Compound identification was based on mass spectral matching using the 2.0 version of the standard NIST library and the linear retention index (LRI) of authentic reference standards. Calibration curves were constructed to calculate the concentration of the volatile compounds. These standard curves were created by graphing the relative area of each compound *versus* concentration. The relative area was calculated by dividing the peak area of the target ion of each compound by the peak area of the target ion of the internal standard. Compounds without a standard were quantified as equivalents using the curve equation of another compound of the same chemical group with the same target ion.

Statistics

All results are expressed as the arithmetic mean ± standard deviation of three replicates. A one-factor analysis of variance (ANOVA) test ($P < 0.05$) was performed with SPSS software (IBM, Barcelona, Spain).

RESULTS AND DISCUSSION

In the present study, two plot-fields planted with Carménère cv., subjected to comparable weather and physiological conditions

during the 2015 season, were selected. They were selected because both plot-fields have no incline, are located very close to each other and are subjected to similar edaphoclimatic conditions. Moreover, both plot-fields were subjected to the same viticultural practices (by the same vine grower), and as a result they show similar vigor, similar yield (per vine) and similar ripening evolution of grapes, but differ in berry size of grapes at harvest. The differences in the berry size of grapes from both plot-fields could be observed in the average mass and volume of 100 berries shown in Table 1. Both plot-fields were harvested on the same date, and grapes from both vineyards reached similar maturity degrees, as observed by the soluble solid and titratable acidity values of grape juice (Table 1). According to these results, it could be asserted that grapes from both plots have the same technological maturity as well as the same phenolic maturity, since the total phenolic index (I_{280}) and total anthocyanins for the initial grape juice (grape juice of de-stemmed and crushed grapes before the onset of alcoholic fermentation) shown in Table 1 were almost the same for grapes from both plot-fields. These data suggest that differences in phenolic, aromatic and polysaccharide extraction from grapes to wine of this study are not due to differences in the degree of ripeness of grapes from the two plot-fields.

Table 1. Physical characterization of grape berries, chemical characterization of grape juice and color and chemical characterization of wines

Sample	Parameter	Plot-field 94N	Plot-field 99S
Grape	V_{100} [a]	85.7 ± 1.5a	133.0 ± 2.0b
	m_{100} [b]	89.3 ± 1.8a	136.8 ± 0.7b
	Seed number$_{100}$ [c]	130 ± 5a	127 ± 2a
	%Skin (m/m)[d]	12.5 ± 0.7a	15.4 ± 0.2b
	%Flesh (m/m)[e]	83.3 ± 0.06b	82.1 ± 0.2a
	%Seed (m/m)[f]	4.2 ± 0.1b	2.4 ± 0.0a
	%Seed (v/v)[g]	4.7 ± 0.2b	2.6 ± 0.1a
	Skin-to-flesh ratio[h]	150 ± 9a	188 ± 3b
	Seed-to-flesh ratio[i]	5.04 ± 0.10b	2.93 ± 0.03a
	Surface-to-volume ratio[j]	5.09 ± 0.03b	4.40 ± 0.02a
	Skin surface[k]	4.88 ± 0.06b	4.27 ± 0.05a
	Skin thickness[l]	25.6 ± 1.7a	36.1 ± 0.8b
Grape juice	SS[m]	24.0 ± 0.2a	23.8 ± 0.4a
	TA[n]	3.93 ± 0.09a	3.98 ± 0.10a
	pH	3.80 ± 0.03a	3.88 ± 0.01b
	Anthocyanins[o]	31.3 ± 4.6a	37.7 ± 0.3a
	I_{280}[p]	12.0 ± 1.6a	11.2 ± 1.2a
Wine	CI[q]	12.6 ± 0.4a	15.3 ± 0.7b
	Tone[r]	0.703 ± 0.020b	0.649 ± 0.002a
	%Red[s]	49.6 ± 1.1a	50.1 ± 0.1a
	%Yellow[t]	34.9 ± 0.3b	32.5 ± 0.1a
	%Blue[u]	15.6 ± 1.0a	17.3 ± 0.2b
	L^*[v]	5.07 ± 1.50b	2.13 ± 0.31a
	a^*[w]	30.29 ± 4.74b	14.84 ± 1.96a
	b^*[x]	8.67 ± 2.53b	3.64 ± 0.48a
	Anthocyanins[o]	577 ± 69a	773 ± 36b
	I_{280}[p]	45.8 ± 0.8a	48.7 ± 1.4b
	Flavanol monomers[y]	23.0 ± 2.2b	13.4 ± 2.4a
	Flavanol dimers[z]	39.2 ± 4.4a	35.9 ± 4.7a

