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Characterization of phenolic profile in dried grape skin of *Vitis vinifera* L. cv. Pinot Blanc with UHPLC-MS/MS and its development during ripening

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ABSTRACT

Comprehensive phenolic composition of dried grape skin from cv. Pinot Blanc and its development during ripening is reported, with particular emphasis on flavonol glycosides profile. Extraction procedure and reversed-phase ultra-high-performance liquid chromatography-triple quadrupole mass spectrometry (UHPLC-QqQ-MS/MS) method were optimized and validated for the determination of 39 phenolic compounds belonging to different groups (flavonols, dihydroflavonols, benzoic and hydroxycinnamic acids, flavan-3-ols). Beside selected reaction monitoring (SRM) for analytes available as standards, flavonols and dihydroflavonols profile was furtherly explored performing precursor ion scan (PIS) with neutral loss (NL) for unavailable compounds. Quercetin-3-*O*-rutinoside occurred as major flavonol component, and rutinosides for kaempferol- and isorhamnetin-structures were also quantitable. Presence of different myricetin derivatives was unveiled, with myricetin-3-*O*-glucoside being quantifiable at all ripening time points. Besides high levels of astilbin, dihydroquercetins profile resulted highly complex. Moreover, ratio between caftaric and coutaric acid and between isomers of coutaric acid at harvest was uncommon if compared to other white cultivars.

1. Introduction

In plants, phenol and polyphenol compounds are secondary metabolites that play crucial physiological roles throughout the plant life cycle. In particular they are produced and accumulated in response to biotic and abiotic stressors, acting as UV-protectants, radical scavengers (antioxidants) and elicitors of cellular responses during plant-pathogen interactions (Sharma et al., 2019; Shalaby and Horwitz, 2015; Cheynier et al., 2013; Treutter, 2005). Following dietary assumption of plant foods, several polyphenols also represent bioactive compounds characterized by an important nutraceutical value, and their effects on human health (antioxidant, vasoprotective, anti-inflammatory and anti-tumoral properties) have been extensively investigated (Pandey and Rizvi, 2009; Duthie et al., 2003).

Grapes (*Vitis vinifera* L.) contain different classes of non-volatile phenols and polyphenols, for most part belonging to flavonoid structures, such as anthocyanins, flavan-3-ols, flavonols, stilbenes, phenolic and hydroxycinnamic acids, which mostly originate from skins and seeds of grape berries and become the main phenolic constituents of wines (Teixeira et al., 2013; Terrier et al., 2009; Singleton, 1988; Somers and Verette, 1988). Polyphenols content in grapes and wines can be deeply affected by grape variety and by both agronomical and pedo-climatic conditions of cultivation and winemaking processes (Garrido and Borges, 2013; Adams, 2006; Downey et al., 2006; Singleton, 1976). Interest is mainly focused on phenolic composition of grape skin since it is part of edible fraction of the fruit and it participates in wine polyphenol and flavors content through release processes during maceration.

As far as white grapes are concerned, the most abundant flavonoid classes in grape skins are flavonols, flavan-3-ols and dihydroflavonols, while non-flavonoid compounds are mainly represented by hydroxycinnamoyl tartaric esters (Ferrandino et al., 2012; Liang et al., 2012; Castillo-Muñoz et al., 2010). Among flavonols, the most expressed are cyanidin-like structures, that is quercetin- and kaempferol-type compounds, together with minor amounts of isorhamnetin derivatives. They mainly occur as glycosides, with different sugar moieties (principally glucuronide, glucoside and galactoside) being almost exclusively linked to 3-*O*- position (present work; Castillo-Muñoz et al., 2010; Mattivi et al., 2006). On the other hand, delphinidine-like glycosides, based on myricetin, larycitrin and syringetin backbones, were absent in the white grape varieties screened so far except at trace levels, so that their biosynthesis appeared to be specific of colored cultivars and has long been considered as precluded by lack of expression for the enzyme

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flavonoid 3′,5′-hydroxylase (F3',5'H; Flamini et al., 2013; Castillo-Muñoz et al., 2010; Mattivi et al., 2006). However, more recent advances reported low levels of myricetin glycosides and/or activation of corresponding biosynthetic pathways in single white grape varieties (Lu et al., 2021; Dal Santo et al., 2016; Ferrandino et al., 2012).

