INVESTIGATION ON 'FREISA' RED GRAPE VARIETY: PHYSICO-CHEMICAL PROPERTIES OF GRAPES FROM FIVE PIEDMONT GROWING AREAS AND OF THE PRODUCED WINES

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ABSTRACT

This study aimed to investigate the physico-chemical characteristics of ''Freisa'' red winegrapes from five growing areas at three ripeness degrees. Wines were produced with grapes from each growing area. Results highlighted that "Freisa" grapes have relatively hard skins (break force>0.8 N), medium anthocyanin content (>800 mg/kg grapes), and high flavanol content (PRO>2500, FVA>1200 mg/kg grapes). Skin thickness, total anthocyanin and flavanol contents were significantly influenced by environmental conditions and ripeness. Grapes from Monferrato area showed the thickest skins and highest contents of anthocyanins but also of seed flavanols. Coherently, wine color characteristics and phenolic composition depended on growing area.

Keywords: phenolic composition, texture parameters, "Freisa" grapes, growing areas, ripeness, minor varieties

1. INTRODUCTION

Vitis vinifera L. cv. "Freisa" is an Italian native and historic red grape variety included in the National register of vine varieties from 1970 (GU 149-17/06/1970). This variety grows mainly in the central part of the Piedmont hills (North-West Italy), in the municipalities of Albugnano (AT), Castelnuovo Don Bosco (AT), Pino d'Asti (AT), and Moncucco Torinese (AT) (AJASSA *et al*., 2015), although vineyards of this grape variety are present also in other zones of the Piedmont region. The first citation of "Freisa" grapes is dated in 1517 in a customs document of Pancalieri town (TO), while the first vine-plantation was near Neive (CN) in 1692 (MAINARDI, 2003). Since then, in the centuries "Freisa" grapevines spread throughout all Piedmont because of its good yield, resistance to late frosts and diseases, *Plasmopara viticola* in particular (PECILE *et al*., 2018). Outside Piedmont, this variety has a little spread in other three regions of Italy (Valle d'Aosta, Lombardia, and Veneto), while abroad some vines are planted in Argentina and in the USA state of California (SCHNEIDER *et al*., 2013).

The Italian National Institute of Statistics (ISTAT) in 1970 reported in Italy 7,410 hectares planted with "Freisa" variety, while the last census in 2010 recorded a decrease of planted surface to 1,041 hectares. Of those, 80% of "Freisa" planted area is located in the Piedmont region, and about 420 hectares in the province of Asti (REGIONE PIEMONTE, 2018). Nowadays, the Italian nursery production of "Freisa" vines is active with a production over 150,000 vine plants in 2017 vintage (PECILE *et al*., 2018).

The strong linkage between this variety and its Piedmont growing area is evidenced by five Designations of Controlled Origin (DOC, *Denominazioni di Origine Controllata*): Freisa d'Asti DOC, Freisa di Chieri DOC, Colli Tortonesi DOC Freisa, Monferrato DOC Freisa, and Langhe DOC Freisa (ROSSI, 2012). Furthermore, from 2014 three of these Designations (Freisa d'Asti DOC, Langhe DOC, and Monferrato DOC) are included in a major wine-producing area of Piedmont that became a UNESCO World Heritage Site (UNESCO ITALIA, 2019).

Over the years, some investigations on "Freisa" variety were done. Regarding viticultural aspects, LISA *et al*. (2005) showed that the most suitable training system for "Freisa" variety is the lateral cordon trellis system, able to satisfy quality and consistent yields. Moreover, ASTEGIANO and CIOLFI (1974), CRAVERO and DI STEFANO (1992), GERBI *et al*. (2005), and ROLLE *et al*. (2008a) studied some aspects of the phenolic composition of "Freisa" grapes and showed that the content of tannins was generally high (> 3500 mg/kg) and of anthocyanins in the skins was satisfactory (> 800 mg/kg). ROLLE *et al*. (2008a) also observed a difficult extraction of anthocyanins from the skin into the must and, on the contrary, a relevant contribution of tannins from the seeds. Furthermore, GERBI *et al*. (2005) and ROLLE and GUIDONI (2007) showed that the anthocyanin profile of "Freisa" grapes was characterized by preponderance of di-substituted forms, cyanidin-3-*O*-glucoside (about 20%) and peonidin-3-*O*-glucoside (about 50%), similarly to "Nebbiolo" grape variety. Indeed, SCHNEIDER *et al*. (2004) and LACOMBE *et al*. (2013) studied the genetic profile of "Freisa" variety and established that it is an offspring of the Italian red grape varieties "Nebbiolo" and "Avanà". Furthermore, SCHNEIDER *et al*. (2004) found that there is a genetic relationship between "Freisa" variety and the white grape variety "Viognier".

Wines produced from "Freisa" grapes have had supporters and critics in the past. Generally, the wine made from ripe "Freisa" grapes has smooth and lightly astringent tannins. However, when the grapes do not reach the optimal ripeness degree, the resulting wines have a high level of acidity and tannicity, leading to sensory perceptions of excessive astringency and bitterness (SCHNEIDER *et al.*, 2013). In order to mitigate this fact and to produce quality wines, both for wines to be consumed young or after an ageing period, the winemaking of "Freisa" grapes often involves the partial removal of the seeds from the fermenting must after at least 48 hours from the beginning of alcoholic fermentation (ROLLE *et al.*, 2008a).

Although some technical information on "Freisa" grapes is already present in scientific literature, to our knowledge, no comprehensive physico-chemical characterization of grapes and wines has already been reported. Therefore, the main aim of this work was to investigate the mechanical properties of berry skin and the total extractable phenolic composition of berry skins and seeds from "Freisa" grapes, taking into consideration five different growing areas in the South-East of Piedmont region and three different grape ripeness levels. Indeed, the study aimed to assess the real impact of the production area and/or ripeness degree on grape characteristics. Finally, monovarietal wines were produced from "Freisa" grapes of each production area in order to propose appropriate enological techniques according to the grape features.

2. MATERIALS AND METHODS

2.1. Vineyards

Grape samples of *Vitis vinifera* L. cv. "Freisa" were collected from different vineyards located in five South-East Piedmont growing areas (North-West Italy): Astigiano (San Paolo Solbrito, AT), Collina Torinese (Chieri, TO), Langhe (Barolo, CN), Tortonese (Monleale, AL), and Monferrato (Casorzo, AT). The production zone was delimited by the following geographical coordinates: 44.374–45.023 N latitude and 7.500–8.974 E longitude, including the five Designations of Controlled Origin linked to "Freisa" wines: Freisa d'Asti DOC, Freisa di Chieri DOC, Langhe DOC Freisa, Colli Tortonesi DOC Freisa, and Monferrato DOC Freisa, respectively. The five mentioned vineyards, chosen each as representative of the respective growing location, were planted for commercial use and met the designation rules: the vines were at least 10 years old, planted on medium slope hills with exposure South (Astigiano, Collina Torinese, and Langhe locations), East (Tortonese location) or South-East (Monferrato location), vertical growth by lateral cordon trellis system, and Guyot pruned. From each production area, "Freisa" grapes were harvested in plastic boxes (maximum capacity of 20 kg to avoid grape crushing during transport) and about 20 kg were randomly selected and transported to the laboratory, while 150 kg were brought to the experimental cellar of the University of Turin for the winemaking process.

2.2. Grape samples and density class selection

Once in the laboratory, the berries were manually separated from the stalk with harvest shears and then placed on paper trays. About 200 berries were randomly taken for the determination of standard chemical parameters and other 200 berries were used for the evaluation of phenolic extractability indices. All the other berries were classified according to their density (i.e., total soluble solid content) by flotation as described by FOURNAND *et al.* (2006) and ROLLE *et al.* (2011b), in order to improve the physiological homogeneity inside the density class, to permit the comparative evaluation between ripeness levels at harvest, and to assess growing area effects.

In brief, for each growing area, the berries were floated and separated using saline solutions ranging from 130 to 190 g/L of sodium chloride, with densities spread between 1088 and 1125 kg/m^3. After flotation, all berries were washed with water and dried using absorbent paper. The three most represented density classes (by weight) were chosen: 1100 kg/m^3 (lower), 1107 kg/m^3 (middle), and 1115 kg/m^3 (higher). For each one, 20 berries were randomly selected for the determination of skin mechanical properties, and three sub-samples of 10 berries were used for the determination of skin and seed phenolic composition.