Different letters in a row indicate significant differences ($P < 0.05$) between plots.
[a] Volume of 100 berries (mL).
[b] Mass of 100 berries (g).
[c] Average number of seeds contained in 100 berries.
[d] Relative proportion of skins expressed as mass percentage of fresh weight.
[e] Relative proportion of flesh expressed as mass percentage of fresh weight.
[f] Relative proportion of seeds expressed as mass percentage of fresh weight.
[g] Relative proportion of seeds expressed as volume percentage.
[h] Measured skin-to-flesh ratio expressed as mg skins g^{-1} berry flesh.
[i] Measured seed-to-flesh ratio expressed as mg seeds g^{-1} berry flesh.
[j] Theoretical ratio between berry surface and volume expressed as cm^2 skin cm^{-3} berry.
[k] Skin surface expressed as cm^2 skin g^{-1} berry fresh weight.
[l] Thickness of skins expressed as mg skin cm^{-2}.
[m] Soluble solid content expressed as °Brix.
[n] Titratable acidity expressed as g tartaric acid equivalent L^{-1}.
[o] Anthocyanin concentration expressed as mg malvidin-3-*O*-glucoside equivalent L^{-1}.
[p] Phenolic index.
[q] Color intensity.
[r] Yellow-to-red component ratio.
[s] Proportion of red component.
[t] Proportion of yellow component.
[u] Proportion of blue component.
[v] Lightness coordinate (CIELab).
[w] Redness coordinate (CIELab).
[x] Yellowness coordinate.
[y] Concentration of monomeric flavanols, extracted using SPE, determined by RP-HPLC-DAD and expressed as mg L^{-1}.
[z] Concentration of dimeric flavanols, extracted using SPE, determined by RP-HPLC-DAD and expressed as mg L^{-1}.

Grape berry characterization

As observed in Table 1, grape berries from 94N were smaller than those from 99S. In the literature, several studies relate berry size to the number of seeds per berry or to the mass of seeds per berry.[14] Our results disagree with those published data, since the average number of seeds per berry is almost the same for grapes from both plots, as indicated in Table 1. Furthermore, the distribution of the number of seeds per berry is also quite similar, although 94N grapes have statistically more berries with two seeds than 99S grapes (the distribution of the number of seeds per berry is shown in supporting information Fig. S1). Moreover, the mass and volume of seeds are quite similar for grapes from 94N and 99S. Hence, in the studied conditions, it appears that seed size does not influence berry size at harvest. The differences in berry size and the similarity of seed size enhance the differences in seed proportion between grapes from the two plots. In fact, the proportion of seeds for 94N grapes is almost 2-fold higher than for 99S grapes (mass proportion and volume proportion), as seen in the seed-to-flesh ratio. Since 94N grapes are smaller than 99S grapes, the berry surface and theoretical surface-to-volume ratio are greater for 94N grapes. These data indicate that the surface of skins in the fermentation tanks of 94N grapes is greater than that for 99S grapes. Despite these values, the (mass) proportion of skin is not greater in 94N grapes, since the skins of 99S grapes are thicker. Therefore the relative proportion of skins and the skin-to-flesh ratio are greater for 99S grapes. According to our results, smaller berries (from 94N plot-field) do not show a higher relative content of skins and lower relative content of seeds, which are the traditional arguments to suggest that smaller grapes lead to better wine. Thus our results appear to indicate that berry size *per se* could not be taken as a quality parameter for wine grapes if they come from different vineyards.

Phenolic extraction during maceration-fermentation

The extraction of phenolics during the maceration-fermentation of grapes was monitored on alternate days. As observed in Fig. 1, higher phenolic extraction occurs during the first stages of maceration, when the tumultuous fermentation phase occurs. In terms

Table 2. Volatile compounds (μg L^{-1}) of grapes, grape juice (before onset of alcoholic fermentation) and wines from both plot-fields

