PAPER

CYCLIC PROANTHOCYANIDINS IN PINOT NOIR WINE

V. MERKYTĖ[1,2], A. DUPAS DE MATOS[1,2], E. LONGO*[1,2], P.F. TCHOUAKEU BETNGA[1,2] and E. BOSELLI[1,2]

[1]Free University of Bozen-Bolzano, Faculty of Science and Technology, Piazza Università 5, 39100 Bolzano, Italy

[2]Oenolab, NOITechPark Alto Adige/Südtirol, Via A. Volta 13B, 39100 Bolzano, Italy

*Corresponding author: edoardo.longo@unibz.it

ABSTRACT

The identification of cyclic (or crown) B-type proanthocyanidins in wine was recently reported; this identification has unlocked new possibilities for their application to wine quality evaluation. Here, cyclic and non-cyclic B-type proanthocyanidins, along with other phenolic compounds as well as sensory and oenological parameters, were characterized in eleven Pinot Noir wines. The wines were produced from grapes harvested in different vineyards and under different winemaking conditions. With Principal Component Analysis (PCA) based on the cyclic proanthocyanidins or their relative proportions, it was possible to differentiate the wines according to specific winemaking conditions. Moreover, cyclic proanthocyanidins were related to the overall sensory quality of Pinot Noir wines.

Keywords: Pinot Noir, cyclic proanthocyanidins, winemaking, phenolic profile, high-resolution mass-spectrometry, sensory analysis

1. INTRODUCTION

Red Pinot Noir wine is a light-to-medium-bodied wine with a complex aroma profile (CASASSA *et al.*, 2018). It is produced in several viticultural areas as well as in South Tyrol (Italy). Several commercial frauds involving the marketing of Pinot Noir have been recorded. For instance, some producers were convicted in 2010 of mislabeling 13.5 million L of Pinot Noir wine that was replaced with cheaper wines made with Merlot and Syrah grape varieties (TAKEOKA *et al.*, 2011). For this reason, assessing the commercial quality of Pinot Noir wines and investigating a wider selection of authenticity markers became advisable. Several studies have been proposed for comparative authenticity assessments of Pinot Noir and other wines. For example, South Tyrolean Pinot Noir wines were differentiated from Cabernet Sauvignon using proton-transfer mass spectrometry analysis (SPITALER *et al.*, 2007). Furthermore, the polyphenol content and antioxidant activity of *nouveau* wines made from Pinot Noir and other grape varieties (PELLEGRINI *et al.*, 2000) were studied. In addition, the comparison of the phenolic and sensory profiles of organic wines made from Pinot Noir grapes and other varieties was performed (LANTE *et al.*, 2004). Pinot Noir showed a content of phenolic compounds (including phenolic acids) comparable to Cabernet Sauvignon and Cabernet Franc (VAN LEEUW *et al.*, 2014). However, Pinot Noir wines are lighter in color compared to other wines because of a lower total anthocyanin content (PETERLUNGER *et al.*, 2002). Also, the content of tannins in Pinot Noir grapes is lower compared to other red wines (CASASSA *et al.*, 2018; HARBERTSON *et al.*, 2008).

Phenolic compounds can be used to differentiate wines according to the winemaking technique (BAIANO *et al.*, 2009, SIREN *et al.*, 2015; ZHANG *et al.*, 2018), grape variety (BOSELLI *et al.*, 2004; PERESTRELO *et al.*, 2018; VAN LEEUW *et al.*, 2014), vintage (BELLOMARINO *et al.*, 2010; GEANA *et al.*, 2016; GIACOSA *et al.*, 2019), and geographical origin (GRANATO *et al.*, 2011; ROCCHETTI *et al.*, 2018; STOCKHAM *et al.*, 2013). The anthocyanin profile is currently one of the most employed parameters for authenticity assessment studies (OIV, 2007; VILLANO *et al.*, 2017). However, anthocyanins as chemical markers have a limited application for several reasons: they can be applied only to red wines, and furthermore, during the aging of wine, anthocyanins are oxidized or transformed into oligomeric and polymeric pigments through condensation reactions with flavanols (HE *et al.*, 2012; ZHANG *et al.*, 2018). Thus, the anthocyanin content decreases in aged wines, and the assessment of the grape varieties used to make red wine may be difficult. For this reason, more stable chemical markers should be identified and investigated for authenticity purposes with respect to the grape variety.

A recent study highlighted the presence of an unconventional cyclic B-type tetrameric procyanidin (also known as 'crown' procyanidin) in Cabernet Sauvignon, providing also its full structural characterization (ZENG *et al.*, 2019). Several studies have also identified the profiles of cyclic B-type tetrameric, pentameric, and hexameric procyanidins and prodelphinidins in red and white wines (LONGO *et al.*, 2018a,b,c; LONGO *et al.*, 2019; MERKYTE *et al.*, 2020), including Pinot Noir. The role of proanthocyanidins (PAC) as chemical markers to evaluate wine quality and authenticity is promising, as their profile and the relative proportions of the different congeners were preliminarily found to be dependent on the grape variety used for winemaking (LONGO *et al.*, 2018c; LONGO *et al.*, 2019). Besides, cyclic proanthocyanidins (C-PAC) showed greater stability towards strongly acidic and depolymerising conditions in comparison to (conventional) non-cyclic proanthocyanidins (NC-PAC) (ZENG *et al.*, 2019). These C-PAC compounds showed also

more resistance than their NC-PAC analogues towards fragmentation during mass spectrometric analysis (LONGO *et al.*, 2018a).
In this report, the profile of C-PAC was studied in eleven Pinot Noir wines from the same winery but produced with different winemaking practices. The aim of this study was to investigate the profile of PAC in these wines in relation to specific winemaking factors, such as the use of raisins or undesired stuck fermentations and the location of the vineyards. In addition, other phenolics and the sensory profiles were discussed. The results shed light on the possible role of C-PAC in relation to the effects of specific winemaking practices or geographical location of the vineyards.

2. MATERIALS AND METHODS

2.1. Wine samples, chemicals, and materials

Eleven red dry wines obtained from 100% Pinot Noir grapes were produced and donated by a local winery (Franz Haas, Montagna, BZ, Italy). The grapes were harvested in 2016 in different vineyards located between 350 and 800 m a.s.l. in Trentino-South Tyrol (Italy). The mass of grapes obtained for each vinification was 3.5 t. The maceration lasted eight days at a constant fermentation temperature of 26°C. The samples differed for aspects such as the altitude, location, and orientation of the vineyards and for the winemaking practices as described in Table 1.