2.3. Mechanical properties of grapes

For the Texture Analysis test, a Universal Testing Machine (UTM) TAxT2i Texture Analyzer (Stable Micro Systems, Godalming, Surrey, UK), equipped with a HDP/90 platform and a 5 kg load cell, was used. The determination of the skin hardness parameters was carried out, on the whole berries placed in a horizontal plane on the metal plate of the UTM, by a puncture test using a SMS P/2 N needle probe (LETAIEF *et al.*, 2008). Berry skin break force (N, as F_{sk}), berry skin break energy (mJ, as W_{sk}), and berry skin Young's modulus (N/mm, as E_{sk}) were measured. Then, berry skin thickness (μm, as Sp_{sk}) was determined, on a portion of skin (ca. 0.25 cm^2) removed by a razor blade from the lateral side of each berry, by a compression test using a 2-mm SMS P/2 flat cylindral probe (RÍO SEGADE *et al.*, 2011a). For each sample, 20 berries were individually analyzed for each test.

2.4. Phenolic compounds extractability trials

For each replicate ($n = 3$), 10 berries were weighed and peeled. The skins and the seeds were manually removed from the pulp using a laboratory spatula, counted, weighed, and quickly immersed separately in 50 mL of a hydroalcoholic buffer solution at pH 3.20 containing 5 g/L of tartaric acid, 2 g/L of $Na_2S_2O_5$, and 12% v/v of ethanol. The skin and seed samples were then placed in an oven at 25°C for 12 h and one week, respectively (DI STEFANO and CRAVERO, 1991; RÍO SEGADE *et al.*, 2014). The skins into the buffer solution were homogenized at 8000 rpm for 1 min with an Ultra-Turrax T25 high-speed homogenizer (IKA Labortechnik, Staufen, Germany), centrifuged for 5 min at 3500 × g at 20°C using a PK 131 centrifuge (ALC International, Milano, Italy), and the supernatant was collected for analysis. In the case of seeds, they were removed from the buffer solution while the extract was used for the determination of the seed phenolic fraction.

2.5. Wine production

In brief, the "Freisa" grapes harvested in each location were separately destemmed, crushed, and the mash was added with 40 mg/L of potassium metabisulfite. After about 8 h, selected yeasts (Lalvin BRL97, Lallemand Inc., Montreal, Canada) were inoculated at a dose of 20 g/hL. Alcoholic and malolactic fermentation were carried out at controlled temperature of 27±2°C and 19±1°C, respectively. At the end of the fermentations, 60 mg/L of potassium metabisulfite were added and wines were cold-stabilized at 0 °C for 2 weeks, filtered (Seitz K300 grade filter sheets, Pall Corporation, Port Washington, NY, USA), and then bottled in glass bottles of 0.75 L with cork stoppers.

2.6. Chemical analysis

2.6.1 Reagents and standards

Solvents of HPLC-gradient grade and all other chemicals of analytical-reagent grade were purchased from Sigma-Aldrich (Milan, Italy). The solutions were prepared in deionized water produced by a Milli-Q system (Merck Millipore, Darmstadt, DE). Chemical standards of malvidin-3-*O*-glucoside chloride and cyanidin chloride were supplied by Extrasynthèse (Genay, France), whereas (+)-catechin was purchased from Sigma-Aldrich.

2.6.2 Standard chemical parameters of grapes and wines

Standard chemical parameters of grape musts, obtained by manual crushing and centrifugation, and wines were determined according to OIV (2016) methods. In particular, the following parameters were determined: grape potential alcohol degree (% v/v; OIV-OENO 466:R2012), must pH (OIV-MA-AS313-15:R2011), total acidity (g/L as tartaric acid; OIV-MA-AS313-01:R2015), wine alcohol content (% v/v; OIV-MA-AS312-01A:R2016), and wine dry net extract (g/L; OIV-MA-AS2-03B:R2012 and OIV-MA-AS311-02:R2009). The contents (g/L) of reducing sugars (as sum of glucose and fructose), tartaric acid and malic acid in grape musts were determined by HPLC (TORCHIO *et al.*, 2010).

2.6.3 Phenolic extractability indices and phenolic composition of grapes and wines

Phenolic extractability indices in grape berries were assessed for each sample in accordance with the procedure described by GLORIES and AUGUSTIN (1993), modified by CAGNASSO *et al.* (2008). The extractant solution at pH 1 was prepared just before use by mixing equal volumes of 1.0 M of hydrochloric acid and 2 g/L of potassium metabisulfite, while the extraction at pH 3.2 was carried out using a buffer solution containing 5 g/L of tartaric acid. The parameters obtained at pH 1 and pH 3.2, namely total phenolic content (A_{280}) and total anthocyanin content (TA_1 and $TA_{3.2}$), were used for the determination of the following extractability indexes: cellular maturity (EA%) and seed maturity (Mp%). The latter index was assessed by taking into consideration the average ratio (TAR) between the skin contents of total phenols (A_{280}) and total anthocyanins (expressed as g/L), equal to the value of 40 (CAGNASSO *et al.*, 2008). The EA% and Mp% indexes were calculated as follows: $EA\% = [(TA_1 - TA_{3.2})/TA_1] \times 100$; $Mp\% = \{[A_{280} - ((TA_{3.2}/1000) \times TAR)] / A_{280}\} \times 100)$.

On berry skin and seed extracts, and on resulting wines, spectrophotometric assessments were done in order to evaluate their phenolic composition. Total anthocyanins index (mg malvidin-3-*O*-glucoside chloride/kg grape or L wine, as TA) was evaluated in berry skin extracts and wines (DI STEFANO and CRAVERO, 1991; TORCHIO *et al.*, 2010), while monomeric anthocyanins index (mg malvidin-3-*O*-glucoside chloride/L wine, as MA) was determined only in wines previous isolation on polyvinylpolypyrrolidone (PVPP) and elution with an ethanol:water:HCl 37% (70:30:1) solution (DI STEFANO *et al.*, 1989). For both skin and seed extracts, and wines, total flavonoids index (mg (+)-catechin/kg grape or L wine, as TF), flavanols vanillin assay (mg (+)-catechin/kg grape or L wine, as FVA), and proanthocyanidins assay based on Bate-Smith reaction (mg cyanidin chloride/kg grape or L wine, as PRO) were evaluated (DI STEFANO and CRAVERO, 1991; TORCHIO *et al.*, 2010). A UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) was used for all analysis. The skin anthocyanin profile was determined by HPLC-

DAD after purification on a 1-g Sep-Pak C18 SPE cartridge (Waters Corporation, Milford, MA, USA) according to the protocol described by RÍO SEGADE *et al.* (2014). The chromatographic separation was performed on a LiChroCART analytical column (25 cm × 0.4 cm i.d.) purchased from Merck (Darmstadt, Germany) and packed with LiChrospher 100 RP-18 (5 μm) particles supplied by Alltech (Deerfield, IL, USA), using formic acid/water (10:90, v/v) and formic acid/methanol/water (10:50:40, v/v) as mobile phases. The different individual anthocyanin forms were expressed as area percentage of total forms.

2.6.4 Wine color parameters

Wine color was evaluated by the CIEL*a*b* parameters including lightness (L*), red/green color coordinate (a*), and yellow/blue color coordinate (b*) according to the method OIV-MA-AS2-11 (OIV, 2016). Furthermore, the color intensity (on 10 mm optical path) and the color hue were calculated using the method OIV-MA-AS2-07B (OIV, 2016). A UV-1800 spectrophotometer (Shimazdu Corporation) was used with 2-mm path length cuvettes.

2.7. Statistical analysis

Statistical analyses were carried out using R Statistics software version 3.4.0 (R Core Team, 2017). Levene's and Shapiro-Wilk's tests were used for assessing the homogeneity of variance and analysis of variance (ANOVA) residuals normality, respectively. In case of heteroscedasticity, we used the ANOVA with Welch's correction, followed by Tamhane's T2 post-hoc test when null hypothesis was rejected. In the case of homoscedasticity, we used one-way ANOVA and the Tukey HSD test for $p < 0.05$ to assess significant differences between groups.

3. RESULTS AND DISCUSSION

3.1. Grape chemical composition at harvest and berry sorting

In all five "Freisa" grape samples harvested from the representative vineyards of each growing area studied, the content of sugars at harvest was suitable to meet the minimum potential alcohol degree [% v/v] indicated in the disciplinary rules of each Designation of Controlled Origin (Table 1).

Regarding the phenolic maturity of unsorted grapes, the cellular maturity index (EA%) and the seed maturity index (Mp%) varied between 35-45% and 77-82%, respectively, depending on the growing area. These values agreed with those previously reported by ROLLE *et al.* (2008a) and confirmed particular varietal characteristics, such as the difficulty for releasing anthocyanins from the skin during maceration (EA% >30) and a high contribution of tannins from the seeds (Mp% >30) (RIBÉREAU-GAYON *et al.*, 2006). Moreover, in the present work, the Astigiano and Tortonese growing areas showed the lowest contents not only of potential anthocyanins (extracted at pH 1) but also of extractable anthocyanins (extracted at pH 3.2), the grapes from Tortonese area also showing the lowest anthocyanin extractability (EA% =45).

Table 1. Grape must standard chemical parameters and phenolic extractability indices of "Freisa" grapes harvested in five Piedmont growing locations.