Chemical family	Plot-field	Grape	Grape juice	Wine
Aldehydes	99S	7115 ± 484c	1934 ± 306b	49 ± 3a
	94N	7201 ± 1561b	1324 ± 231a	54 ± 2a
Alcohols	99S	864 ± 149a*	1776 ± 163a	251 737 ± 20 094b
	94N	582 ± 59a	1696 ± 182a	270 871 ± 16 603b
Acids	99S	147 ± 11a*	52 ± 3a*	4355 ± 199b*
	94N	103 ± 17a	70 ± 2a	3654 ± 46b
Acetate esters	99S	0 ± 0a	0 ± 0a	951 ± 128b*
	94N	0 ± 0a	0 ± 0a	361 ± 35b
Ethyl esters	99S	0 ± 0a	0 ± 0a	2159 ± 192b
	94N	0 ± 0a	0 ± 0a	2036 ± 85b
Isoamyl esters	99S	0 ± 0a	0 ± 0a	4483 ± 836b
	94N	0 ± 0a	0 ± 0a	4652 ± 279b
Total esters	99S	0.20 ± 0.22a	0.96 ± 0.00a	6862 ± 969b
	94N	0 ± 0a	0.96 ± 0.00a	6850 ± 291b
Terpenes	99S	0 ± 0a	0.15 ± 0.04b	3.84 ± 0.11c*
	94N	0 ± 0a	0.22 ± 0.02a	3.17 ± 0.41b
Norisoprenoids	99S	0.02 ± 0.01a	0.02 ± 0.01a	1.39 ± 0.31b
	94N	0.07 ± 0.04a	0.01 ± 0.01a	1.12 ± 0.22b
Total	94N	8126 ± 443a	3763 ± 383a	263 009 ± 19 175b
	99S	7886 ± 1492a	3092 ± 346a	281 434 ± 16 929b

Values are expressed as mean ± standard deviation of three replicates. Different letters in a row indicate significant differences ($P < 0.05$) within type of sample (from grapes to wines). Asterisks (*) indicate significant differences ($P < 0.05$) between plot-fields for each chemical family and type of sample.

of phenolic content, the levels in 99S grape juice/wine samples were higher than in 94N samples during all maceration processes, but significant differences were only observed for two fermentation points (8 and 10 days of maceration). Those differences were reduced during the lasts days of maceration, and the finished (unstable) wines from both plot-fields contained almost the same concentration of total phenols. Wines were subjected to cold stabilization treatment and bottled and stored in a dark cellar until analysis. The 99S stabilized wines had statistically higher total phenol content (I_{280}) than the 94N stabilized wines (Table 1). Thus it appears that during stabilization the loss of phenolics is higher for 94N wines than for 99S wines. In contrast, the anthocyanin content of 99S samples was statistically higher than that of 94N samples from day 4 of maceration until the end of the process. As anthocyanins come from grape skins,[19] our results appear to indicate that a higher extraction of anthocyanins is more likely related to skin proportion (or skin-to-flesh ratio, Table 1) than to the relative surface of berries (related to the theoretical surface-to-volume ratio).

Volatile compounds

A total of 38 volatile compounds were monitored from the grape and through the maceration-fermentation process on alternate days. Among them were 19 esters, nine alcohols, four aldehydes, three acids, two terpenes and a C13 norisoprenoid. The results for volatile families of grapes, grape juice (before the onset of alcoholic fermentation) and finished wines are shown in Table 2. The volatile families determined in the samples during maceration-fermentation are shown in Fig. 2, and the results for each individual volatile compound throughout winemaking can be found in supporting information Table S1.

Aldehydes are usually present in the grape juice due to the enzymatic oxidation of the grape lipids during grape crushing.[30] They were the compounds present in higher quantity in the grape juice and, as expected, drastically decreased after 2 days of fermentation because they were reduced by yeast in the corresponding alcohols. No significant difference was found between the two samples regarding aldehyde content. Moreover, the most abundant aldehydes, hexanal and *E*-2-hexenal, formed in grape juice by enzymatic oxidation of linoleic acid, decreased in a more significant way in 94N samples. It should be noted that the majority of grape volatile compounds are located in the skin, some in their glycosylated form.[3,31] They are composed of a glucopyranosyl (sugar moiety) and an aglycone (non-sugar moiety) joined by a β-glycosidic linkage. These non-aromatic precursors release the odoriferous part of the molecule, for example through the β-glycosidase activity of yeast during fermentation. Most aldehydes are located in the skins,[3,32] especially in their glycosylated form. Therefore a possible explanation for the smaller decrease in 99S during the crushing of grapes was a continuous release of aldehydes from skins, since 99S grapes have a higher skin proportion, and this release compensates the natural decay of the total aldehyde content. From the second day of maceration-fermentation until the finished wines, only phenyl acetaldehyde remained in the samples, and no differences in its content were observed between samples from the two origins.