Table 1. Description of the eleven Pinot Noir wines in terms of vineyard, altitude, location, orientation, and winemaking techniques.

Wine	Vineyard	Altitude (a.s.l./m)	Location (orientation)	Winemaking technique
1	A	400	Pinzano (BZ) (South West)	Grape mass 3.5 t; 8 days maceration, 25-26°C fermentation temperature
2	A	400	Pinzano (BZ) (South West)	As wine 1, but a thermal maceration at 42°C was applied for 8 hours prior to alcoholic fermentation held at 20°C
3	A	400	Pinzano (BZ) (South West)	As wine 1, but it underwent a stuck fermentation followed by a second inoculation with supplementary addition of SO_2
4	B	780	Trentino (South East)	As wine 1
5	C	750-800	Aldino (BZ) (South)	As wine 1 (grapes have been treated with a leaf fertilizer)
6	C	750-800	Aldino (BZ) (South)	As wine 1
7	D	650	Gleno (BZ) (South West)	As wine 1
8	E	350	Mazzon (BZ) (North West)	As wine 1
9	E	350	Mazzon (BZ) (North West)	As wine 1
10	E	350	Mazzon (BZ) (North West)	As wine 1, but with 20% of non-destemmed grapes
11	E	350	Mazzon (BZ) (North West)	As wine 1, but using 100% raisins

2.2. HPLC-DAD-HRMS/MS analysis

Solvents and standard compounds for the HPLC-HRMS/MS analysis were purchased from Sigma-Aldrich Ltd. All chemicals were LC-MS grade. The preparation of wine samples and the HPLC-HRMS/MS analysis were performed according to the procedure reported by LONGO *et al*., 2018a with slight modifications. Briefly, 20 mL of each wine were concentrated under low pressure (11 mbar) at 40°C. Then, a gentle N_2 flux was applied for 30 min and the samples were re-dissolved (with a sonication for 5 min) to a final concentration 10 times higher. Finally, all samples were filtered (0.2 μm) before HPLC injection.

A Q-Exactive HRMS instrument (Thermo Fisher Scientific, Rodano, Milano, Italy) was coupled to an Agilent 1260 HPLC (Agilent Technologies Italia S.p.A., Cernusco sul Naviglio, Milano, Italy) with a 16 channel DAD detector. The chromatographic separation was carried out using an ODS Hypersyl C18 LC column (125 mm × 4.6 mm i.d., 5 μm, Thermo Fisher Scientific), which was protected with a HPLC pre-column filter (ODS Hypersil, 5 μm pore size, 10 x 4 mm drop-in guards, Thermo Fisher Scientific) at a flow rate of 1 $mL \cdot min^{-1}$. The mobile phase consisted of solvent A (0.1% v/v formic acid in 0.02 $mol \cdot L^{-1}$ ammonium formate in water) and solvent B (0.1% v/v formic acid in saturated ammonium formate acetonitrile). The gradient program of solvent B was as follows: from 0 to 21 min 5%, 21 to 22 min 25%, 22 to 27 min 95%, 27 to 28 min 5%, followed by a re-equilibration step (5% B) from 28 to 35 min. The DAD spectra were recorded from 210 to 600 nm and provided real-time monitoring at 280 nm, 320 nm, 365 nm, 420 nm and 520 nm (+/- 4 nm). A post-column flow splitter valve (Upchurch Scientific) was used to feed both analyzers in parallel (DAD and HRMS) at a fixed ratio. For the Full MS analysis, the HESI source was operated in positive ionization mode for the analysis of proanthocyanidins and in negative ionization mode during the analysis of the phenolic profile. The following conditions were used: sheath gas at 20 (arbitrary units), auxiliary gas at 5 (arbitrary units), auxiliary gas temperature at 250°C, spray voltage at +3,500 kV, capillary temperature at 320°C and RF S-lens at 70 (arbitrary units). The mass range was from m/z 500 to 2,000 with the Full MS set resolution of 70,000 (@200 m/z), AGC target at 3.106, max injection time of 300. Full MS parameters were: MS/MS AGC target 106, max. injection time 300, FT-MS set resolution 35,000, loop count 5, isolation window 2 or 3 m/z with 1 m/z offset, normalized collision energy 15 eV (positive mode) and from 30 to 60 eV (negative mode). For data-dependent settings: minimum AGC target 3.103, apex trigger from 2 to 8 sec, charge exclusion from 3 to 8 and higher, dynamic exclusion 3 sec, "if idle" tool set to "pick others." Lock masses were constantly employed to correct mass deviations across the Full MS acquisition range throughout the experiments.

The HPLC-DAD data were collected and analyzed by the OpenLab software while the HPLC-MS data were collected and analyzed with Xcalibur 3.1 software and Compound Discoverer 2.0 (Thermo Fisher Scientific). Simple phenolic compounds quantitation was achieved at HPLC-DAD with external calibration and with injection of standard compounds (peaks integration at 280 nm).

2.3. Standard oenological characterization

Acetic acid, glucose and fructose, free and total SO_2 were measured using an automatic multi-parametric analyzer – Miura One (Exacta+Optech Labcenter S.p.A., San Prospero, Italy). All samples were filtered (0.2 μm, cellulose acetate filter) before the analysis without any specific sample preparation. Reagents for the enzymatic analysis of wines were

purchased from Exacta+Optech Labcenter S.p.A. (San Prospero, Italy). The total acidity was measured according to OIV (OIV, 2015a). The alcohol content was measured with a Malligand ebulliometer.