	Location				
Parameter	**Astigiano**	**Collina Torinese**	**Langhe**	**Tortonese**	**Monferrato**
Designation of Origin	*Freisa d'Asti DOC*	*Freisa di Chieri DOC*	*Langhe DOC Freisa*	*Colli Tortonesi DOC Freisa*	*Monferrato DOC Freisa*
Minimum potential alcohol content [% v/v]	*10.5*	*10.5*	*10.5*	*11.0*	*10.0*
Density at 20°C	1.102	1.106	1.097	1.110	1.106
Sugars content [g/L]	239	249	227	258	247
Potential alcohol degree [% v/v]	14.2	14.8	13.5	15.3	14.6
pH	3.57	3.44	3.40	3.61	3.59
Total acidity [g/L as tartaric acid]	5.51	6.30	6.65	6.27	6.43
Tartaric acid [g/L]	5.54	6.81	6.61	6.76	5.18
Malic acid [g/L]	2.33	2.00	2.35	2.23	3.31
EA%	37	35	40	45	40
Mp%	82	78	77	82	77
$TA_{3.2}$ [mg/kg malvidin-3-*O*-glucoside chloride]	605	762	758	571	766
A_{280} on extract at pH 3.2	132	137	130	125	135
TA_1 [mg/kg malvidin-3-*O*-glucoside chloride]	959	1169	1269	1032	1273

EA% = cellular maturity index; Mp% = seed maturity index; $TA_{3.2}$ = total anthocyanins extracted at pH 3.2; A_{280} = absorbance at 280 nm; TA_1 = total anthocyanins extracted at pH 1.

The distribution percentages of all "Freisa" grape berries in different density classes at harvest were determined for the five vineyard locations. In agreement with data reported in scientific literature (KONTOUDAKIS *et al.*, 2011; ROLLE *et al.*, 2012), bell-shaped distributions were found (data not shown). In general, as already reported by FOURNAND *et al.* (2006), the difference in the total sugar content of the berries belonging to two consecutive density classes was ~17 g/L (i.e. 1% v/v potential alcohol). For all the growing areas, the three more representative density classes were 1100 kg/m^3, 1107 kg/m^3, and 1115 kg/m^3. More than 80% of the berries were belonging to these three density groups. In Table 2 some berry physical traits are shown for the three considered density groups. In order to evaluate the real impact of the growing area and ripeness degree, two comparisons were done by statistical analysis: the first among the five locations considering the same density class, the second among the three density classes inside each growing area. The first comparison showed that the grapes from Astigiano location presented, in two density classes out of three (the middle and the higher), the highest values of both berry and skin weight while, on the contrary, the grapes from Collina Torinese had the lowest berry weight values. For these two parameters, the second comparison showed that the ratio of skin and berry weight increases with increasing the sugar contents. This ratio ranged between 7.3 and 9.1% in the low density class, 8.2 and 8.9% in the middle density class, and 9.6 and 12.2% in the high density class.

Table 2. Physical parameters of "Freisa" grapes harvested in five Piedmont growing locations and sorted according to density.

Density class	Location	Berry weight (g)	Skin weight (g)	Seeds (n)	Single seed weight (g)
1100 kg/m^3	Astigiano	$2.21^{ab,\alpha}$	$0.17^{ab,\alpha}$	2.0^{a}	0.04^{ab}
	Collina Torinese	$1.93^{a,\alpha\beta}$	0.14^{a}	$2.5^{ab,\alpha\beta}$	0.04^{a}
	Langhe	2.18^{ab}	0.18^{bc}	2.3^{ab}	0.04^{ab}
	Tortonese	2.18^{ab}	$0.18^{ab,\alpha}$	$2.1^{a,\beta}$	0.05^{b}
	Monferrato	$2.41^{b,\beta}$	$0.22^{c,\beta}$	$2.9^{b,\beta}$	0.04^{ab}
	Sign (1)	*	***	**	*
1107 kg/m^3	Astigiano	$2.44^{b,\beta}$	$0.20^{b,\beta}$	2.4^{b}	0.04
	Collina Torinese	$2.03^{a,\beta}$	0.18^{ab}	$2.7^{b,\beta}$	0.04
	Langhe	2.19^{ab}	0.19^{ab}	2.5^{b}	0.04
	Tortonese	1.97^{a}	$0.17^{a,\alpha}$	$1.7^{a,\alpha}$	0.05
	Monferrato	$2.10^{a,\alpha}$	$0.18^{ab,\alpha}$	$2.6^{b,\alpha\beta}$	0.04
	Sign (1)	**	*	**	ns
1115 kg/m^3	Astigiano	$2.29^{c,\alpha}$	$0.24^{c,\gamma}$	2.6	0.04
	Collina Torinese	$1.78^{a,\alpha}$	0.17^{a}	2.1^{α}	0.04
	Langhe	1.90^{ab}	0.21^{ab}	2.1	0.04
	Tortonese	1.97^{ab}	$0.24^{bc,\beta}$	$2.0^{\alpha\beta}$	0.04
	Monferrato	$2.03^{b,\alpha}$	$0.21^{bc,\beta}$	2.4^{α}	0.04
	Sign (1)	***	***	ns	ns
	Sign (2)	**, *, ns, ns, *	***, ns, ns, **, **	ns, *, ns, *, *	ns, ns, ns, ns, ns

Values are expressed as average ($n = 3$). Different Latin letters within the same column indicate significant differences (1) among zones for the same berry density (Tukey HSD test; $p < 0.05$). Different Greek letters within the same column indicate significant differences (2) among the three density classes for the same zone (Tukey HSD test; $p < 0.05$). Sign: *, **, ***, and "ns" indicate significance at $p < 0.05$, 0.01, 0.001, and not significant, respectively.

Regarding seeds, although their weight was similar among the locations and the density classes, the variability of their number influenced the ratio between seeds and berry weight, the highest percentage value (5.4%) being in the grapes from Collina Torinese location belonging to the middle density class and the lowest value (3.6%) being in the grapes from Astigiano and Tortonese location belonging to the lower and the middle density class.

Grape berries of the same diameter and/or fresh weight have different total soluble solid concentrations as a consequence of the functional relationship among berry sugar accumulation, transpiration, and water accumulation (ŠUKLJE *et al*., 2012). Indeed, they may belong to different density classes. This aspect could be related not only to the environmental conditions of the vineyard but also to the position of a specific berry in a bunch and the relative position of a bunch in the vine. All these factors are of great importance because they influence the relative skin and seed weight and therefore berry phenolic composition (ROBY *et al*., 2004).

3.2. Berry skin mechanical properties

The berry skin mechanical parameters measured on "Freisa" grapes from the five different locations and sorted by density are available in Table 3. The data showed high values of skin break force ($F_{sk} \geq 0.824$ N) and skin break energy ($W_{sk} \geq 0.782$ mJ), in agreement with LETAIEF *et al.* (2008) who reported higher values of skin hardness parameters for "Freisa" grapes with relation to other six grapevine varieties, including the genetically related "Nebbiolo" grape variety. Although "Freisa" can be considered a grape variety with a 'hard' skin, when compared with other varieties growing in the same vineyard in a single vintage (i.e. same bioclimatic indexes), the skin hardness parameters (F_{sk} and W_{sk}) for "Freisa" berries did not show the highest values (ROLLE *et al.*, 2011a). In this sense, "Becuet" (variety grown in mountain environment) and some varieties of "Teinturier" (cultivar with coloured pulp) were characterized by harder skins.

Table 3. Berry skin mechanical properties of "Freisa" grapes harvested in five Piedmont growing locations and sorted according to density.