In terms of alcohols, the grapes contained hexanol, *cis*-3-hexenol and *trans*-2-hexenol, three major alcohols previously described in grapes.[33] These alcohols are formed from the corresponding C6 aldehydes by alcohol dehydrogenase enzymes. Both C6 alcohols and C6 aldehydes are described as having a fresh green aroma that can cause leafy-grassy off-odors in wine[34] or may not have an impact on the global aroma of the resulting wine, depending on their concentration.[35] The common trend was an increase in these C6 alcohols during maceration-fermentation, as previously reported by other authors.[36] The results showed that the concentration of volatile alcohols was higher in the 99S grapes, mainly due

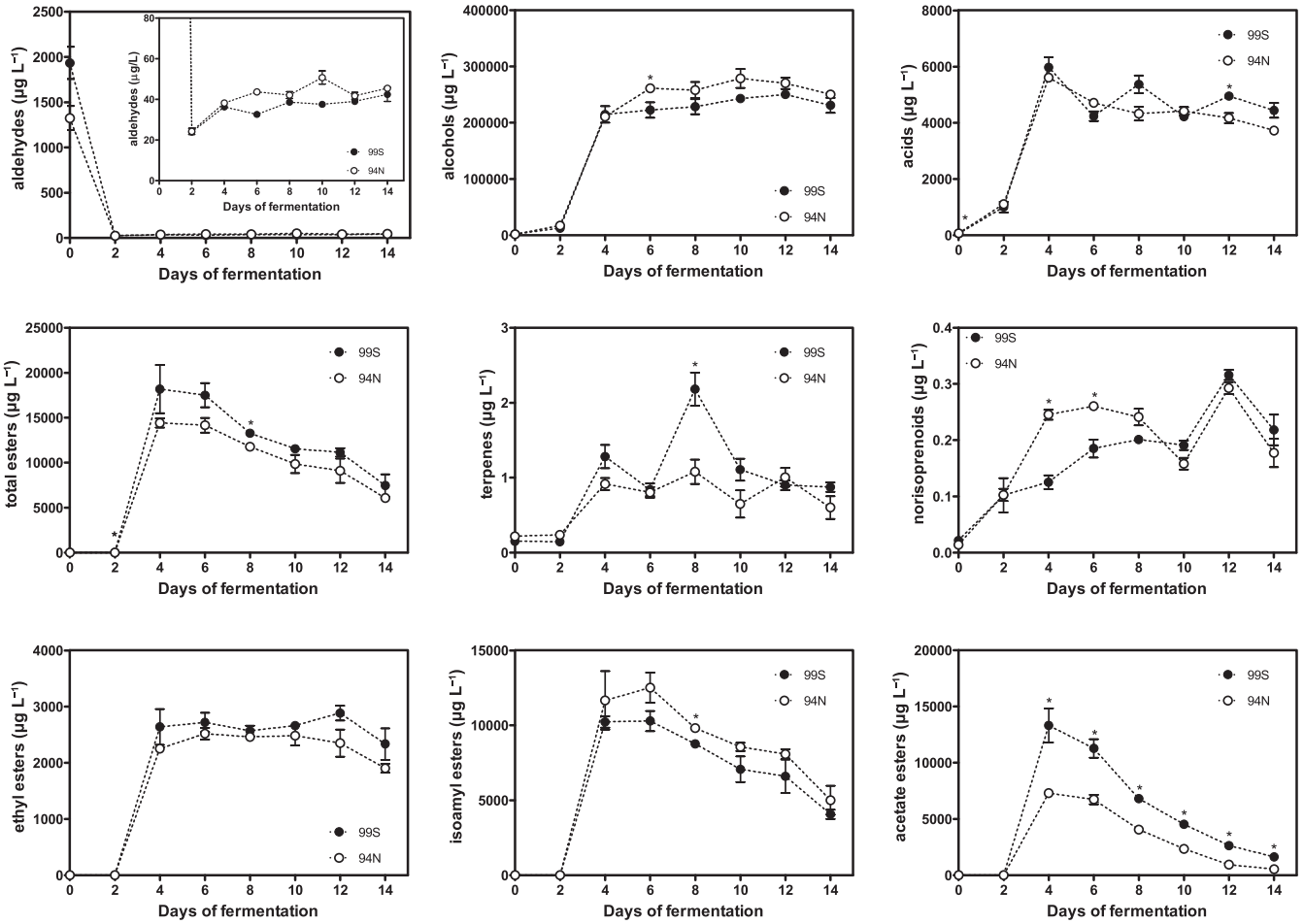

Figure 2. Extraction of aromatic compounds during maceration-fermentation of grapes from 94N and 99S plot-fields. Asterisks (*) indicate significant differences ($P < 0.05$) between grape origins for each chemical family at each fermentation stage.