2.4. Sensory evaluation

A group of eight trained panelists (4 females and 4 males) aged from 30 to 50 years were recruited at Free University of Bozen-Bolzano, Faculty of Science and Technology. An initial qualitative analysis phase consisted in presenting the wine samples in order to define a common vocabulary of the sensory descriptors for Pinot Noir wines. Then, nineteen sensory descriptors were identified and evaluated with the procedure of the round table (YASAR *et al*., 2018). The visual descriptors were clarity, hue, and color intensity. The olfactory descriptors were olfactory intensity, floral, fruity, herbaceous, spicy, liquor, maderized, caramelized aromas, and solvent. The gustatory descriptors were alcoholic, softness, sweetness, acidity, sapidity, tannicity, and balance. Each descriptor was evaluated using a 10-point scale (1 = no perception, 10 = high intensity). The bottles were opened just before each sensory session and 30 mL of wine were offered randomly to the panelists in ISO glasses codified with 3-digit number at around 18°C. The presentation order of the samples was counterbalanced between and within participants. The participants were provided with mineral water to rinse their mouths between samples. At the end of the session, an overall quality judgment was also requested.

2.5. Statistical analysis

Principal Component Analysis was performed using XLStat (version 2019.2.2.59417, Addinsoft, Paris, France). NIPALS (Non-Linear Iterative Partial Least Squares) algorithm was preliminary applied to account for sparse missing values in the chemical datasets (WOLD *et al*., 1984). The relative abundances of non-cyclic and cyclic proanthocyanidins and their relative ratios were auto-scaled (mean-centered followed by division of each column - i.e. variable - by the standard deviation of that column). The average ratings of each sensory descriptor were instead only mean-centred as they all shared the same 10-point scale for the evaluation. 'Overall judgment' was used as supplementary variable (non-active) in the sensory analysis.

3. RESULTS AND DISCUSSION

In Table 1, the information on each analyzed Pinot Noir sample is reported. Samples 1, 4, 5, 6, 7, 8, and 9 were produced with the same winemaking procedure (mass of 3.5 t for each sample; 8 d maceration, 25-26°C fermentation temperature). The main differences among the cited samples were the altitude and the geographical orientation of the vineyards. Samples 1, 2, and 3 differed for the winemaking practice used: to produce wine 2, a thermal maceration at 42°C was applied for 8 h before the alcoholic fermentation; wine 3 instead underwent an unwanted stuck fermentation; thus, it was re-inoculated with selected yeast and then added with supplementary SO_2 to prevent off-fermentations (DI MATTIA *et al*., 2015). Wine 11 was obtained from grapes harvested in the same vineyard (E) of wines 8, 9, and 10, but using 100% raisin grapes obtained by cutting some vine shoots and leaving the clusters hanging on the plants for a few days. Wine 10 was made

with 20% of whole clusters (non-destemmed and uncrushed) that were left in the must during maceration/fermentation.

3.1. Oenological parameters

The standard oenological results are presented in Table 2. The alcohol content in Pinot Noir wines ranged from 12.8% (sample 4) to 15.4% (sample 11). As expected, wines 4, 5, and 6 obtained from the vineyards located in the highest sites showed the lowest alcohol content due to the lowest degree of grape ripeness whereas wines 1-3 and 8-11 showed the highest alcohol content since the grapes were cultivated in lower vineyards (Table 2). The highest alcohol content of sample 11 compared to the other Pinot Noir wines could be expected since this wine was made with 100% raisins (with higher sugar content). The pH ranged from 3.2 (sample 4) to 3.5 (sample 6). The first four wines had lower pH compared to the others. The pH fitted the usual pH range of red wines (3.0 – 4.0) (JACOBSON, 2006). The total acidity measured in samples 1-3, 5, and 7- 9 was 5.6 gL^{-1} tartaric acid. Samples 4, 6, 10, and 11 had a higher total acidity (6.2 – 6.8 gL^{-1} tartaric acid). All Pinot Noir wines had low acetic acid content (within the legal threshold of 1.2 gL^{-1} acetic acid equivalents, OIV, 2015b and OIV, 2012). All the wines were dry and most of them showed a residual sugar content ranging from 0.06 gL^{-1} (wines 3 and 6) to 0.44 gL^{-1} (wine 7) (FERNANDEZ-NOVALES *et al.*, 2009). Wine 11 (made with 100% raisin grapes) contained the highest residual sugar content (1.63 gL^{-1}). Interestingly, wines 5 and 6 had the lowest glucose-fructose levels (0.07 and 0.06 gL^{-1}, respectively). The free SO_2 levels were relatively low (12 – 18 mgL^{-1}) and the total SO_2 (73 – 108 mgL^{-1}) was within the legal limits (OIV, 2012).

Table 2. Oenological parameters of the eleven Pinot Noir wines.

Wine	[1]ABV (%)	pH	[2]total acidity (gL^{-1})	acetic acid (gL^{-1})	[3]Gl-Fr (gL^{-1})	[4]fSO_2 (mgL^{-1})	[5]tSO_2 (mgL^{-1})
1	14.4	3.38	5.6	0.21	0.19	14	107
2	14.4	3.33	5.6	0.24	0.17	14	93
3	13.7	3.25	5.6	0.41	0.06	13	108
4	12.8	3.21	6.8	0.40	0.14	12	88
5	13.1	3.48	5.6	0.25	0.07	14	79
6	13.4	3.54	6.2	0.32	0.06	13	82
7	14.7	3.42	5.6	0.36	0.44	12	83
8	14.8	3.41	5.6	0.31	0.31	15	73
9	14	3.48	5.6	0.30	0.22	14	90
10	14.5	3.46	6.5	0.39	0.30	18	91
11	15.4	3.50	6.8	0.40	1.63	18	90

[1]ABV: alcohol by volume (% v/v); [2]g/l tartaric acid; [3]gl-fr: glucose-fructose (gL^{-1}); [4]fso_2: free sulphur dioxide (mgL^{-1}); [5]tso_2: total sulphur dioxide (mgL^{-1}).