Density class	Location	Berry skin break force [F_{sk}. N]	Berry skin break energy [W_{sk}. mJ]	Berry skin Young's modulus [E_{sk}. N/mm]	Berry skin thickness [Sp_{sk}. μm]
1100 kg/m^3	Astigiano	0.855±0.182	0.862±0.278	0.386 ± 0.082^{ab}	190 ± 35^{a}
	Collina Torinese	0.907±0.121	0.817±0.178	0.440 ± 0.058^{b}	192 ± 34^{a}
	Langhe	0.892±0.120	0.868±0.173	$0.400\pm0.055^{b.\beta}$	$188\pm29^{a.\alpha}$
	Tortonese	0.869±0.141	0.884±0.256	$0.386\pm0.057^{ab.\beta}$	215 ± 25^{a}
	Monferrato	0.879±0.201	0.893±0.272	0.342 ± 0.061^{a}	244 ± 35^{b}
	Sign (1)	ns	ns	***	***
1107 kg/m^3	Astigiano	0.875±0.175	0.896±0.268	0.380±0.074	199 ± 29^{a}
	Collina Torinese	0.892±0.148	0.880±0.236	0.391±0.062	213 ± 24^{ab}
	Langhe	0.897±0.137	0.906±0.256	$0.380\pm0.050^{\alpha\beta}$	$218\pm27^{ab.\beta}$
	Tortonese	0.838±0.128	0.877±0.211	$0.332\pm0.035^{\alpha}$	230 ± 23^{b}
	Monferrato	0.835±0.229	0.822±0.280	0.354±0.099	225 ± 34^{b}
	Sign (1)	ns	ns	ns	**
1115 kg/m^3	Astigiano	0.879±0.130	0.934±0.217	0.340 ± 0.038^{a}	209±22
	Collina Torinese	0.870±0.162	0.782±0.217	0.417 ± 0.090^{b}	211±28
	Langhe	0.857±0.156	0.860±0.223	$0.356\pm0.064^{a.\alpha}$	$224\pm31^{\beta}$
	Tortonese	0.824±0.160	0.872±0.247	$0.339\pm0.058^{a.\alpha}$	231±17
	Monferrato	0.945±0.137	0.953±0.219	0.387 ± 0.066^{ab}	223±43
	Sign (1)	ns	ns	***	ns
	Sign (2)	ns, ns, ns, ns, ns	ns, ns, ns, ns, ns	ns, ns, *, **, ns	ns, ns, ***, ns, ns

Values are expressed as average ± standard deviation (n = 20). Different Latin letters within the same column indicate significant differences (1) among zone for the same berry density (Tukey HSD test; $p < 0.05$). Different Greek letters within the same column indicate significant differences (2) among the three density classes for the same zone (Tukey HSD test; $p < 0.05$). Sign: *, **, ***, and "ns" indicate significance at $p < 0.05$, 0.01, 0.001, and not significant, respectively.

No significant differences in F_{sk} and W_{sk} were observed among density classes and growing areas. Although it is normal to observe high coefficients of variation for these parameters in berry skin analysis, this behaviour may highlight the assumption that the berry skin hardness traits (F_{sk} and W_{sk}) are firstly variety dependent and not markedly influenced by the ripeness degree and the growing area. In fact, ROLLE *et al.* (2008b) have observed similar values of F_{sk} and W_{sk} at the last weeks during ripening of "Nebbiolo" variety. These skin mechanical parameters give high resistance to "Freisa" grapes against fungal pathogens (ROSENQUIST and MORRISON, 1988) and to handling injury during harvest, transport, and postharvest treatments (KÖK and ÇELIK, 2004). From a technological point of view, a higher skin hardness is generally associated to a slower anthocyanin release into the must-wine during maceration-fermentation but, with a longer maceration, the anthocyanin extraction yield is higher (ROLLE *et al.*, 2008b). This aspect is particularly important and favorable for "Freisa" grapes and for all the wine grapes varieties rich in 3'-hydroxylated anthocyanins because these pigments are extracted preferentially during the initial phase of maceration and may be easily oxidized by the enzymes present in the juice (GONZÁLEZ-NEVES *et al.*, 2008).

Regarding other berry skin hardness-derived parameter, significant differences were found for Young's modulus (E_{sk}) that measures the rigidity or stiffness of tissues. Inside each density class, zone effects were observed. Particularly, the grapes of Collina Torinese location had the highest values of E_{sk} (> 0.390 N/mm). Furthermore, ripeness effects were evidenced in some growing areas (Langhe and Tortonese), with a tendency to decrease E_{sk} values when increasing the density class, as observed by TORCHIO *et al.* (2010) on "Barbera" grapes.

For skin thickness (Sp_{sk}, Table 3), the values obtained in the present study agreed with the reported by LETAIEF *et al.* (2008) for "Freisa" grapes. In this study, some significant differences were found among growing areas and density classes. Monferrato location showed a significantly higher value of skin thickness with respect to other locations when considering the grapes belonging to the lowest density class considered (244 μm). Furthermore, also in the other density groups this location evidenced high skin thickness values (225 and 223 μm, respectively, in the middle and in the higher density class). On the other hand, Astigiano location was characterized by thinner skins (190-209 μm). Previous studies have highlighted that precipitation indices, which could strongly influence berry water availability in the last ripening weeks, are responsible for differences in skin mechanical parameters among production areas (ROLLE *et al.*, 2011a). Moreover, the influence of rain on skin thickness has been already reported for Mondeuse grapes during on-vine withering (ROLLE *et al.*, 2009).

Significant differences in this skin mechanical parameter among the density groups inside each location were found only for Langhe samples ($p < 0.001$), with an increasing trend from 1100 kg/m^3 class to the higher density classes. A slight increasing tendency was found also for other locations and similarly observed by TORCHIO *et al.* (2010) on "Barbera" grapes, by RÍO SEGADE *et al.* (2011b) on Galician grapes, and by ROLLE *et al.* (2012) on "Nebbiolo". Moreover, ROLLE *et al.* (2011b) showed on "Nebbiolo" grapes that stiffer and thicker berry skins allowed respectively the higher accumulation and extraction of proanthocyanidins, while harder skins provided higher concentration and extraction of oligomeric flavanols. VILLANGÓ *et al.* (2015) evidenced that thicker skins had the highest content of anthocyanins in "Syrah", while RÍO SEGADE *et al.* (2011a) found on "Mencía" grapes that thinner skins were characterized by a higher release of anthocyanins.

Therefore, although the changes in the skin mechanical properties during ripening are generally limited, the preliminary knowledge of these parameters could help the planning of the harvest time (i.e. selection among different vineyards) and the strategy of maceration process, in order to guarantee the rapid degradation of the grape skin and an improved extraction of its components (RÍO SEGADE *et al.*, 2014, RÍO SEGADE *et al.*, 2015).

3.3. Skin anthocyanin content and profile

The skin total anthocyanin content (TA_{sk}) and profile of "Freisa" samples are shown in Table 4. The first comparison shows that there were differences in total anthocyanin content among growing areas for all the density classes considered ($p < 0.05$ for density class of 1100 kg/m^3, and $p < 0.001$ for 1107 and 1115 kg/m^3). This behaviour highlighted a zone effect on grape total anthocyanin content because climate indices, sunlight and soil conditions are factors of great relevance on anthocyanin biosynthesis (SPAYD *et al.*, 2002). The lowest and highest contents of anthocyanins were found in the grapes from Astigiano and Monferrato locations, respectively, belonging to the three density classes, even though the differences between the two locations were more relevant for the grapes belonging to the highest density classes (156, 418, and 475 mg/kg for 1100, 1107, and 1115 kg/m^3, respectively). Although probably there is not an only influential factor, the South-East exposure of the Monferrato vineyard could favour the biosynthesis of anthocyanins or reduce their degradation, with respect to the South orientation of the Astigiano vineyard, as a consequence of temperature and/or sunlight effects.

These same samples had also the lowest and highest values of Sp_{sk}, respectively, in accordance with VILLANGÓ *et al.* (2015) that observed on "Syrah" grapes a higher concentration of anthocyanins in the thicker skins. The second comparison showed that total extractable concentrations of anthocyanins almost always increased with increasing berry density, with significance at $p < 0.05$ in three cases (Collina Torinese, Langhe, and Tortonese locations) and at $p < 0.01$ in the case of Monferrato location. A density effect on anthocyanins concentration is evident and confirms the assumptions of TORCHIO *et al.* (2010), who previously observed this behaviour on "Barbera" grapes, and of ROLLE *et al.* (2011b) on "Nebbiolo" grapes.

In this study, "Freisa" grapes were characterized by a high average percentage of simple anthocyanin glucosides (95.3% of total forms on average), with a higher content of free di-substituted anthocyanins (66.0% on average) with respect to free tri-substituted free forms (29.3% on average), as already reported in literature (ROLLE and GUIDONI, 2007; FERRANDINO *et al.*, 2012). In the present study, peonidin-3-*O*-glucoside was the major anthocyanin compound found, with an average content of 48.1%, followed by malvidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside with an average percentage of 19.2 and 17.8% of total forms, respectively. A similar anthocyanin profile was evidenced for "Nebbiolo" grapes and for other minor, ancient grape varieties diffused in North-West Italy close to the Alps such as "Avanà", "Doux d'Henry", "Grignolino", "Neretto di Bairo", "Pelaverga Piccolo", and "Rastajola", which showed a percentage of peonidin-3-*O*-glucoside of about 45-55% on total anthocyanin forms (GERBI *et al.*, 2005; FERRANDINO *et al.*, 2012).

Some differences in the anthocyanin profile among locations and density classes were observed, even if the variations are not easily attributable to environmental or ripeness factors, as already discussed by ORTEGA-REGULES *et al.* (2006). In the lower density class, the grapes from Collina Torinese location had the lowest content of di-substituted anthocyanins (cyanidin- and peonidin-3-*O*-glucoside, 56.1%) and the highest content of tri-substituted ones (delphinidin-, petunidin-, and malvidin-3-*O*-glucoside, 38.8%).