Flavonoids biosynthetic pathways and their expression have been well characterized both in red and white grapes (Braidot et al., 2008; Bogs et al., 2006; Boss et al., 1996). Due to the genetic control the flavonoid patterns, especially the anthocyanin profile in red grapes, are relatively stable for each variety but can vary considerably among varieties. The amount ratio of target molecules tends to be retained, despite absolute amounts are influenced by environmental conditions, like accumulation due to sun exposure (Downey et al., 2006). For this reason, anthocyanins (acylated and non-acylated) and flavonols have been proposed as chemical markers for chemotaxonomical classification, cultivar differentiation and authenticity purposes of red grapes (Figueiredo-González et al., 2012; Dimitrovska et al., 2011; Jaitz et al., 2010; Garcia-Beneytez et al., 2002; Carreño et al., 1997). The phenolic characterization of white grapes mostly relays on the specific flavonol and hydroxycinnamate profiles, which have shown high statistical differentiation among varieties, but dihydroflavonols and flavan-3-ols composition has also been proposed for the purpose (Ferrandino et al., 2012; Castillo-Muñoz et al., 2010; Masa et al., 2007; Montealegre et al., 2006; Mattivi et al., 2006).

Determination of phenolic pattern, including molecules at both high and low amount levels, can help to elucidate peculiarities of single cultivars and derived products, allowing their differentiation. Moreover, the development of phenolics during berry development can support the assessment and verification of enzymatic activities for their biosynthesis. Grape polyphenols also play a fundamental role to determine wine quality and stability, due to their impact on wine pigmentation, sensory properties and structure, and wine evolution during aging (Colombo et al., 2021; Ferrer-Gallego et al., 2016; Heras-Roger et al., 2016; Ma et al., 2014; Terrier at al, 2009; Boulton, 2001; Kallithraka et al., 1997). Consequently, monitoring polyphenolic composition in grapes along fruit development and ripeness, or in musts during maceration, can assist agronomical and winemaking processes, including development and calibration of non-destructive approaches for in-field direct determinations of technological or phenolic maturity, which were developed and gained interest in the last decade for different red and white grapes (Ferrandino et al., 2017; Agati et al., 2013; Cerovic et al., 2008). Nevertheless, only few works reported comprehensive polyphenol composition at different stages of ripening in white grapes (Ferreira et al., 2017; Dal Santo et al., 2016; Friedel et al., 2015; Liang et al., 2012; Perestrelo et al., 2012). Methodologies for extraction and analysis of grape skins phenolic fractions can also assist processing of by-product from winemaking industry like grape pomace, mainly composed by grape skins, which represent sources of phenolics and flavonoids for cosmetics, foods and nutraceuticals (Fontana et al., 2013).

Reversed-phase high-performance liquid chromatography (RP-HPLC) is the most common approach for the analysis of flavonoids and other phenolics from plant extracts including grapes and wines. Chromatography at ultra-high performance levels (UHPLC) is often needed for higher selectivity and separation of isomeric structures, while coupling with mass spectrometry yielding fragmentation (MS/MS or MS^n) provides higher specificity of analytical methodologies allowing accurate quali-quantitative simultaneous determinations of different compounds.