3.2. Profiles of proanthocyanidins

The proanthocyanidins (PAC) profile was analyzed by means of HPLC-HRMS and the results are reported in Table 3. Both non-cyclic procyanidins (NC-PC) and cyclic procyanidins (C-PC) were found in higher concentrations in Pinot Noir samples, compared to prodelphinidins (PD). All wines except sample 3 had a high content of dimeric procyanidins (NC-2 PCs). The abundances of NC-PC decreased at a higher degree of polymerization (DP). The highest amount of NC-6 PC (non-cyclic hexameric procyanidin) was present in wine 11. Wine 3 had instead the lowest amount of C-PAC. Also, wines 10 and 11 stood out with a higher content of C-6 PC (cyclic hexameric procyanidin) with respect to other samples. Furthermore, wine 11 had almost twice as much of C-5 PD (cyclic pentameric prodelphinidin) compared to wines 7 and 8.
Principal Component Analysis was performed using auto-scaled PAC variables, to highlight trends within the dataset that may suggest relationships between the PAC profiles and the different factors involved. In previous studies on the distribution of procyanidins (LONGO *et al.*, 2019) and prodelphinidins (LONGO *et al.*, 2018c) in wines, the relative (%) ratios were applied: these showed clear dependency upon the grape variety, but no study has yet addressed their relationship with the winemaking practices or the geographical origin. These ratios correspond to the proportions (%) of any cyclic congener over the total amount of cyclic + non-cyclic congeners by number and composition of monomers as reported in previous reports (LONGO *et al.*, 2018c; LONGO *et al.*, 2019). The PCA bi-plot of these ratios is shown in Fig. 1.
The total variance explained by the first two principal components is 84.0% (PC1: 69.6% + PC2: 14.4%). All variables are in positive correlation with the first principal component, except for the ratio of C-PD (cyclic prodelphinidins) with one and three (epi)gallocatechin units (indicated as %C-4-1-OH and %C-4-3-OH respectively). All %C-PC (relative (%) ratios of procyanidins) showed strong correlations among each other and also with most of the PD. Wine 3 is well separated from the other wines, which are clustered in the central area of the bi-plot. This is probably caused by the occurrence of a stuck fermentation: namely, as the fermentation halted prematurely, the extraction of the polyphenols from the berry skins was hampered, since the reached concentration of ethanol was lower in comparison to the other samples. After that event, sample 3 was racked before being re-inoculated with the yeast. Removing the skins at an early stage of maceration presumably prevented the completion of the extraction of polyphenols. However, this also slowed down the extraction of the non-cyclic congeners, since these are less polar compounds than the cyclic ones and require higher percentages of ethanol for their extraction. Instead, the cyclic compounds were still extracted in higher proportions (as evidenced in Fig. 1). Hence, the relative ratios (%) of cyclic congeners were "over-expressed" in sample 3. Notably, these percentages do not represent absolute concentrations, but instead they are just the relative proportions (%) of C-PAC over C-PAC *plus* NC-PAC (by DP and composition). Indeed, the data in Table 3 show that the peak areas in sample 3 are lower for all compounds than in the other samples. Notably, a recent study on the kinetics of skin extraction for C-PC in Cabernet Sauvignon showed that these compounds are extracted almost completely at the beginning of maceration (JOUIN *et al.*, 2019), while NC-PC are only extracted over time with the increasing formation of ethanol.

Table 3. Relative abundances (integrated total ion current) of non-cyclic and cyclic proanthocyanidins in the eleven Pinot Noir wines.

PAC		NC-2 PC I	NC-2 PC II	NC-2 PC III	NC-2 PC IV	NC-2 PC V	NC-3 PC	NC-4 PC	C-4 PC	NC-5 PC
m/z		579.1497	579.1497	579.1497	579.1497	579.1497	867.2124	1155.2760	1153.2604	1443.3392
Wine samples	1	22274956	22306888	22323575	22374250	22325218	7750638	2404025	162851	480128
	2	37425812	37336457	37336457	37354174	37354706	19392028	7135274	183528	1925495
	3	639543	639543	630124	639543	639543	79649	3475	25201	3698
	4	33026883	33012707	33004346	33012578	32999883	15520019	5166134	84876	1522658
	5	49790473	49787516	49753715	49788501	49788582	23452844	8328293	157309	2525998
	6	34515047	34495827	34503463	34515042	34499696	11871865	4004473	104562	967950
	7	58252407	58023701	58083779	58145139	58145139	26156359	7954429	316117	1873445
	8	45458622	45411956	45413310	45358853	45368291	25007492	8781802	301711	2467726
	9	12463012	12487106	12463722	12467415	12462662	4193665	1141355	131150	242130
	10	26662439	26665699	26664558	26668416	26646611	11066048	4442351	174339	1332144
	11	29720456	29692215	29720858	29720262	29720035	20466723	9604520	67151	3235409

PAC		C-5 PC	NC-6 PC	C-6 PC	NC-2 PD 1-galloc	NC-3 PD 1-galloc	NC-3 PD 2-galloc	NC-3 PD 3-galloc	NC-4 PD 1-galloc
m/z		1441.3213	1731.4010	1729.3870	595.1446	883.2072	899.2021	915.1970	1171.2710
Wine samples	1	168545	28905	8308	0	103469	12973	787	638805
	2	145329	348388	16802	565	71849	7113	0	1081774
	3	22871	1632	990	0	427	0	230	221433
	4	114903	251288	24875	0	80485	7434	0	1301338
	5	174167	467959	35912	575	231898	30436	514	2028349
	6	118992	155456	9765	0	157400	25428	5041	1023981
	7	263641	272430	13032	308	268426	45048	1024	1960693
	8	365229	446402	43924	0	164426	19409	1344	2336156
	9	117526	18048	4672	0	47356	6993	349	432706
	10	211433	307633	36639	0	107349	26470	1365	2719
	11	111594	784272	38554	0	39650	3466	0	1874952

PAC		NC-4 PD 2-galloc	NC-4 PD 3-galloc	NC-4 PD 4-galloc	C-4 PD 1-galloc	C-4 PD 2-galloc	C-4 PD 3-galloc	C-4 PD 4-galloc	NC-5 PD 1-galloc
m/z		1187.2660	1203.2605	1219.2550	1169.2557	1185.2507	1201.2456	1217.2405	1459.3343
Wine samples	1	40168	7470	1317	34465	2686	3725	0	60521
	2	45131	2996	735	60435	3717	14213	0	238163
	3	0	289	308	1277	198	0	0	0
	4	73318	10593	3446	61911	4280	21048	1941	369331
	5	331689	34976	4516	88724	8139	6740	1307	632792
	6	160249	20578	1964	42105	6604	5050	10487	232513
	7	225155	29024	3917	109277	22825	14161	3913	383685
	8	226537	17780	4891	99430	18169	4590	482	618359
	9	17701	10183	3886	21521	1577	18515	397	27818
	10	109507	17819	2251	49828	11919	17315	448	295178
	11	183308	4883	1081	210398	23102	3371	0	756973