Table 4. Total extractable anthocyanins content and relative profile of "Freisa" grapes harvested in five Piedmont growing locations and sorted according to density.

Density class	Location	Total anthocyanins TA_{sk}	Delphinidin-3-O-glucoside [%]	Cyanidin-3-O-glucoside [%]	Petunidin-3-O-glucoside [%]	Peonidin-3-O-glucoside [%]	Malvidin-3-O-glucoside [%]	Acetyl-3-O-glucosides [%]	Cinnamoyl-3-O-glucosides [%]
1100 kg/m^3	Astigiano	834 ± 53^{ab}	3.93 ± 0.11^{a}	$19.43 \pm 0.61^{b.\alpha}$	5.44 ± 0.06^{a}	$49.17 \pm 1.02^{b.\alpha}$	$17.06 \pm 0.36^{a.\beta}$	1.28 ± 0.13^{b}	$3.69 \pm 0.05^{ab.\beta}$
	Collina Torinese	$961 \pm 69^{ab.\alpha\beta}$	$5.23 \pm 0.30^{b.\beta}$	14.02 ± 0.93^{a}	$7.09 \pm 0.29^{b.\beta}$	$42.06 \pm 2.27^{a.\alpha}$	$26.52 \pm 2.18^{b.\beta}$	0.31 ± 0.05^{b}	3.77 ± 0.39^{b}
	Langhe	$924 \pm 76^{ab.\alpha}$	3.54 ± 0.36^{a}	20.00 ± 1.81^{b}	$4.93 \pm 0.18^{a.\alpha}$	50.99 ± 1.36^{b}	16.60 ± 0.69^{a}	$0.95 \pm 0.03^{a.\alpha}$	$2.99 \pm 0.31^{a.\alpha}$
	Tortonese	$777 \pm 16^{a.\alpha}$	$3.24 \pm 0.65^{a.\alpha}$	17.57 ± 0.66^{ab}	$4.57 \pm 0.55^{a.\alpha}$	$53.17 \pm 3.34^{b.\beta}$	$16.45 \pm 1.39^{a.\alpha}$	$1.32 \pm 0.04^{b.\alpha}$	3.69 ± 0.22^{ab}
	Monferrato	$990 \pm 100^{b.\alpha}$	$3.17 \pm 0.52^{a.\alpha}$	18.51 ± 2.21^{b}	$4.79 \pm 0.62^{a.\alpha}$	$51.63 \pm 1.78^{b.\beta}$	17.24 ± 2.58^{a}	1.34 ± 0.11^{b}	3.32 ± 0.24^{ab}
	Sign (1)	*	***	**	***	***	***	***	*
1107 kg/m^3	Astigiano	773 ± 75^{a}	3.53 ± 0.96	$21.35 \pm 0.60^{b.\beta}$	5.05 ± 0.95	$50.47 \pm 5.09^{\alpha\beta}$	$14.96 \pm 2.70^{\alpha\beta}$	1.28 ± 0.13	$3.36 \pm 0.69^{\alpha\beta}$
	Collina Torinese	$869 \pm 52^{a.\alpha}$	$3.99 \pm 0.59^{\alpha}$	15.67 ± 2.09^{a}	$5.69 \pm 0.35^{\alpha}$	$50.52 \pm 2.59^{\beta}$	$19.76 \pm 1.41^{\alpha}$	1.14 ± 0.09	3.23 ± 0.23
	Langhe	$1122 \pm 44^{b.\beta}$	4.67 ± 0.42	18.89 ± 2.07^{ab}	$6.16 \pm 0.40^{\beta}$	44.81 ± 3.49	20.78 ± 3.71	$1.30 \pm 0.31^{\alpha\beta}$	$3.39 \pm 0.66^{\alpha\beta}$
	Tortonese	$847 \pm 45^{a.\alpha\beta}$	$4.22 \pm 0.38^{\alpha\beta}$	17.65 ± 1.09^{ab}	$5.57 \pm 0.44^{\alpha\beta}$	$48.67 \pm 1.77^{\alpha\beta}$	$19.00 \pm 2.00^{\alpha\beta}$	$1.32 \pm 0.11^{\alpha}$	3.56 ± 0.30
	Monferrato	$1191 \pm 41^{b.\beta}$	$4.87 \pm 0.66^{\beta}$	15.47 ± 0.91^{b}	$6.64 \pm 0.69^{\beta}$	$47.75 \pm 2.57^{\alpha\beta}$	20.88 ± 1.76	1.35 ± 0.13	3.34 ± 0.24
	Sign (1)	***	ns	**	ns	ns	ns	ns	ns
1115 kg/m^3	Astigiano	785 ± 50^{a}	3.25 ± 0.14^{a}	$22.42 \pm 0.24^{b.\beta}$	4.57 ± 0.09^{a}	$54.07 \pm 0.86^{b.\beta}$	$11.90 \pm 0.34^{a.\alpha}$	1.05 ± 0.02^{a}	$2.74 \pm 0.20^{a.\alpha}$
	Collina Torinese	$1050 \pm 50^{b.\beta}$	$4.88 \pm 0.42^{b.\alpha\beta}$	15.97 ± 0.90^{a}	$6.61 \pm 0.31^{b.\beta}$	$46.77 \pm 2.09^{ab.\alpha\beta}$	$21.23 \pm 0.68^{b.\alpha}$	1.11 ± 0.15^{a}	3.43 ± 0.31^{b}
	Langhe	$1042 \pm 74^{b.\alpha\beta}$	4.94 ± 0.88^{b}	17.65 ± 2.19^{ab}	$6.51 \pm 0.90^{ab.\beta}$	43.25 ± 4.87^{a}	22.13 ± 4.97^{ab}	$1.52 \pm 0.16^{b.\beta}$	$4.01 \pm 0.14^{c.\beta}$
	Tortonese	$936 \pm 77^{ab.\beta}$	$5.40 \pm 0.76^{b.\beta}$	16.31 ± 0.18^{a}	$6.87 \pm 0.70^{ab.\beta}$	$43.67 \pm 3.85^{a.\alpha}$	$22.24 \pm 2.36^{b.\beta}$	$1.54 \pm 0.06^{b.\beta}$	3.97 ± 0.27^{c}
	Monferrato	$1260 \pm 29^{c.\beta}$	$5.28 \pm 0.31^{b.\beta}$	16.28 ± 0.96^{a}	$7.13 \pm 0.50^{b.\beta}$	$45.45 \pm 2.06^{a.\alpha}$	21.13 ± 1.87^{b}	1.41 ± 0.08^{b}	3.33 ± 0.17^{b}
	Sign (1)	***	**	***	**	**	***	**	**
	Sign (2)	ns,*,*,*,**	ns,*,ns,*,**	**,ns,ns,ns,ns	ns,**,*,**,**	**,*,ns,*,*	***,**,ns,*,ns	ns,ns,*,*,ns	*,ns,*,ns,ns

Values are expressed as average ± standard deviation ($n = 3$). Different Latin letters within the same column indicate significant differences (1) among zone for the same berry density (Tukey HSD test; $p < 0.05$). Different Greek letters within the same column indicate significant differences (2) among the three density classes for the same zone (Tukey HSD test; $p < 0.05$). Sign: *, **, ***, and "ns" indicate significance at $p < 0.05$, 0.01, 0.001, and not significantly respectively.
TA_{sk} = total anthocyanins (mg malvidin-3-*O*-glucoside chloride/kg berries).

On the contrary, in the higher density class, the grapes from Astigiano location had the highest content of di-substituted anthocyanins (76.5%) and the lowest content of tri-substituted forms (19.7%). When the ripeness effect was evaluated for each location, in the grapes from Astigiano (and partly Collina Torinese) location there was a relative increase of di-substituted anthocyanins and a decrease of tri-substituted ones when increasing the density class considered, while in the grapes from Langhe, Tortonese, and Monferrato locations the opposite effect was observed. As previously commented for total anthocyanins, the South orientation of the Astigiano vineyard seems to influence negatively the percentage of the most stable forms, namely acylated- and malvidin-derivatives. It also can be hypothesized that higher rainfall and lower temperatures during the pre-veraison period could probably affect positively the F3′H activity, resulting in a higher di-substituted flavonoid accumulation, such as peonidin- and cyanidin-3-*O*-glucoside, and negatively the F3′5′H activity with a lower tri-substituted flavonoid accumulation, such as delphinidin-, petunidin- and malvidin-3-*O*-glucoside (FERRANDINO and GUIDONI, 2010).