'Pinot Blanc' grape, that arose as an independent somatic mutation of 'Pinot Noir', is a colorless berry mutant due to the encompassing deletion of both the *VvMybA1* and *VvMybA2* genes, which are involved in the regulation of anthocyanin biosynthesis in *V. vinifera* (Vezzulli et al., 2012; Fournier-Level et al., 2010). This cultivar firstly appeared at the end of the 19th century in Bourgogne (France), and its world production has raised up to 15 thousand hectares (Philipp et al., 2019, 2017; OIV., 2017; Maul and Töpfer, 2015; Schultz and Stoll, 2015; Robinson et al., 2013). It is cultivated in Argentina, Brazil, Canada, China, New Zealand, South Africa, United States and Uruguay. In Europe, the 'Pinot Blanc' cultivation is experiencing an unexpected development in Austria, Germany and South Tyrol, the latter being an important historical winegrowing region located in the Central Alps (northern Italy) for which 'Pinot Blanc' has become a leader cultivar (Anonymous, 2020; Pedri and Pertoll, 2013). Although 'Pinot Blanc' is widely cultivated, its chemical characterization is still at the early stages. Nowadays, only little information about the phenolic profile of 'Pinot Blanc' grape and its development is available (Ferreira et al., 2017; Niu et al., 2017; Bachteler et al., 2013; Lenk et al., 2007; Vrhovšek, 1998; Lee and Jaworski, 1989; Singleton, 1986a, 1986b; Joslyn and Dittmar, 1967).

The aim of the present study was the characterization of phenolic composition in 'Pinot Blanc' grapes skin at different berry development stages (from pre-veraison to technological maturity) with particular emphasis on the flavonols profile. Sample preparation and UHPLC-MS/MS analysis were optimized for this purpose, using targeted metabolomics approach based on selected reaction monitoring (SRM) for available standards, and precursor ion scan (PIS) with neutral loss scan (NL) for unavailable compounds. Peculiarities of the data obtained, with respect to literature available on white grapes composition, and their possible role in the differentiation of 'Pinot Blanc' from other white grape cultivars are highlighted. Possible implications on flavonols biosynthesis and its regulation in white grapes are also discussed. To our knowledge this is the first comprehensive phenolic profile characterization of 'Pinot Blanc' grape skin.

2. Materials and methods

2.1. Chemicals and standard solutions

Acetonitrile (LC-MS grade) was from Panreac (Barcelona, ES), formic acid (LC-MS grade) was from Merck (Darmstadt, DE), methanol (LC-MS grade) was from J.T. Baker (Phillipsburg, US) and methanol (gradient grade) was from VWR (Fontenay-sous-Bois, FR). Ultrapure deionized water was from Millipore MilliQ apparatus (Burlington, US). *trans*-*p*-coumaric acid, isorhamnetin, isorhamnetin-3-*O*-glucoside, kaempferol, kaempferol-3-*O*-glucuronide, kaempferol-3-*O*-rutinoside, myricetin-3-*O*-glucoside, myricetin-3-*O*-rhamnoside, quercetin, quercetin-3-*O*-galactoside, quercetin-3-*O*-rhamnoside, quercetin-3,4'-*O*-diglucoside and *trans*-resveratrol were purchased from Extrasynthese (Genay, FR), (-)-epicatechin, (-)-epigallocatechin, (+)-gallocatechin, astilbin, *trans*-caftaric acid, *trans*-coutaric acid, isorhamnetin-3-*O*-rutinoside, kaempferol-3-*O*-glucoside, procyanidin B1, procyanidin B2, procyanidin C1, protocatechuic acid, quercetin-3-*O*-glucuronide, quercetin-4'-*O*-glucoside and taxifolin were from Phytolab (Vestenbergsgreuth, DE), (+)-catechin, *trans*-caffeic acid, *trans*-ferulic acid, myricetin, myricetin-3-*O*-galactoside, quercetin-3-*O*-arabinoside and quercetin-3-glucoside were from Sigma-Aldrich (St. Louis, US), gallic acid and quercetin-3-*O*-rutinoside were from Roth (Karlsruhe, DE) and kaempferol-3-*O*-galactoside was from ChemFaces (Wuhan, CN). All reference compounds were of analytical grade. Mother standard solutions of analytes and internal standard (I.S.: quercetin-4'-*O*-glucoside) were prepared at 400 μg/mL by weighing 2.00 mg and dissolving in H_2O-MeOH 50:50 (v/v) in 5.0 mL volumetric flask. Calibration solutions, each spiked with 1.00 μg/mL I.S., were prepared by direct dilutions with deionized water at exact concentrations between 5.000 and 0.001 μg/mL).