PAC		NC-5 PD 2-galloc	NC-5 PD 3-galloc	NC-5 PD 4-galloc	C-5 PD 1-galloc	C-5 PD 2-galloc	C-5 PD 3-galloc	C-5 PD 4-galloc	C-5 PD 5-galloc
m/z		1475.3291	1491.3240	1507.3189	1457.3191	1473.3140	1489.3090	1505.3039	1521.2988
Wine samples	1	7874	0	0	49574	10550	1964	565	0
	2	9260	790	0	53667	5422	1422	294	0
	3	676	306	0	3030	886	0	0	0
	4	23878	3065	0	45778	5108	1889	0	0
	5	106745	16166	0	75154	16454	1808	0	0
	6	36370	7013	601	43342	8661	4357	0	0
	7	44404	3795	0	95602	26777	5586	295	0
	8	73686	4936	0	123419	30556	3078	0	257
	9	2255	1900	0	36030	6991	1400	0	0
	10	31967	4855	0	68776	19911	4301	0	0
	11	88643	7073	0	123989	13498	0	0	0

abbreviations: nc – non-cyclic; c – cyclic; numbers after nc or c indicates the number of monomer units of catechin or epicatechin (e.g. nc-2 is non-cyclic dimer); pc – procyanidins, pd – prodelphinidin; the last number in prodelphinidins indicates the number of gallocatechins in the oligomeric chain.

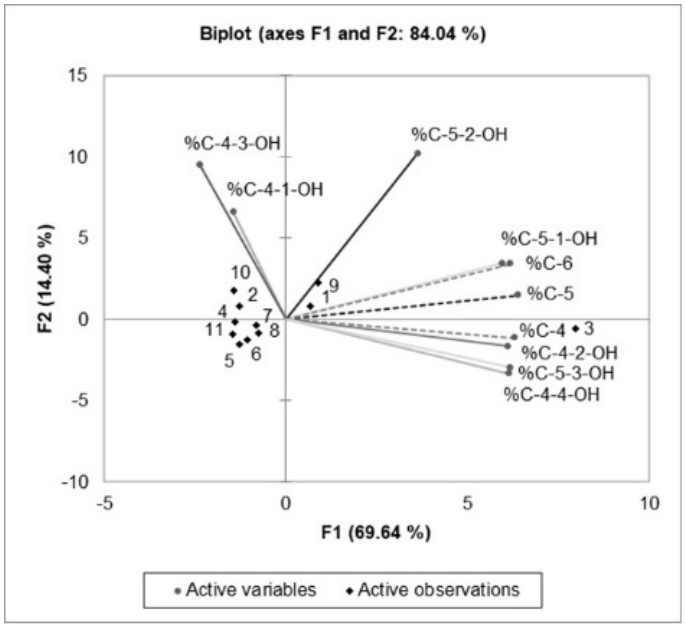

Figure 1. PCA bi-plot of the relative ratios of proanthocyanidins (%) of the eleven Pinot Noir wines. The vectors of the ratios of procyanidins are dashed, whereas the vectors for prodelphinidins are full. The first number in the abbreviations indicates the number of the monomers forming the proanthocyanidin. The second number shows the number of gallocatechin units in the oligomeric chain of prodelphinidins. F1 and F2, Principal Components. %C-N-M-X: ratio of relative abundance of a cyclic oligomer over the sum of relative abundances for cyclic and non-cyclic, considering the same relative compositions in (epi)catechins and (epi)gallocatechins and number of composing monomeric units. In the formula: C = cyclic, N = number of monomeric units, M = number of (epi)gallocatechins in the structure, X = -OH if the compound is a prodelphinidin or empty if it is a procyanidin.

In Fig. 2, the PCA models, which were elaborated over the relative abundances of NC-PAC (2A) and C-PAC (2B) are shown separately. The lack of NC-PAC in wine 3 is again confirmed in Fig. 2A (84.4% of total variance), where wine 3 is situated on the opposite side of PC1 with respect to all the variables. Wine 11 had higher concentrations of NC-PAC and the highest concentrations of residual sugars and alcohol (Table 2).
In fact, the grapes used for winemaking of sample 11 had been cut and left to dry hanging on the vine before the harvest, which had the effect of concentrating even further the polyphenols besides the sugars. Notably, in Figs. 2A and 2B the values used represent absolute abundances, as they are integrated peak values obtained with the HPLC-HRMS analysis (Table 3). Wines 3 and 11 are clearly separated from the others in 2A and 2B respectively, and the trends for the variables are shown: wine 3 was on the opposite part of most descriptors, while wine 11 was driven by the C-PD with one or two (epi)gallocatechin units.