to hexanol. However, during the maceration-fermentation process, the concentrations of samples from both plot-fields reached similar levels. After 2 days of fermentation, significant differences were observed for alcohols, and the 99S sample had higher levels of hexanol and *cis*-3-hexenol. It is possible that a thicker skin provokes a slower but constant release throughout maceration-fermentation for 99S samples, probably due to the release of hexanol from the glycoconjugates located in the skins. This superiority in the concentration of alcohols in 99S continued until the wines were finished. Moreover, *trans*-2-hexenol disappeared very quickly after 2 days of fermentation, possibly due to a reduction of this compound to hexanol during fermentation.[37] However, the typical alcohols produced by the action of the yeast arose from the second day of fermentation, namely isobutanol and isoamyl alcohol. On the fourth day, butanol, 3-methyl-1-pentanol, 2-phenylethanol and octanol were added to the alcohols group. Between the second and fourth day of fermentation, the production of this alcohol was explosive in 99S and more modest in 94N. However, this behavior is inverted between the fourth and sixth day of this process, mainly due to the important increase in 2-phenylethanol. This alcohol is produced by the yeast and is characterized by a rose-like aroma. The concentration in final wines from 94N grapes is almost 25% above the concentration of the 99S wines, and typically both are significantly above the odor threshold of this aromatic alcohol (14 000 µg L^{-1}).[38] Hence it appears that wines from 99S grapes had more alcohols from grapes but less alcohol content directly produced by the yeast during fermentation-maceration compared with the 94N wines.

Esters represent the chemical family of compounds with the major concentrations in wines following alcohols and enhance sweet-fruity aromas in wines. These compounds usually appear at negligible concentrations in grapes, the majority of them being formed during alcoholic fermentation by the action of yeasts. In our conditions, esters began to appear after the second day of maceration-fermentation and, as observed in Fig. 2, increased dramatically between day 2 and day 4 of maceration-fermentation. The evolution of these compounds was similar during the maceration-fermentation of 94N and 99S grapes, with no significant differences in the total esters. For acetate esters, it was observed that 99S wines had a higher concentration than 94N wines, mainly due to the higher concentration of isoamyl acetate. This ester has a typical fruity/pear/banana flavor and is produced from the reaction between isoamyl alcohol and acetyl coenzyme A, which is catalyzed by the enzyme isoamyl alcohol acetyl transferase.

As mentioned above, terpenes are molecules that are present as non-volatile compounds joined to a sugar moiety. This family of compounds has a strong influence on the aromatic profile of white Muscat wines primarily because they have a very low odor threshold.[39,40] Linalool and α-terpineol were determined in

both 94N and 99S samples. However, the amounts of these terpenes present in 99S samples (grapes with a higher proportion of skins) were statistically higher when compared with those of 94N wines. This is consistent with the results observed by Gomez *et al.*,[3] who found that all terpenes were located in the skin in Cabernet Sauvignon grapes, and Park *et al.*,[31] who observed that 46% of the total amount of glycosylated monoterpenes was also located in the skins. Hence it appears that grapes with a higher skin proportion contain greater amounts of terpenes. Related to terpenes, it is important to note the evolution of β-damascenone throughout the maceration-fermentation process. This compound is a C13 norisoprenoid that decreases the detection threshold of linalool when present in a matrix.[41] In a wine matrix, the odor threshold for damascenone is 4–7 $\mu g\,L^{-1}$, and the literature suggests that β-damascenone has more of an indirect than a direct impact on red wine aroma by enhancing other aromatic notes.[42] This C13 norisoprenoid is described as having honey/apple/caramel aromas.[43] In País cv., it was found as a glycoside aroma precursor located mainly in the pulp of the grape.[32] Since this compound was located mainly in the pulp, it seems quite logical that 94N grapes (with a higher flesh proportion) release a higher amount of β-damascenone after crushing, with significant differences during the first days of maceration-fermentation. However, these significant differences almost disappear from the eighth day of maceration-fermentation until the end, since at the end of the maceration process the wines showed a similar concentration of β-damascenone.

For all of the above, it can be generally asserted that higher numbers of volatile compounds were produced during the first stages of maceration, especially between days 2 and 4 of the fermentation-maceration process. The exception is that aldehydes decreased dramatically before the second day of fermentation to be transformed mainly into hexanol. Some significant differences ($P < 0.05$) were found between the 94N and 99S samples during maceration-fermentation, but the amounts of total aldehydes, alcohols, esters and norisoprenoids (Table 2) were almost the same for the finished wines from both plot-fields. In contrast, the amounts of total acids, total terpenes and acetate esters of finished wines were higher for 99S samples compared with 94N wines. The acids and terpenes were primarily located in the grape skins, thus it is logical that 99S grapes (with a higher proportion of skins) lead to higher amounts of these compounds in the finished wines. However, acetate esters are mainly produced by yeasts during alcoholic fermentation from the alcohols present in the fermentation matrix. Hence the higher amount of acetate esters and lower amount of alcohols for 99S samples could be related to higher ester production by yeast during winemaking, especially from the fourth day of maceration-fermentation, leading to finished wines with a greater fruity character.