2.2. Sampling and extraction of phenolics

Berries samples were collected in 2018 vintage from day of year (DOY) 186–246 from a single vineyard (Termeno (BZ), Italy, 270 m a.s.l.) adapting the protocol reported by Tomaz et al. (2019). A total of 36 berries samples divided into seven sampling dates were analyzed, three performed from DOY 186 to DOY 208 (bunch closure-veraison period) and four from DOY 212 to DOY 246 (berry ripening). The veraison stage

(BBCH) was recorded at DOY 202. Each sample was composed of 20–25 berries, that were quickly stored at −30 °C. Before extraction, the frozen samples were dipped in liquid nitrogen to prevent melting. Berry skin was completely removed using a scalpel and thoroughly cleaned from pulp residue with cotton swabs on a glass plate. Fresh weight was measured, then samples were dried in oven at 45 °C for 72 h (until constant weight). Each dried sample was weighed and totally transferred into 1 or 2 flip-cap plastic vials (2.0 mL). The samples were reduced to a fine powder (150 s at 30 Hz) with a 400 MM ball mill (Retsch, Haan, DE). The powdered material was kept in a desiccator (under vacuum, in the dark) until short-term further processing (one week).

For each sample, 20.0 mg of powder were weighted in a 15 mL plastic tube and spiked with 30 μL of internal standard mother solution, then 12 mL of methanol-deionized water (1:1) solution with formic acid 1 % (v/v) were added (1.0 ppm I.S. final). Each sample was prepared in triplicate (technical replicates). Tubes were vortexed for 20 s, then extraction was performed in two following steps: 1) Rotary mixer PTR-60 (Grant Intruments, Shepreth, EN), multiple cycles at room temperature (orbital: 100 rpm for 20 s; reciprocal: 45° for 40 s; vibro: 5° for 5 s; total time 20 min); 2) Ultrasonic Cleaner (VWR, Radnor, US): 10 min at room temperature. Samples were centrifuged (4000 rpm for 20 min, 4 °C), then supernatants were transferred in glass vials and stored at −80 °C until analysis.

2.3. Liquid chromatography–mass spectrometry analysis

High-performance liquid chromatography-mass spectrometry (HPLC-MS) analysis of extracts was conducted on Accela 1250 UHPLC system equipped with a diode array detector (DAD) and connected to a TSQ Quantum Access Max triple-quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, US). Separation was performed on

Table 1
Instrumental and analytical parameters of the compounds investigated in 'Pinot Blanc' grape skin from South Tyrol.