3.4. Total flavonoids and flavanol composition of skins and seeds

The skin and seeds flavonoid content and flavanol composition of "Freisa" grapes harvested in the five different locations and sorted by flotation are shown in Table 5. Regarding grape skins, significantly higher contents of total flavonoids (TF_{sk}) were found in the grapes from Langhe and Monferrato locations when belonging to the middle and higher density classes ($p < 0.001$). However, an increase of TF_{sk} was observed with the increase of sugar level for all locations with exception of Astigiano, as also occurred for TA_{sk} content. Differently, few differences were found in skin proanthocyanidin and oligomeric flavanol contents (represented by PRO_{sk} and FVA_{sk} parameters, respectively) among locations for each density class and no differences were observed among the berries belonging to the different density classes for the same growing area. A similar behaviour of PRO_{sk} and FVA_{sk} was previously observed in "Nebbiolo" grapes with berry density (ROLLE *et al.*, 2012). Particularly, in this study, the highest contents of both larger and smaller molecular mass flavanols were found in the grapes skins from Astigiano, especially in the middle density class [PRO_{sk}, 2849 mg cyanidin chloride/kg berries; FVA_{sk}, 1122 mg (+)–catechin/kg berries]. This location had also the highest values of FVA_{sk}/PRO_{sk} ratio (0.36 and 0.39, respectively, in the lower and in the middle density class), thus evidencing an important contribution of the oligomeric flavanol fraction assessed by FVA_{sk} parameter.

Regarding seeds, a remarkable aspect of "Freisa" grapes is the high content of flavanols reactive to vanillin (FVA_s) with respect to proanthocyanidins (PRO_s) and, as a consequence, the high FVA_s/PRO_s ratio (> 0.57). ROLLE *et al.* (2008a) have also observed a similar relationship between oligomeric and polymeric flavanols in "Freisa" grapes and therefore a varietal behaviour is evident. This could represent an issue when seeds flavanol extraction into the must during the maceration is high, with the risk of producing unbalanced wines for bitterness and astringency. In fact, flavanols vanillin assay (FVA) quantifies monomeric and oligomeric flavanols, which are more bitter than the polymeric ones, while the astringency is positively correlated to mean degree of polymerization (mDP) and galloylation degree (VIDAL *et al.*, 2003, CHEYNIER *et al.*, 2006).

Table 5. Skin and seeds total extractable phenolic composition of "Freisa" grapes harvested in five Piedmont growing locations and sorted according to density.

Density class	Location	SKINS				SEEDS			
		Total flavonoids [TF_{sk}]	Proanthocyanidins [PRO_{sk}]	Vanillin assay [FVA_{sk}]	FVA_{sk}/PRO_{sk} ratio	Total flavonoids [TF_s]	Proanthocyanidins [PRO_s]	Vanillin assay [FVA_s]	FVA_s/PRO_s ratio
1100 kg/m^3	Astigiano	3770±154	2800±88	999±117^{b}	0.36±0.03^{b}	1310±115^{bc}	1476±5^{b}	847±69^{a}	0.57±0.03^{a}
	Collina Torinese	3802±29$^{\alpha}$	2204±253	649±138^{a}	0.29±0.03^{ab}	860±49^{a}	961±22^{a}	621±70^{a}	0.65±0.06^{a}
	Langhe	3816±160$^{\alpha}$	2305±161	750±18^{ab}	0.33±0.03^{ab}	1011±137^{ab}	981±126^{a}	758±125^{a}	0.77±0.03^{b}
	Tortonese	3482±70$^{\alpha}$	2217±181	691±147^{ab}	0.31±0.04^{ab}	780±154^{a}	986±145^{a}	593±115^{a}	0.60±0.03^{a}
	Monferrato	3710±210$^{\alpha}$	2501±380	648±122^{a}	0.26±0.01^{a}	1425±69$^{c.\beta}$	1684±87$^{c.\beta}$	1327±88$^{b.\beta}$	0.79±0.02^{b}
	Sign (1)	ns	ns	*	*	***	***	***	***
1107 kg/m^3	Astigiano	3481±104^{a}	2849±140^{b}	1122±206^{b}	0.39±0.06^{b}	1205±142^{cd}	1278±163^{c}	942±131^{cd}	0.74±0.05^{b}
	Collina Torinese	3736±149$^{a.\alpha}$	2054±183^{a}	584±39^{a}	0.28±0.01^{ab}	1059±125^{bc}	1187±224^{bc}	771±96^{bc}	0.65±0.04^{ab}
	Langhe	4249±162$^{b.\beta}$	2787±73^{b}	878±242^{ab}	0.32±0.09^{ab}	898±25^{b}	903±49^{ab}	663±21^{ab}	0.74±0.06^{b}
	Tortonese	3813±24$^{a.\beta}$	2516±211^{ab}	722±135^{ab}	0.29±0.04^{ab}	642±111^{a}	800±89^{a}	470±89^{a}	0.58±0.04^{a}
	Monferrato	4241±212$^{b.\beta}$	2809±372^{b}	650±76^{a}	0.23±0.04^{a}	1384±49$^{d.\beta}$	1644±112$^{d.\beta}$	1137±116$^{d.\beta}$	0.69±0.08^{ab}
	Sign (1)	***	**	*	*	***	***	***	*
1115 kg/m^3	Astigiano	3541±210^{a}	2651±82	796±207	0.30±0.08	979±100^{b}	1075±134	693±78^{ab}	0.64±0.01
	Collina Torinese	4326±180$^{bc.\beta}$	2317±255	605±35	0.26±0.03	868±39^{ab}	1014±86	575±42^{ab}	0.57±0.01
	Langhe	4204±53$^{bc.\beta}$	2727±328	778±83	0.29±0.04	947±102^{b}	1042±126	698±53^{ab}	0.67±0.03
	Tortonese	3863±263$^{ab.\beta}$	2287±442	672±118	0.30±0.03	708±53^{a}	861±18	520±57^{a}	0.60±0.06
	Monferrato	4567±125$^{c.\beta}$	2885±141	819±53	0.28±0.03	949±111$^{b.\alpha}$	1111±160$^{\alpha}$	707±94$^{b.\alpha}$	0.64±0.07
	Sign (1)	***	ns	ns	ns	*	ns	*	ns
	Sign (2)	ns,**,*,*,**	ns,ns,ns,ns,ns	ns,ns,ns,ns,ns	ns,ns,ns,ns,ns	ns,ns,ns,ns,***	ns,ns,ns,ns,**	ns,ns,ns,ns,***	ns,ns,ns,ns,ns

Values are expressed as average ± standard deviation ($n = 3$). Different Latin letters within the same column indicate significant differences (1) among zone for the same berry density (Tukey HSD test, $p < 0,05$). Different Greek letters within the same column indicate significant differences (2) among the three density classes for the same zone (Tukey HSD test; $p < 0.05$). Sign: *, **, ***, and "ns" indicate significance at $p < 0.05$, 0.01, 0.001, and not significant, respectively. $TF_{sk,s}$ = total flavonoids (mg (+) – catechin/kg berries); $PRO_{sk,s}$ = proanthocyanidins (mg cyanidin chloride/kg berries); $FVA_{sk,s}$ = flavanols vanillin assay (mg (+) – catechin/kg berries).

The content of flavanols in "Freisa" seeds showed differences mainly induced by the growing area, with the highest content of TF_s, FVA_s and PRO_s in the grapes from Monferrato location and belonging to the lower and middle density classes [PRO_s, 1684 and 1644 mg cyanidin chloride/kg berries; FVA_s, 1327 and 1137 mg (+)–catechin/kg berries, respectively]. Among density classes, a decreasing tendency of flavanols content from the lower to higher density class was observed, only being significantly relevant in the grapes from Monferrato location. These results agreed with the higher seed number found in the berries from the Monferrato vineyard, particularly in those belonging to the lower density class. Indeed, it is widely recognised that during the berry development the concentration of monomeric flavanols decreases rapidly (GONZÁLEZ-MANZANO *et al.*, 2004) and that the histological and histochemical modifications (i.e. solidification of the cells rich in tannins) occurring during grape ripeness influence negatively the aptitude for the extraction of these compounds (CADOT *et al.*, 2006).

3.5. "Freisa" experimental wines assessment

Monovarietal wines were produced for each single "Freisa" Designation of Origin considered in this study, and then analysed according to their general chemical composition, chromatic characteristics, and phenolic content (Table 6).

As expected, the alcohol content in all five "Freisa" wines were higher than the minimum limits imposed by disciplinary rules and always higher than 13% v/v. In the same way, also total acidity and dry net extract were satisfactory with values higher than 5.27 g/L as tartaric acid and 25.8 g/L, respectively. The wines produced by grapes from Astigiano and Collina Torinese locations had the highest values of total acidity (6.26 and 6.27 g/L as tartaric acid, respectively), with the latter presenting also the highest alcohol content (14.6% v/v). Wines produced by grapes from Tortonese location showed the highest value of dry net extract (28.1 g/L).

Regarding colour characteristics, the highest values of lightness (L*), red/green colour coordinate (a*), and yellow/blue colour coordinate (b*), related to the lowest value of colour intensity, were found in the wine from Astigiano location that also had the lowest content of total anthocyanins (TA_{sk}). As opposed, the wine from Monferrato location showed the lowest values of L* and of the coordinates a* and b*, related to the highest value of colour intensity, and a high value of colour hue (more red-orange nuances).