Wine color

As observed in Table 1, 99S wines have a deeper color than 94N wines, since 99S wines have greater CI and lower L^*. In terms of wine color nuances, 94N wines showed a higher yellow component (as observed by tone, %yellow and b^* values), while 99S wines showed a higher blue component (as observed by %blue and b^* values). For the red component, no significant differences were observed for %red analyzed using the traditional method, but the redness (a^*) of 99S wines was almost double that of 94N wines. Hence the wines from 99S grapes, which were larger than grapes from the 94N plot-field, had a deeper and bluish color. Thus it appears that wine color potential was more related to the skin proportion of grapes than to grape berry size at harvest in this case.

Flavanol monomers, dimers and polymers

The flavanols of the finished wines were fractionated by SPE into two different fractions: a low-molecular-mass fraction (primarily monomers and dimers) and a polymeric fraction (polymers with more than three units of flavanol). For the low-molecular-mass fraction, two monomers and seven dimers were identified and quantified by RP-HPLC with diode array detection (DAD), and the results are shown in Table 1. The higher content of monomeric flavanols in the 94N wines could be related to the higher proportion of seeds, since seeds have been described as the source of these compounds.[44] In contrast, no differences were observed for dimeric flavanols between wines from the 94N and 99S plot-fields. The polymeric fraction of flavanols was analyzed using two different approaches: GPC by means of a GPC column installed into an HPLC-DAD system, and analysis by RP-HPLC-DAD of adducts formed after acidic depolymerization in the presence of excess phloroglucinol (hereafter referred to as phloroglucinolysis). As observed in Table 3, when the polymeric fraction was analyzed by GPC (a non-hydrolytic technique), wines from 99S and 94N grapes did not show significant differences. In contrast, when the polymeric fraction is analyzed by a hydrolytic technique such as phloroglucinolysis, the 94N wines show a higher amount of polymeric flavanols than the 99S wines. The mismatch between the two employed methods appears to indicate that both wines contain similar amounts of polymeric flavanols, but the 99S wines had a greater amount of complex polymers that could not be hydrolyzed (or did not lead to simple and quantifiable adducts after hydrolysis) and consequently were not observed by phloroglucinolysis.[45] Hence, for the hydrolysable polymeric flavanol composition determined via phloroglucinolysis, no differences were observed between wines from 94N and 99S grapes in the proportion of prodelphinidins (i.e. proportion of flavanols with trihydroxylated B-ring) and the proportion of galloylated units. According to the literature, it is expected that wines from grapes with a higher skin proportion contain a higher amount of prodelphinidins, while wines from grapes with a higher seed proportion contain a higher amount of galloylated units.[44] The lack of differences in prodelphinidins and the galloylation of wine polymeric flavanols under our conditions (despite differences in the skin and seed proportion of grapes) could be related to the low yield of phloroglucinolysis, which masks the true monomeric composition of polymers. Finally, significant differences were observed for the mean degree of polymerization (mDP) of polymeric flavanols between wines from the two origins. The 94N wines show higher mDP values for polymeric flavanols than the 99S wines. However, the GPC profiles do not support the idea that 94N contains more high-molecular-mass compounds. Hence, once again, the differences in mDP could be due to the technique employed, and the lower mDP for 99S polymeric flavanols could be related to the greater amount of complex non-hydrolyzable polymers, which does not release single monomeric flavanols as terminal units and consequently alters the estimated value for mDP.

In addition to the analyses of the flavanol fractions discussed above, the total proanthocyanidins (entire flavanol content, excluding monomeric flavanols) of wines were also analyzed using four different methods: the methylcellulose precipitation method, the LA method (also known as the Bate-Smith method) and the same two methods discussed above, i.e. GPC

Table 3. Wine proanthocyanidins

Method	Parameter	Total proanthocyanidins[a] 94N	Total proanthocyanidins[a] 99S	Polymeric flavanols[b] 94N	Polymeric flavanols[b] 99S
MC[c]	Conc.[g]	768 ± 123a	755 ± 140a		
GPC[d]	Conc.	843 ± 153a	981 ± 73a	533 ± 59A	617 ± 23A
LA[e]	Conc.	1862 ± 114b	1655 ± 29a		
PHGL[f]	Conc.	213.6 ± 36.0b	82.2 ± 14.7a	134.1 ± 10.1B	56.6 ± 2.9A
	mDP[h]	12.5 ± 0.7b	10.3 ± 0.6a	20.3 ± 5.9B	9.8 ± 1.6A
	%PD[i]	25.1 ± 0.5b	20.7 ± 0.8a	29.7 ± 1.2A	28.4 ± 3.6A
	%Gal[j]	4.9 ± 0.6a	5.4 ± 1.0a	1.4 ± 0.7A	4.4 ± 2.5A