Compound	Rt	Polarity	Q1 *precursor*	Q3 *quantifier*	Q3 *qualifier*	Regression			Linear Range		ILOQ	RSD[a]
	[min]		*m/z*	*m/z*	*m/z*	r^2	offset	slope	[μg/mL] min	[μg/mL] max	[μg/mL]	(%)
Flavonols												
Isorhamnetin	11.58	+	317.1	302	153	0.9821	-0.0004	0.099	0.005	1.000	0.005	n.d.
isorhamnetin-3-glucoside	11.04	+	479.1	317	302	0.9994	0.0082	1.317	0.001	0.500	<0.001	4.45
isorhamnetin-3-rutinoside	10.66	+	625.2	317	479	0.9976	-0.0002	0.017	0.005	5.000	0.005	n.d.
Kaempferol	11.56	+	287.1	153	165	0.9853	-0.0002	0.088	0.005	2.000	0.005	9.80
kaempferol-3-glucoside	10.66	+	449.1	287	153	0.9996	+0.0010	1.328	0.001	5.000	<0.001	3.04
kaempferol-3-galactoside	10.20	+	449.1	287	153	0.9997	-0,0042	1.126	0.001	5.000	<0.001	2.49
kaempferol-3-glucuronide	10.69	+	463.1	287	153	0.9991	+0.0029	1.172	0.001	5.000	<0.001	2.97
kaempferol-3-rutinoside	10.34	+	595.2	287	449	0.9987	+0.0003	0.9134	0.001	5.000	<0.001	4.34
Myricetin	11.15	+	319.1	245	165	0.9863	-0.0015	0.039	0.050	1.000	0.05	n.d.
mirycetin-3-glucoside	8.40	+	481.1	319	153	0.9985	-0.006	1.264	0.001	5.000	<0.001	7.03
myricetin-3-galactoside	8.25	+	481.1	319	153	0.9996	-0.0005	1.180	0.001	5.000	<0.001	n.d.
myricetin-3-rhamnoside	9.23	+	465.0	319	153	0.9989	0.0053	1.118	0.002	5.000	0.002	n.d.
Quercetin	11.43	+	303.1	153	225	0.9947	-0.0013	0.065	0.010	1.000	0.01	6.43
quercetin-3-*O*-glucoside	9.73	+	465.1	303	229	0.9981	-0.0148	1.254	0.001	5.000	0.001	3.85
quercetin-3-*O*-galactoside	9.33	+	465.1	303	2299	0.9995	-0.0003	1.507	0.001	5.000	<0.001	2.82
quercetin-3,4′-*O*-diglucoside	8.09	+	627.2	303	465	0.9993	-0.0002	0.705	0.002	5.000	0.002	5.64
quercetin-3-*O*-glucuronide	9.53	+	479.1	303	257	0.9992	+0.0124	0.954	0.001	5.000	<0.001	3.59
quercetin-3-*O*-arabinoside	10.13	+	435.1	303	229	0.9996	-0.0081	1.173	0.001	1.000	0.001	4.68
quercetin-3-*O*-rhamnoside	10.73	+	449.2	303	71	0.9969	0.0014	0.411	0.002	2.000	0.002	5.38
quercetin-3-*O*-rutinoside	9.33	+	611.2	303	465	0.9996	-0.0007	0.745	0.001	5.000	0.001	3.25
quercetin-4′-*O*-glucoside (I.S.)	11.05	+	463.2	303	257	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.14
Dihydroflavonols												
Astilbin	9.65	+	451.1	305	129	0.9977	+0.0005	0.043	0.005	2.000	0.005	3.96
Taxifolin	9.00	+	305.0	259	231	0.9951	+0.0026	0.158	0.050	5.000	0.050	n.d.
Benzoic and hydroxycinnamic acids/esters												
gallic acid	1.18	–	169.1	125	79	0.09911	-0.0001	0.044	0.002	0.2	0.002	6.87
protocatechuic acid	2.44	–	153.1	109	108	0.9959	+0.0013	0.031	0.020	2.0	0.02	n.d.
cis-coutaric acid[b]	4.63	–	295	163	119	0.9983	-0.0011	0.046	0.001	5.0	0.001	3.95
trans-coutaric acid	4.93	–	295	163	119	0.9983	-0.0011	0.046	0.001	5.0	0.001	4.62
caftaric acid[c]	3.49	–	311	179	149	0.9981	+0.0013	0.062	0.002	5.0	0.002	3.96
trans-caffeic acid	5.40	–	179.1	135	134	0.9908	+0.0009	0.087	0.010	2.0	0.01	n.d.
trans-ferulic acid	9.50	–	195.1	177	145	0.9951	+0.0049	0.269	0.010	5.0	0.01	n.d.
trans-p-coumaric acid	7.40	–	165.1	119	91	0.9927	-0.0009	0.042	0.100	5.0	0.1	n.d.
Flavan-3-ols												
(+)-catechin	4.85	+	291	139	123	0.9974	0.0001	0.191	0.001	2.0	<0.001	3.82
(-)-epicatechin	6.75	+	291	139	123	0.9965	0.0009	0.334	0.001	5.0	<0.001	11.83
(+)-gallocatechin	2.37	+	307	139	163	0.9969	0.0011	0.360	0.001	5.0	<0.001	5.86
(-)-epigallocatechin	4.50	+	307	139	163	0.9952	0.0020	0.243	0.001	2.0	<0.001	n.d.
procyanidin B1	4.30	+	579.1	427	127	0.9987	-0.0003	0.074	0.001	5.0	0.001	7.80
procyanidin B2	6.08	+	579.1	427	127	0.9973	+0.0002	0.119	0.001	5.0	0.001	n.d.
procyanidin C1	7.52	+	868.2	580	287	0.9982	-0.0001	0.033	0.001	5.0	<0.001	n.d.
Others												
t-resveratrol	11.29	+	229.1	107	135	0.9951	-0.0008	0.031	0.05	2.0	0.05	n.d.