The anthocyanin content of "Freisa" wines ranged between 198 and 303 mg malvidin-3-*O*-glucoside chloride/L. It is well known that anthocyanins are strongly related to wine chromatic characteristics, but PAISSONI *et al.* (2018) demonstrated that they also can contribute to in-mouth properties in function of their content and acylation. These authors estimated the perception threshold of total anthocyanins at 255 mg/L, and evidenced that acetylated and cinnamoylated anthocyanins contribute to in-mouth sensory properties more than glucoside forms. Regarding "Freisa" wines analysed in the present study, only in two wines out of five (Langhe DOC Freisa and Monferrato DOC Freisa) the content of total anthocyanins was higher than the proposed threshold. Taking into account that "Freisa" grapes had a low amount of acylated forms (Table 4), it may be hypothesized that the wine contains limited acylated anthocyanins and, therefore, presents a very small contribution to in-mouth sensory traits. For these reasons, the contribution of anthocyanins to in-mouth sensory properties of "Freisa" wines can be excluded in most cases.

Table 6. Compositional characteristics of wines produced from "Freisa" grapes harvested in five Piedmont growing locations.

	Location				
Wine parameter	**Astigiano**	**Collina Torinese**	**Langhe**	**Tortonese**	**Monferrato**
Designation of Origin	*Freisa d'Asti DOC*	*Freisa di Chieri DOC*	*Langhe DOC Freisa*	*Colli Tortonesi DOC Freisa*	*Monferrato DOC Freisa*
Minimum alcohol content [% v/v]	*11.0*	*11.0*	*11.0*	*11.0*	*11.0*
Minimum total acidity content [g/L as tartaric acid]	*5.50*	*4.50*	*4.50*	*4.50*	*5.00*
Minimum dry net extract [g/L]	*19.0*	*19.0*	*19.0*	*20.0*	*20.0*
Alcohol content [% v/v]	13.7	14.6	14.1	14.1	14.1
Total acidity [g/L as tartaric acid]	6.26	6.27	5.85	5.27	5.48
Dry net extract [g/L]	25.8	26.7	27.0	28.1	27.3
L*	20.7	17.7	19.1	18.8	16.9
a*	55.1	52.4	54.0	52.7	51.1
b*	51.3	49.6	51.7	49.9	49.4
Color intensity [A.U., 10 mm optical path]	17.6	18.2	18.1	18.5	19.6
Color hue	0.87	0.79	0.78	0.92	0.90
TA$_w$ [mg malvidin-3-*O*-glucoside chloride/L]	198	238	303	237	276
MA$_w$ [mg malvidin-3-*O*-glucoside chloride/L]	64	83	135	85	107
MA$_w$/TA$_w$ [%]	32	35	45	36	39
TF$_w$ [mg (+)-catechin/L]	2217	2050	2048	2449	2155
FVA$_w$ [mg (+)-catechin/L]	2136	1679	1580	2209	1665
PRO$_w$ [mg cyanidin chloride/L]	3654	2875	3038	4044	3213
FVA$_w$/PRO$_w$	0.58	0.58	0.52	0.55	0.52

The values in the first three numerical rows are the levels to achieve in the wines for each appellation, in the latter three are the levels achieved in the produced wines. L* = lightness; a* = red/green color coordinate; b* = yellow/blue color coordinate; TA$_w$ = total anthocyanins; MA$_w$= monomeric anthocyanins; TF$_w$ = total flavonoids; PRO$_w$ = proanthocyanidins; FVA$_w$ = flavanols vanillin assay.

The highest percentage of monomeric anthocyanins on the total content (45%) was found in Langhe DOC Freisa wine, which had also the highest content of total anthocyanins (303 mg malvidin-3-*O*-glucoside chloride/L). On the contrary, the lowest monomeric/total anthocyanin ratio (32%) and total anthocyanin content (198 mg malvidin-3-*O*-glucoside chloride/L) were found in Freisa d'Asti DOC wine. In both wines the data confirmed the trends found in the grape anthocyanins (Table 1).

About wine flavanols, the two highest contents of smaller molecular mass tannins [FVA$_w$, 2209 and 2136 mg (+)-catechin/L] were found in the wines from Tortonese and Astigiano locations, respectively. These two wines were produced from the grapes with high values of seed maturity index (Mp%=82, Table 1). As previously mentioned, smaller molecular mass tannins (flavanol monomers and oligomers) are perceived as more bitter, and therefore high contents of FVA$_w$ in wines could be involved in high bitter sensations. For this purpose, some production strategies could be useful: for instance, when both technological and phenolic maturity are not satisfactory, the grape dehydration could aid to reach a better sugars/acids ratio and a higher level of seeds lignification with a lower flavanols release. Furthermore, when technological maturity is satisfactory, the removal of the seeds from the must 48-96 h after the beginning of the fermentation process could limit the extraction of flavanols during maceration-fermentation (ROLLE *et al*., 2008a).

4. CONCLUSIONS

The physico-chemical characteristics of the Piedmont minor variety *Vitis vinifera* L. cv. "Freisa" were comprehensively studied, considering grapes from five different growing areas and at three ripeness levels defined by density sorting. Mechanical properties and phenolic composition of "Freisa" grapes were differently affected by these factors, and some parameters showed a strong varietal character. Particularly, berry skin hardness parameters (break force and break energy), which influence extraction kinetics of phenolic compounds, were slightly affected by environmental conditions and ripeness degree. Instead, the other two mechanical parameters, namely skin Young's modulus and thickness, varied among the locations and berry density classes considered. The first one decreased with increasing the ripeness degree while, on the contrary, the second one showed an increasing tendency that affects positively the skin extractable content of phenolic compounds as follows. Berry skin total anthocyanin content increased significantly with increasing the density class, in agreement with higher values of skin thickness, and also was significantly affected by the growing area. Nevertheless, berry skin flavanol contents (monomeric - oligomeric and polymeric) varied only among locations, while seed flavanol contents varied among the locations and the density classes with a tendency to decrease from the less ripe to the ripest grapes berries considered. Grapes from Monferrato growing area showed the thickest skins, and coherently the highest contents of total anthocyanins but also of seed flavanols.

The crucial point of "Freisa" grapes has been confirmed to be the high release of flavanols from the seeds during winemaking. This aspect highlights the importance of a careful management of the maceration process in cellar and the possibility of using process strategies such as partial grape dehydration, when both technological and phenolic maturity are not satisfactory to avoid an excessive alcoholic degree, or the seeds removal from the must to reduce an excessive extraction. This may be important especially when "Freisa" grapes have not achieved a satisfactory ripeness degree, and hence the risk to produce unbalanced wines for bitterness and astringency sensory traits is high.

REFERENCES

Ajassa R., Caviglia C., Destefanis E., Mandrone G. and Masciocco L. 2015. A Study for Preserving the "Freisa" Terroir (Central Piedmont-Northwestern Italy) from Soil Erosion. In: Engineering Geology for Society and Territory, 8, 427-429, Springer, Cham.

Astegiano V. and Ciolfi G. 1974. Costituenti antocianici dei vini rossi piemontesi. Riv. Vitic. Enol., 11-12, 473-479, 497-507.

Cadot Y., Minana Castello M.T. and Chevalier M. 2006. Flavan-3-ols compositonal changes in grape berries (*Vitis vinifera* L. cv Cabernet Franc) before veraison, using two complementary analytical approaches, HPLC reversed phase and histochemistry. Anal. Chim. Acta, 563:65-75.

Cagnasso E., Rolle L., Caudana A. and Gerbi V. 2008. Relationship between grape phenolic maturity and red wine phenolic composition. *Ital. J. Food Sci.*, 20:365-380.

Cheynier V., Duenas-Paton M., Salas E., Maury C., Souquet J.M., Sarni-Manchado P. and Fulcrand H. 2006. Structure and properties of wine pigments and tannins. Am. J. Enol. Vitic., 57:298-305.

Cravero M.C. and Di Stefano R. 1992. Composizione fenolica di alcune varietà di vite coltivate in Piemonte. Vignevini, 19:47-54.

Di Stefano R., Cravero M.C. and Gentilini N. 1989. Metodi per lo studio dei polifenoli dei vini. *L'Enotecnico*, 25:83-89.

Di Stefano R. and Cravero M.C. 1991. Metodi per lo studio dei polifenoli dei vini. Riv. Vitic. Enol., 2:37-45.

Ferrandino A., Carra A., Rolle L., Schneider A. and Schubert A. 2012. Profiling of hydroxycinnamoyl tartrates and acylated anthocyanins in the skin of 34 *Vitis vinifera* genotypes. J. Agric. Food Chem., 60:4931-4945.