Different lowercase letters in a row indicate significant differences ($P < 0.05$) between plots for total proanthocyanidin analyses. Different capital letters in a row indicate significant differences ($P < 0.05$) between plots for polymeric flavanol fraction analyses.
[a] Characterization of proanthocyanidin fraction (excluding flavanol monomers).
[b] Characterization of high-molecular-weight proanthocyanidins (excluding flavanol monomers and oligomers).
[c] Methylcellulose method; results are expressed as mg epicatechin equivalent L^{-1}.
[d] Gel permeation chromatography method; results are expressed as mg gallic acid equivalent L^{-1}.
[e] LA method; results are expressed as mg catechin equivalent L^{-1}.
[f] Phloroglucinolysis method; results for concentration are expressed as mg proanthocyanidins L^{-1}.
[g] Concentration.
[h] Mean degree of polymerization.
[i] Prodelphinidin fraction expressed as percentage of total proanthocyanidins.
[j] Galloylated fraction expressed as percentage of total proanthocyanidins.

and phloroglucinolysis. As observed in Table 3, the total proanthocyanidin content of wines from 94N and 99S grapes did not show statistical differences when non-hydrolytic techniques were applied (methylcellulose and GPC methods). In contrast, the total proanthocyanidin content was higher for 94N wines when hydrolytic techniques were applied (LA and phloroglucinolysis methods). Once again, the mismatch among the methods used for proanthocyanidin analyses appears to indicate that 99S wines contain higher amounts of complex non-hydrolyzable proanthocyanidins, while the total amount of proanthocyanidins estimated for non-hydrolytic techniques is almost the same for wines from both origins. According to the literature, grape seeds primarily release flavanol monomers and dimers into the wines, while grape skins release flavanol polymers with higher mDP.[44] Moreover, during winemaking, proanthocyanidins from grapes undergo several rearrangements, with or without the intervention of acetaldehyde.[19] In addition, anthocyanins can be incorporated into flavanol polymers, contributing to the formation of new polymeric pigments.[46] Furthermore, several oxidation processes have also been described to occur in wine proanthocyanidins during winemaking,[47] leading to some oxidized non-hydrolyzable proanthocyanidins. The conversion yield of skin tannins has been described as very low when analyzed by phloroglucinolysis, since they contain certain structures (e.g. A-type proanthocyanidins and oxidized proanthocyanidins) that are not depolymerizable by phloroglucinol reagent.[45] Thus, as 99S wines come from grapes with a higher proportion of grape skins, it appears logical that 99S wines contain a higher amount of proanthocyanidins from skins, which in turn could contain a higher proportion of non-depolymerizable proanthocyanidins.

Soluble polysaccharides of grape skins and finished wines

Wine polysaccharides have gained popularity in recent decades, since it has been observed that they have technological implications for winemaking, as well as wine stability and wine sensory perception.[48] Wine polysaccharides are derived from yeast cell walls or grape cell walls.[48,49] It has been noted that red wines contain higher amounts of polysaccharides than white wines due to a continuous release of these compounds from grape skins to the wine during maceration.[49,50] Hence, owing to differences in skin thickness between grapes from the 99S and 94N plot-fields, it could be interesting to investigate whether there are differences in the polysaccharide extractability of grape skins from the two origins. Thus skins from both the 99S and 94N plot-fields were extracted with an oxalate solution and with a wine-like solution. The results are shown in Table 4. For grape skins, soluble polysaccharides from two different fractions were quantified: F1 (greater than 23–30 kDa) and F3 (between 23–30 and 2.5 kDa). When polysaccharides were extracted with a buffer solution of oxalate, the polysaccharide profile showed three different fractions, but two fractions with a higher molecular mass were taken together to compare the extraction solvents. The differences in the polysaccharide profiles obtained by HRSEC-RID can be observed in supporting information Fig. S2. Thus we quantified a polysaccharide fraction (F1) and an oligosaccharide fraction (F3). The 99S grape skins released more F1 and less F3 when a wine-like solution was used as the extraction solvent. However, the total extracted polysaccharides (when F1 and F3 were taken together) showed no statistically significant differences between grape skins from the 94N and 99S plot-fields. Since the results were expressed as $\mu g\ g^{-1}$ skin fresh weight, several considerations must be taken into account. First, for the same mass of skins extracted, the 94N skins contained a greater total skin surface, because 94N grapes have thinner skins. In light of the results, it is possible that thinner skins could release more oligosaccharides or that a higher skin surface favors the extraction of those oligosaccharides. Second, since the proportion of grape skins was higher for 99S grapes, the higher extraction rate of F1 for 99S skins will likely be enhanced if the berry size and skin proportion are considered. Thus it appears that 99S grape skins have a higher potential to release polysaccharides than 94N grape skins.