[a] : calculated as coefficient of variation on $n = 11$ runs of QC sample; analytes content lower than LOQ is reported as "n.d."
[b] : *cis*-coutaric acid was calculated as *trans*-coutaric acid equivalents.
[c] : caftaric acid amount was calculated as the sum of the *cis* + *trans* isomers.

a RSLC Acclaim C18 column (100 × 2.1 mm, 2.2 μm particle size, Thermo Fisher Scientific, Waltham, US) with filter guard column. The mobile phase consisted of 0.1 % formic acid in water (A), and 0.1 % formic acid in acetonitrile (B), and linear gradient elution was applied as follows: 0.0–1.0 min 4.0 % (B), 3.7–4.3 min 9.0 % (B), 9.0–10.0 min 19.0 % (B), 11.0–14.5 min 95.0 % (B), then 15.0–18.0 min 4.0 % (B). Flow rate was set to 0.5 mL/min and column was thermostated at 40 °C. Detection wavelength was 360 nm (UV–VIS spectra in the range 200–750 nm were acquired during the whole run). The injection volume was 5 μL.

For quali-quantitative analysis, TSQ Quantum Access Max triple-quadrupole mass spectrometer equipped with heated electrospray ionization (HESI) source was used (Thermo Fisher Scientific, Waltham, USA). Both positive and negative ion mode were applied. The ion source settings were as follows: spray voltage 3000 V (positive polarity) - 3000 V (negative polarity), vaporizer temperature 450 °C, sheet gas pressure 60 arbitrary units (Arb), ion sweep gas pressure 0 Arb, auxiliary gas pressure 35 Arb, capillary temperature 250 °C, skimmer offset 0 V. Mass spectrometry data were acquired with Q1 and Q3 resolution set at 0.7 amu. Collision-induced fragmentation was obtained with argon (1.5 mTorr pressure). Retention time (Rt) and ion ratio in selected reaction monitoring (SRM) were used for compound identification. Discrimination of structural isomers (same parent and product ions) was obtained by their retention times. Analytical parameters for each compound are summarized in Table 1.

Technical replicates for each sample were injected in single replicate, while calibration solutions were injected in triplicate. A quality control sample (QC), prepared by pooling all the samples, was injected every 15 analyses to verify absence of chromatographic drift and check instrumental response. Quantitative determination was based on comparison of analyte/internal standard area ratio in samples and calibration solutions, with external calibration curve for each reference compound. Calibration curves were obtained by plotting the analyte/internal standard area ratio against the concentration injected (μg/mL), and regression factor (r^2) was calculated by means of least-square analysis for linearity evaluation. Instrumental limit of detection (ILOD) and quantification (ILOQ) were established as the lowest standard level providing 3- and 10-times signal-to-noise ratio, respectively. Instrumental precision (intraday repeatability) was expressed as the coefficient of variation (CV %) in content of each analyte from 11 injections of QC in the same day. Official guidelines were followed for method validation (Magnusson and Örnemark, 2014). Quantitative data were corrected following pureness of standard reference compounds. Results are expressed as mg/kg (dry weight).