Ferrandino A. and Guidoni S. 2010. Anthocyanins, flavonols and hydroxycinnamates: an attempt to use them to discriminate *Vitis vinifera* L. cv 'Barbera' clones. Eur. Food Res. Technol., 230:417-427.

Fournand D., Vicens A., Sidhoum L., Souquet J.M., Moutounet M. and Cheynier V. 2006. Accumulation and exractability of grape skin tannins and anthocyanins at different advanced physiological stages. J. Agric. Food Chem., 54:7331-7338.

Gerbi V., Rolle L., Zeppa G., Guidoni S. and Schneider A. 2005. Indagine sul profilo antocianico di vitigni autoctoni piemontesi. Industrie delle bevande, 34:*23-27.*

Glories Y. and Augustin M. 1993. Maturité phénolique du raisin, consequences technologiques: application aux millésimes 1991 et 1992. Actes du Colloque 'Journée technique du CIVB', Bordeaux, France, 56-61.

González-Manzano S., Rivas-Gonzalo J.C. and Santos-Buelga C. 2004. Extraction of flavan-3-ols from grape seed and skin into wine using simulated maceration. Anal. Chim. Acta, 513:283-289.

González-Neves G., Gil G. and Barreiro L. 2008. Influence of grape variety on the extraction of anthocyanins during the fermentation on skins. Eur. Food Res. Technol., 226:1349-1355.

Kök D. and Çelik S. 2004. Determination of characteristics of grape berry skin in some table grape cultivars (*V. vinifera* L.*). J. Agron.*, 3:141-146.

Kontoudakis N., Esteruelas M., Fort F., Canals J.M., De Freitas V. and Zamora F. 2011. Influence of the heterogeneity of grape phenolic maturity on wine composition and quality. Food Chem., 124:767-774.

Lacombe T., Boursiquot J.M., Laucou V., Di Vecchi-Staraz M., Péros J.P. and This P. 2013. Large-scale parentage analysis in an extended set of grapevine cultivars (*Vitis vinifera* L.), Theor. Appl. Genet., 126:401-414.

Letaief H., Rolle L. and Gerbi V. 2008. Mechanical behavior of winegrapes under compression tests. Am. J. Enol. Vitic., 59:323-329.

Lisa L., Lisa L. and Parena S. 2005. Forme di allevamento per vitigni autoctoni piemontesi. Informatore Agrario, 47:43-49.

Mainardi G. 2003. Le storiche colline della 'Freisa'. Vignevini, 3:91-94.

OIV. 2016. Recueil international des méthodes d' analyse des vins et des moûts. Organisation Internationale de la Vigne et du Vin, Paris, France.

Ortega-Regules A., Romero-Cascales I., López-Roca J-M., Ros-García J-M. and Gómez-Plaza E. 2006. Anthocyanin fingerprint of grapes: environmental and genetic variations. J. Sci. Food Agric., 86:1460-1467.

Paissoni M.A., Waffo-Teguo P., Ma W., Jourdes M., Rolle L. and Teissedre P.L. 2018. Chemical and sensorial investigation of in-mouth sensory properties of grape anthocyanins. Sci. Rep., 8:17098, 1-13.

Pecile M., Zavaglia C. and Ciardi A. 2018. "Freisa" - Scheda della varietà. In: Registro Nazionale delle Varietà di Vite. Ministero delle politiche agricole alimentari, forestali e del turismo, Rome, Italy.

Regione Piemonte. 2018. Sistema Piemonte data on viticultural productions: "Freisa". www.sistemapiemonte.it.

Ribéreau-Gayon P., Glories Y., Maujean A. and Dubourdieu D. 2006. The Chemistry of Wine Stabilization and Treatments. In: Handbook of Enology, Volume 2. John Wiley & Sons, 2nd Edition, England.

Río Segade, S., Giacosa S., Gerbi V. and Rolle L. 2011a. Berry skin thickness as main texture parameter to predict anthocyanin extractability in winegrapes. LWT-Food Sci. Technol., 44:392-398.

Río Segade S., Orriols I., Giacosa S., Rolle L. 2011b. Instrumental texture analysis parameters as winegrapes varietal markers and ripeness predictors. Int. J. Food Prop., 14:1318-1329.

Río Segade S., Pace C., Torchio F., Giacosa S., Gerbi V. and Rolle L. 2015. Impact of maceration enzymes on skin softening and relationship with anthocyanin extraction in wine grapes with different anthocyanin profiles. Food Res. Int., 71:50-57.

Río Segade, S., Torchio, F., Giacosa, S., Ricauda Aimonino, D., Gay, P., Lambri, M., Dordoni, R., Gerbi, V. and Rolle, L. 2014. Impact of several pre-treatments on the extraction of phenolic compounds in winegrape varieties with different anthocyanin profiles and skin mechanical properties. J. Agric. Food Chem., 62:8437-8451.

Roby G., Harbertson J. F., Adams D. A. and Matthews M. A. 2004. Berry size and vine water deficits as factors in winegrape composition: anthocyanins and tannins. Aust. J. Grape Wine Res., 10:100-107.

Rolle L., Torchio F., Giacosa S. and Gerbi V. 2009. Modification of mechanical characteristic and phenolic composition in berry skins and seeds of Mondeuse winegrapes throughout the on-vine drying process J. Sci. Food Agric., 89:1973-1980.

Rolle L., Torchio F., Giacosa S., Río Segade S., Cagnasso E. and Gerbi V. 2012. Assessment of physicochemical differences in "Nebbiolo" grape berries from different production areas sorted by flotation. Am. J. Enol. Vitic., 63:195-204.

Rolle L., Gerbi V., Schneider A., Spanna F. and Río Segade S. 2011a. Varietal relationship between instrumental skin hardness and climate for grapevines (*Vitis vinifera* L.). J. Agric. Food. Chem., 59:10624-10634.

Rolle L., Río Segade S., Torchio F., Giacosa S., Cagnasso E., Marengo F. and Gerbi V. 2011b. Influence of Grape Density and Harvest Date on Changes in Phenolic Composition, Phenol Extractability Indices, and Instrumental Texture Properties during Ripening. J. Agric. Food Chem., 59:8796-8805.

Rolle L., Caudana A. and Gerbi V. 2008a. Tecniche di vinificazione per la valorizzazione del vitigno Freisa. 31° Congresso mondiale della vite e del vino - VI assemblea generale dell'OIV. 15-20 giugno 2008, Verona, Italy.

Rolle L. and Guidoni S. 2007. Color and anthocyanin evaluation of red winegrapes by CIE L*, a*, b* parameters. J. Int. Sci. Vigne Vin., 41:193-201.

Rolle L., Torchio F., Zeppa G. and Gerbi V. 2008b. Anthocyanin extractability assessment of grape skins by texture analysis. J. Int. Sci. Vigne Vin., 42:157-162.

Rosenquist, J.K. and Morrison J.C. 1988. The development of the cuticle and epicuticular wax of the grape berry. *Vitis*, 27:63-70.

Rossi A. 2012. Codice della Vite e del Vino. Unione Italiana Vini. ISBN 978-88-900836-4-8.

Schneider A., Torello Marinoni D. and Raimondi S. 2013. 'Freisa'. In: Italian Vitis Database, www.vitisdb.it, ISSN 2282-006X.

Schneider A., Boccacci P., Torello Marinoni D., Botta R., Akkak A. and Vouillamoz J. 2004. The genetic variability and unexpected parentage of "Nebbiolo". First International Conference on "Nebbiolo" grapes, Sondrio, Italy.

Spayd S. E., Tarara J. M., Mee D. L. and Ferguson J. C. 2002. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. Am. J. Enol. Vitic., 53:171-182.

Šuklje K., Lisjak K., Baša Česnik H., Janeš L., Du Toit W., Coetzee Z., Vanzo A. and Deloire A. 2012. Classification of grape berries according to diameter and total soluble solids to study the effect of light and temperature on methoxypyrazine, glutathione, and hydroxycinnamate evolution during ripening of Sauvignon blanc (*Vitis vinifera* L.). J. Agric. Food Chem., 60:9454-9461.

Torchio F., Cagnasso E., Gerbi V. and Rolle L. 2010. Mechanical properties of, phenolic composition and extractability indices of “Barbera” grapes of different soluble solids contents from several growing areas. Anal. Chim. Acta, 660:183-189.

Unesco Italia. 2019. Paesaggi vitivinicoli del Piemonte: Langhe-Roero e Monferrato. www.unesco.it.

Vidal S., Francis L., Guyot S., Marnet N., Kwiatkowski M., Gawel R., Cheynier V. and Waters E. 2003. The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. J. Sci. Food Agr., 83:564-573.

Villangó S., Pásti G., Kállay M., Leskó A., Balga I., Donkó A., Ladányi M., Pálfi Z. and Zsófi Z. 2015. Enhancing Phenolic Maturity of Syrah with the Application of a New Foliar Spray. S. Afr. J. Enol. Vitic., 36:304-315.

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