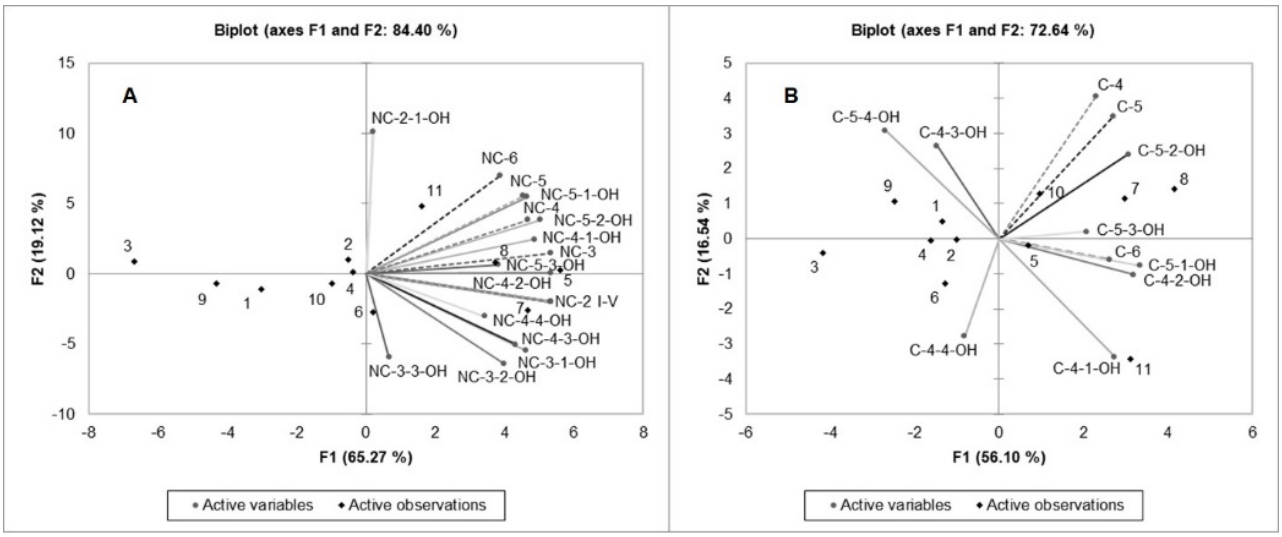

Figure 2. PCA bi-plots of non-cyclic proanthocyanidins (A) and cyclic proanthocyanidins (B) in Pinot Noir wines. NC - non-cyclic, C - cyclic. The vectors of procyanidins are dashed, whereas the vectors for prodelphinidins are full. The first number in the abbreviations indicates the number of the monomers. The second number shows the number of gallocatechin units in the oligomeric chain of prodelphinidins. F1 and F2, Principal Components.

3.3. Profiles of simple phenolics

Overall, none of the evaluated simple phenolic variables could distinguish significantly groups of samples; therefore, they were not included in the previous statistical analysis (*data not shown*). Instead, they are just mentioned qualitatively.
Seven monomeric phenolic compounds (gallic acid, protocatechuic acid, 4-hydroxybenzoic acid, vanillic acid, catechin, caffeic acid, ferulic acid) were identified (Table 4), and concentrations were evaluated by standard injection according to LONGO *et al*. (2017) for phenolic compounds. Gallic, vanillic and caffeic acids were present in all samples. The highest amount of gallic acid was shown in wine 10, vanillic acid in wine 11, and caffeic acid in wines 7, 10 and 11. Wine 1 showed a higher content of protocatechuic acid; wine 3 was higher in ferulic acid; wines 10 and 11 in 4-hydroxybenzoic acid; wines 7 and 8 in catechin.

3.4. Sensory evaluation of Pinot Noir wines

Fig. 3 shows the PCA bi-plot for the sensory data. The first two components explained 46.2% of the total variance. The first principal component (26.6% of the total variance) was correlated with wine balance and the overall judgment on wine quality. Besides, PC1 was correlated with softness, sweetness, herbaceous, floral and fruity aromas. The second principal component (19.6%) was correlated with clarity, tannicity (astringency), and caramelized descriptors, which were inversely correlated with a maderized descriptor. As shown in Fig. 3**,** wines 1, 2, and 3 (vineyard A) were clustered on the left part of the graph. Samples 1 and 2 showed a very similar trend; thus the thermal maceration of wine 2 did not remarkably affect the sensory properties. However, wine 3 was characterized more by alcoholic, liquor, and maderized variables, and it was lacking in tannicity. Wines 5 and 6 (vineyard C) were situated in the center of the plot. Wines 8, 9, and 10 (vineyard E) were

situated on the same side as wine 11 (vineyard E). The wines 9, 10, and 11 were the most balanced and with a high overall judgment assigned by the panelists. Finally, the other two wines – 4 (vineyard B) and 7 (vineyard D) – were well separated from the other samples.

Table 4. Concentration of simple phenolic compounds in the eleven Pinot Noir wines evaluated by HPLC-DAD (280 nm) standard injections. Calibration curves with $R^2 = 0.999$ for evaluated compounds.

Wine	Gallic acid (μM)	Protocatechuic acid (μM)	*p*-hydroxybenzoic acid (μM)	Vanillic acid (μM)	(+)-catechin (μM)	Caffeic acid (μM)	Ferulic acid (μM)
1	171	672	0	206	5	14	2
2	229	1	0	440	2	24	1
3	225	63	0	304	5	20	4
4	203	56	2	300	3	22	0
5	220	40	0	319	2	21	0
6	213	47	0	308	1	20	0
7	221	1	2	348	21	30	0
8	153	1	0	404	31	29	0
9	253	1	4	256	3	16	0
10	311	1	6	314	0	31	1
11	180	1	6	482	0	30	1

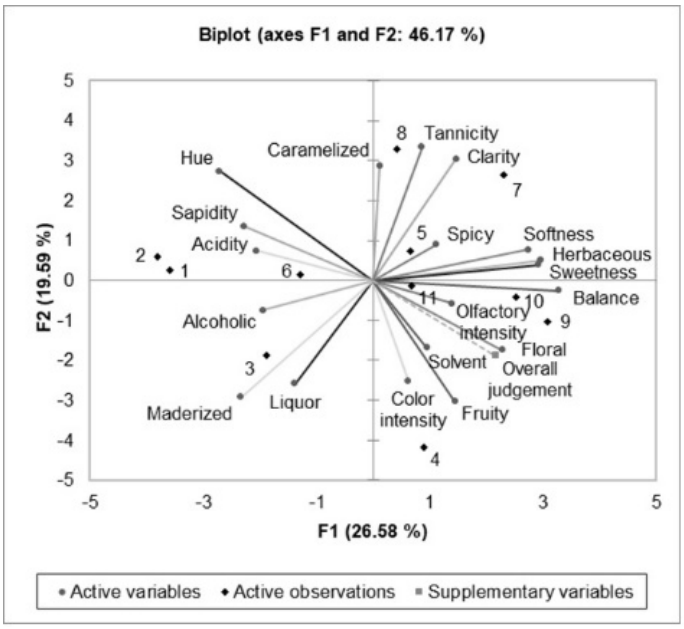

Figure 3. PCA bi-plot of the sensory data across the eleven Pinot Noir wines. Overall judgment was used as a supplementary variable. F1 and F2, Principal Components.

4. CONCLUSION

Using the profile of cyclic and non-cyclic proanthocyanidins, the separation of the most different samples of Pinot Noir wines, such as sample 3 (that had experienced a stuck fermentation) and sample 11 (that was produced using raisin grapes) was similar to that achieved with sensory analysis. Sample 3, with low proanthocyanidins concentration (including the cyclic ones), was described by the panel as highly maderized and lacking in tannins. Conversely, wine 11 (made with raisin grapes) contained the highest amount of cyclic tetrameric prodelphinidins and it was described as a balanced wine with a high overall quality judgment by the panel. The ratios between cyclic and non-cyclic proanthocyanidins confirmed the different solubility and extractability of these compounds and did reflect the occurrence of a stuck fermentation followed by racking and re-inoculation. Thus, the profile of cyclic and non-cyclic proanthocyanidins was affected by specific factors, such as the stuck fermentation or the use of 100% raisins. Both of these factors were related to the sensory quality judgement of Pinot Noir wines.