3. Results and discussion

3.1. Extraction and UHPLC-MS method optimization

Water-methanol mixtures were already reported to provide highest extraction yield of phenolics from grape pomace compared to other solvent mixtures (Corte-Real et al., 2021; Jara-Palacios et al., 2014). Extraction of different phenolics using water-methanol acidic solutions, assisted by sonication and mixing under mild conditions (room temperature), was already developed in our lab for freeze-dried apple pulps and skins, with very good recoveries for dihydrochalcones, flavan-3-ols and phenolic acids including chlorogenic acid (Serni et al., 2020b; Valls et al., 2017). Optimization of extraction procedure of flavonols glycosides from Pinot Blanc grape skin was initially conducted measuring extraction yield of 3-*O*-glucoside and 3-*O*-glucuronide of quercetin and kaempferol since these were reported to be majoritarian in flavonol fraction of most white grape skin investigated (Ferrandino et al., 2017; Agati et al., 2013; Castillo-Muñoz et al., 2010; Mattivi et al., 2006). Solvent composition, sample weight/extraction volume ratio and extraction time and temperature were optimized to achieve full extractability of target analytes after one-cycle procedure. Indeed, the protocol described allowed over 99 % extraction of both quercetin and kaempferol 3-*O*-glucuronides and 3-*O*-glucosides after the first cycle (compared with total yield after further extraction on sample residue with same protocol), resulting in relatively fast and easy-to-practice procedure.

SRM parameters were obtained by direct infusion of each single compound, with all flavonol/dihydroflavonol glycosides showing pseudomolecular ion ($[M+H]^+$) as highest precursor signal in positive mode, as already verified in recent works (Serni et al., 2020a; Ceci et al., 2021). ESI-source parameters, like vaporizer and capillary temperature, are crucial in LC/MS analysis of glycosidic derivatives due to their degradation with loss of sugar moieties, so that they also were optimized for signal-to-noise ratio to get maximized (see Table 1 for parameters list). Quercetin-4'-*O*-glucoside proved to be an appropriate internal standard since it could be clearly separated from its most abundant isomers (quercetin-3-*O*-glucoside and quercetin-3-*O*-galactoside) and it existed in non-spiked extracts at non-quantifiable levels. Finally, compared to products with similar specifications, the chromatographic column under investigation was chosen since it provided sufficient selectivity towards structural isomers couples (e.g. 3-*O*-glucoside vs. 3-*O*-galactoside of all the four flavonols hence considered). Mobile phase gradient elution and column temperature were then optimized to obtain best resolution of isomers within shortest time. The repeatability was evaluated by the coefficient of variation (CV%) for peaks areas of the QC sample solution. Values ranged between 2.49 % and 11.83 %, with all flavonols glycosides below 5.00 % except for myricetin-3-O-glucoside and highest values regarding molecules close to LOQ value. This confirms high robustness of the method proposed.

3.2. Screening for flavonols glycosides

In several white grape varieties, occurrence of quercetin and kaempferol glycosides is generally reported, together with minor levels of single isorhamnetin derivatives, with − 3-*O*-glucuronide and − 3-*O*-glucoside forms being the most abundant. Largest pool of reference compounds at our disposal, belonging to these three structures and all featured of 3-*O*-glycoside linkage with different sugar moieties, was thus set for screening in SRM mode to be as complete as possible (Table 1). Then, occurrence of quercetin, kaempferol and isorhamnetin glycosides in addiction to those available as reference compounds was checked through precursor ion scan (PIS) and neutral loss (NL) of corresponding aglycones and/or mono-glycosides in QC sample for best completeness. The main signals obtained from scan of quercetin aglicone in mono- and di-glycosides mass range corresponded to reference compounds in possess and already screened in SRM. The same was valid also for kaempferol aglycone in both mass ranges scans. For this reason, screening for quercetin and kaempferol glycosides was considered satisfactory. This also confirmed that − 3-*O*- linkage is far the main expressed for flavonol derivatives in white grape skin. Scan of isorhamnetin aglycone molecular ion precursor (m/z = 317.1, $[M+H]^+$) in monoglycosides range (m/z = 445.0–495.0, Fig. S1) showed two main peaks with signals at m/z = 479.1, coherent with monohexosides isomers ($[M+H]^+$): the second (Rt = 10.95) was identified through comparison with reference compound in SRM mode as isorhamnetin-3-glucoside, while the first (Rt = 10.67) was tentatively identified as isorhamnetin-3-galactoside by analogy with data from literature and with behavior of corresponding glycosides for quercetin and kaempferol (Fig. S2). Following by intensity, two peaks with signals at, respectively, m/z = 451.1 (Rt = 9.57, not identified) and m/z = 493.2 (Rt = 9.45, probably $[M+H]^+$ of 3-*O*-glucuronide) were also present in the chromatogram. In the range of diglycosides precursors (600.0–660.0; Fig. S3), one peak yielded signal at m/z = 625.2 (Rt = 10.59) and was confirmed with reference compound in SRM mode as isorhamnetin-3-rutinoside (Fig. S4). A peak cluster at Rt = 8.00–8.10 showed unidentified signals at m/z = 616.2–617.1 and 648.2, while peak at Rt = 7.40 yielded signal consistent with isorhamnetin diglucoside (m/z = 641.0)