For the wine soluble polysaccharides, four fractions were characterized from HRSEC-RID profiles, and the results are shown in Table 4. The 99S wines contain a statistically higher amount of total

Table 4. Soluble polysaccharides of grape skins and finished wines analyzed by HRSEC-RID

Sample	Plot-field	Parameter	F1	F2	F3	F4	Total
Skins OX[a]	94N	Conc.[d]	213 ± 77	–	225 ± 39	–	438 ± 110
		Range[e]	>30.1	–	30.1–2.4	–	>2.4
	99S	Conc.	210 ± 73	–	154 ± 32	–	407 ± 108
		Range	>28.9	–	28.9–2.5	–	>2.5
Skins EtOH[b]	94N	Conc.	210 ± 8*	–	170 ± 36*	–	381 ± 31
		Range	>29.9	–	29.9–2.4	–	>2.4
	99S	Conc.	270 ± 31	–	75 ± 24	–	345 ± 49
		Range	>23.3	–	23.3–2.6	–	>2.6
Wine[c]	94N	Conc.	202 ± 6*	272 ± 18*	211 ± 15*	17 ± 3	702 ± 41*
		Range	1430–102	102–18	18–3.2	3.2–2.3	>2.3
		M_n[f]	164.4 ± 0.6	62.4 ± 1.0*	11.5 ± 0.1	3.4 ± 0.4	–
	99S	Conc.	160 ± 22	362 ± 12	272 ± 12	22 ± 3	816 ± 33
		Range	801–119	119–18	18–3.2	3.2–2.3	>2.3
		M_n	162.8 ± 3.4	50.4 ± 3.5	11.5 ± 0.1	2.8 ± 0.4	–

Asterisks (*) indicate significant differences ($P < 0.05$) between plot-field origins for the same parameter.
[a] Soluble polysaccharides from grape skins extracted with an oxalate solution.
[b] Soluble polysaccharides from grape skins extracted with a wine-like solution.
[c] Soluble polysaccharides from wines.
[d] Concentration. Concentration of soluble polysaccharides from grape skins expressed as μg g^{-1} fresh skins. Concentration of soluble polysaccharides of wines expressed as mg L^{-1}.
[e] Range of molecular masses for each fraction expressed as kDa.
[f] Number average molecular weight for each fraction expressed as kDa.

soluble polysaccharides due to a higher content of fractions F1, F2 and F3. In contrast, no differences were observed for F4 (i.e. a fraction containing small peptides of approximately 0.4–3.0 kDa) for wines from both 94N and 99S plot-fields. The technique employed to estimate polysaccharide concentrations cannot confirm that differences in soluble polysaccharide content are due to polysaccharides from grape skins, since yeast releases polysaccharides during alcoholic fermentation and afterwards during the autolysis process.[49,50] Moreover, the yeast culture used for fermentation was the same for all vinifications at the outset, thus the contribution of yeast to the soluble polysaccharides of both wines was expected to be almost the same. However, for Cabernet Sauvignon red wines, it was found that a higher proportion of grape cell wall polysaccharides was correlated with a lower proportion of yeast polysaccharides.[50] Hence yeast polysaccharide release during winemaking can smooth differences in total soluble polysaccharide content. Considering all of these data, the higher levels of soluble polysaccharides of 99S wines and the suggested greater potential for polysaccharide release of 99S skins, it appears quite logical that a higher proportion of grape skins and/or thicker skins could lead to wines with higher polysaccharide content. Once again, there was no evidence of greater release of polysaccharides from smaller berries, since wines with higher polysaccharide content are those made from the larger grape berries from the 99S plot-field.

CONCLUSIONS

In light of the obtained results, it appears that the hypothesis that small grape berries produce more concentrated wines must be reconsidered. Our results indicate that the potential extractability of grape berries is more related to the skin mass proportion (or the real skin-to-flesh ratio (m/m)) than to berry size. Hence, in our conditions, more concentrated wines with a deeper color were obtained from larger grapes, although the proportion of skins was greater than for smaller berries. Thus skin thickness cannot be neglected, and it disrupts the popular, classical postulate of grape berry size. In conclusion, berry size at harvest *per se* could not be used as a grape quality parameter for winemakers, since winemaking with smaller berries does not ensure wines with deep color and higher sensory active compound content, even if they were from the same variety, reached the same degree of ripeness and came from vines subjected to similar conditions during the season.

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SUPPORTING INFORMATION

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

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