ACKNOWLEDGEMENTS

The authors would like to thank the winery FRANZ HAAS Srl (Montagna, South Tyrol, Italy) for supplying the Pinot Noir wines. This work was supported financially by the Provincia di Bolzano (Italy) (Beschluss der Landesregierung Nr. 1472, 07.10.2013) and is part of the WineID (Wine Identity Card) interdisciplinary project of UNIBZ (no. 3666).

ABBREVIATIONS

ABV – alcohol by volume; PAC – proanthocyanidins; PC – procyanidin; PD – prodelphinidin; C- – cyclic oligomer; NC- – non-cyclic oligomer; C-*n* PC – cyclic *n*-meric (procyanidin); C-*n* PD – cyclic *n*-meric (prodelphinidins); C-*n* PD *m*-galloc – cyclic *n*-meric prodelphinidin with *m* (epi)gallocatechin units; PCA – Principal Component Analysis; NIPALS: Nonlinear Iterative Partial Least Square; PC*n*: *n* principal component; fSO_2: free SO_2; tSO_2: total SO_2.

REFERENCES

Baiano A., Terracone C., Gambacorta G. and La Notte E. 2009. Phenolic Content and Antioxidant Activity of Primitivo Wine: Comparison among Winemaking Technologies. Journal of Food Science, 74(3):258-267. DOI: doi.org/10.1111/j.1750-3841.2009.01101.x

Bellomarino S.A., Parker R.M., Conlan X.A., Barnetta N.W. and Adams. M.J. 2010. Partial least squares and principal components analysis of wine vintage by high performance liquid chromatography with chemiluminescence detection. Analytica Chimica Acta, 678(1):34-38. DOI: doi.org/10.1016/j.aca.2010.08.021

Boselli E., Boulton R.B., Thorngate J.H. and Frega N.G. 2004. Chemical and Sensory Characterization of DOC Red Wines from Marche (Italy) Related to Vintage and Grape Cultivars. Journal of Agricultural and Food Chemistry, 52:3843-3854. DOI: doi.org/10.1021/jf035457h

Casassa L.F., Sar, S.E., Bolcato E.A., Diaz-Sambueza M.A., Catania A.A., Fanzone M.L., Raco, F. and Barda N. 2018. Chemical and Sensory Effects of Cold Soak, Whole Cluster, Fermentation, and Stem Additions in Pinot noir Wines. American Journal of Enology and Viticulture, 70:19-33. DOI: doi.org/10.5344/ajev.2018.18014

Di Mattia C.D., Piva A., Martuscelli M., Mastrocola D. and Sacchetti G. 2015. Effect of sulfites on the in vitro antioxidant activity of wines. Italian Journal of Food Science, 27(4):505-512. DOI: doi.org/10.14674/1120-1770/ijfs.v381

Fernandez-Novales J., Lopez M.I., Sanchez M.T., Morales J. and Gonzalez-Caballero V. 2009. Shortwave-near infrared spectroscopy for determination of reducing sugar content during grape ripening, winemaking, and aging of white and red wines. Food Research International, 42:285-291. DOI: doi.org/10.1016/j.foodres.2008.11.008

Geana E.I., Popescu R., Costinel D., Dinca O. R., Ionete R.E., Stefanescu I., Artem V. and Bala C. 2016. Classification of red wines using suitable markers coupled with multivariate statistic analysis. Food Chemistry, 192:1015-1024. DOI: doi.org/10.1016/j.foodchem.2015.07.112

Giacosa S., Ossola C., Botto R., Rio Segade S., Paissoni M.A., Pollon M., Gerbi V. and Rolle L. 2019. Impact of specific inactive dry yeast application on grape skin mechanical properties, phenolic compounds extractability, and wine composition. Food Research International, 116:1084-1093. DOI: doi.org/10.1016/j.foodres.2018.09.051

Granato D., KatayamaInar F.C.U. and De Castro I.A. 2011. Phenolic composition of South American red wines classified according to their antioxidant activity, retail price and sensory quality. Food Chemistry, 129:366-373. DOI: doi.org/10.1016/j.foodchem.2011.04.085

Harbertson J.F., Hodgins R.E., Thurston L.N., Schaffer L.J., Reid M.S., Landon J.L., Ross C.F. and Douglas A.O. 2008. Variability of Tannin Concentration in Red Wines. American Journal of Enology and Viticulture, 59:210-214.

He F., Liang N.N., Mu L., Pan Q.H., Wang J. and Reeves M.J. 2012. Anthocyanins and their variation in red wines II. Anthocyanin derived pigments and their colour evolution. Molecules, 17(2):1483-1519. DOI: doi.org/10.3390/molecules17021483

Jacobson J.L. 2006. Introduction to Wine Laboratory Practices and Procedures, Springer, New York, ISBN 978-0-387-24377-1.

Jouin A. 2019. Evolution of the crown procyanidins during wine making and aging in bottle. ŒNOIVAS 2019-11th International Symposium of Œnology, 25-28th June, Bordeaux (France), p. 86.

Lante A., Crapisi A., Lomolino G. and Spettoli P. 2004. Chemical parameters, biologically active polyphenols and sensory characteristics of some Italian organic wines. Journal of Wine Research, 15(3):203-209. DOI: doi.org/10.1080/09571260500142054

Longo E., Morozova K., Loizzo M.R., Tundis R., Savini S., Foligni R., Mozzon M., Martin-Vertedor D., Scampicchio M. and Boselli E. 2017. High resolution mass approach to characterize refrigerated black truffles stored under different storage atmospheres. Food research international, 102:526-535. DOI: doi.org/10.1016/j.foodres.2017.09.025

Longo E., Rossetti F., Scampicchio M. and Boselli E. 2018a. Isotopic exchange HPLC-HRMS/MS applied to cyclic proanthocyanidins in wine and cranberries. Journal of the American Society for Mass Spectrometry, 29(4):663-674. DOI: doi.org/10.1007/s13361-017-1876-8

Longo E., Rossetti F., Merkyte V. and Boselli E. 2018b. Disambiguation of Isomeric Procyanidins with Cyclic B-Type and Non-cyclic A-Type Structures from Wine and Peanut Skin with HPLC-HDX-HRMS/MS. Journal of the American Society for Mass Spectrometry, 29(11):2268-2277. DOI: doi.org/10.1007/s13361-018-2044-5

Longo E., Merkyte V., Rossetti F., Teissedre P.L., Jourdes M. and Boselli E. 2018c. Relative abundances of novel cyclic prodelphinidins in wine depending on the grape variety. Journal of Mass Spectrometry, 53:1116-1125. DOI: doi.org/10.1002/jms.4280

Longo E., Rossetti F., Jouin A., Teissedre P.L., Jourdes M. and Boselli E. 2019. Distribution of crown hexameric procyanidin and its tetrameric and pentameric congeners in red and white wines. Food Chemistry, 299:125125. DOI: doi.org/10.1016/j.foodchem.2019.125125

Merkytė V., Longo E., Jourdes M., Jouin A., Teissedre P.L. and Boselli E. 2020. High-Performance Liquid Chromatography–Hydrogen/Deuterium Exchange–High-Resolution Mass Spectrometry Partial Identification of a Series of Tetra- and Pentameric Cyclic Procyanidins and Prodelphinidins in Wine Extracts. Journal of Agricultural and Food Chemistry, 68(11):3312-3321. DOI: doi.org/10.1021/acs.jafc.9b06195.

OIV. 2007. Determination of nine major anthocyanins in red and rose wines using HPLC. OENO, OIV-MA-AS315-11.

OIV. 2012. Maximum acceptable limits of various substances contained in wine. OENO, OIV-MA-C1-01.

OIV. 2015a. Total acidity (Oeno 551/2015). OENO, OIV-MA-AS313-01.

OIV. 2015b. Volatile acidity (A 11, revised by 377/2009). OENO, OIV-MA-AS313-02: R2015.

Pellegrini N., Simonetti P., Gardana C., Brenna O., Brighenti F. and Pietta P. 2000. Polyphenol Content and Total Antioxidant Activity of Vini Novelli (Young Red Wines). Journal of Agricultural and Food Chemistry, 48:732−735. DOI: doi.org/10.1021/jf990251v

Perestrelo R., Silva C., Silva P. and Camara J.S. 2018. Unraveling Vitis vinifera L. grape maturity markers based on integration of terpenic pattern and chemometric methods. Microchemical Journal, 142:367-376. DOI: doi.org/10.1016/j.microc.2018.07.017

Peterlunger E., Celotti E., Da Dalt G., Stefanelli S., Gollino G. and Zironi R. 2002. Effect of Training System on Pinot noir Grape and Wine Composition. American Journal of Enology and Viticulture, 53(1):14-18.

Rocchetti G., Gatti M., Bavaresco L. and Lucini L. 2018. Untargeted metabolomics to investigate the phenolic composition of Chardonnay wines from different origins. Journal of Food Composition and Analysis, 71:87-93. DOI: doi.org/10.1016/j.jfca.2018.05.010

Siren H., Siren K. and Siren J. 2015. Evaluation of organic and inorganic compounds levels of red wines processed from Pinot Noir grapes. Analytical Chemistry Research, 3:26-36. DOI: doi.org/10.1016/j.ancr.2014.10.002

Spitaler R., Araghipour N., Mikoviny T., Wisthaler A., Dalla Via J. and Mark T.D. 2017. PTR-MS in enology: Advances in analytics and data analysis. International Journal of Mass Spectrometry, 266:1-7. DOI: doi.org/10.1016/j.ijms.2007.05.013

Stockham K., Sheard A., Paimin R., Buddhadasa S., Duong S., Orbell J.D. and Murdoch T. 2013. Comparative studies on the antioxidant properties and polyphenolic content of wine from different growing regions and vintages, a pilot study to investigate chemical markers for climate change. Food Chemistry, 140(3):500-506. DOI: doi.org/10.1016/j.foodchem.2013.01.006

Takeoka G. and Ebeler S. 2011, November. Progress in authentication of food and wine. American Chemical Society Symposium Series.

Van Leeuw R., Kevers C., Pincemail J., Defraigne J.O. and Dommes J. 2014. Antioxidant capacity and phenolic composition of red wines from various grape varieties: Specificity of Pinot Noir. Journal of Food Composition and Analysis, 36:40-50. DOI: doi.org/10.1016/j.jfca.2014.07.001

Villano C., Lisanti M.T., Gambuti A., Vecchio R., Moio L., Frusciante L., Aversano R. and Carputo D. 2017. Wine varietal authentication based on phenolics, volatiles and DNA markers: State of the art, perspectives and drawbacks. Food Control, 80:1-10. DOI: doi.org/10.1016/j.foodcont.2017.04.020

Wold S., Ruhe A., Wold H. and Dunn III W.J. 1984. The collinearity problem in linear regression. The partial least squares (PLS) approach to generalized inverses. SIAM Journal on Scientific and Statistical Computing, 5(3):735-743. DOI: doi.org/10.1137/0905052

Yasar S., Boselli E., Rossetti F. and Gok M S. 2018. Effect of fermented cereals, probiotics, and phytase on the sensory quality of poultry meat. Scientia Agriculturae Bohemica, 49(3):225-235. DOI: doi.org/10.2478/sab-2018-0029

Zhang X., He F., Zhang B., Reeves M.J., Liu Y., Zhao X. and Duan C. 2018. The effect of prefermentative addition of gallic acid and ellagic acid on the red wine color, copigmentation and phenolic profiles during wine aging. Food Research International, 106:568-579. DOI: doi.org/10.1016/j.foodres.2017.12.054

Zeng L., Pons-Mercadé P., Richard T., Krisa S., Teissèdre P.L. and Jourdes M. 2019. Crown Procyanidin Tetramer: A Procyanidin with an Unusual Cyclic Skeleton with a Potent Protective Effect against Amyloid-β-Induced Toxicity. Molecules, 24(10):1915-192. DOI: doi.org/10.3390/molecules241019

Paper Received September 6, 2019 Accepted January 13, 2020