plus unidentified signal at $m/z = 643.9$.

Basically, both PIS experiments and SRM analyses asserted the occurrence of different isorhamnetin glycosidic derivatives at measurable level in Pinot Blanc grape skin extract.

Since currently available literature lacks a detailed description of 'Pinot Blanc' grape skin flavonols, and being this white variety genetically derived from the coloured ancestor 'Pinot Noir', a more detailed characterization of flavonols pattern was considered worth. Therefore, glycosides of delphinidine-like flavonols myricetin, larycitrin and syringetin were also tested with the same approach.

Comparison with blank injection showed no significant peak for larycitrin and syringetin derivatives in the QC sample (Figs. S5–S6). On the contrary, screening for precursors of myricetin aglycone (m/z = 319.1, $[M+H]^+$) in the mass range of monoglycosides (m/z = 440.0–500.0; Fig. S7) yielded two double peaks (Rt = 7.80–8.00 and 8.15–8.50) with signals compatible with monohexosydes and monoglucuronides, at $m/z = 481.1$ and 495.0, resp. ($[M+H]^+$). Occurrence of both − 3-*O*-glucoside and − 3-*O*-galactoside in QC was confirmed by injection in SRM mode of corresponding reference compounds (Fig. S8).

Since myricetin-3-glucuronide as reference compound was missing, signals at $m/z = 495.0$ were extracted from PIS chromatogram and Rt compared to signals generated after neutral loss of glucuronide moiety ($m/z = 176.0$) in the range $m/z = 490.0–500.0$. This led to the detection of two compounds (Fig. S9). Absence at detectable level of myricetin-3-*O*-rhamnoside ($m/z = 465.0$) was also confirmed by injection of reference compound in SRM (Rt = 9.23; Fig. S10). Screening for precursors of aglycone and/or monoglucosides at higher mass range (m/z = 610.0–660.0) showed no significant peaks on the chromatogram (Fig. S11).

These results confirmed the presence of myricetin in the skin extract with different forms of glycosylation, and enzymatic production of myricetin as an effective part of flavonoid biosynthetic pathway in Pinot Blanc grape variety. Hence, lack of expression of the enzyme flavonoid 3′,5′-hydroxylase in white grapes cannot be firmly asserted in a general manner.

3.3. Screening for dihydroflavonols glycosides

Dihydroflavonols profile has already been reported to vary among different white grape cultivars, with dihydroquercetin derivatives being the most expressed and therefore proposed as chemical markers for cultivar differentiation (Masa et al., 2007). For this reason, scan for dihydroquercetins was performed as well.

Parent ion scan of dihydroquercetin (taxifolin) at m/z = 305.0 ($[M+H]^+$) in the range of monoglycosides ($m/z = 425.0–485.0$) yielded one main peak at Rt = 9.59 (m/z = 450.9) corresponding to monorhamnoside, which is confirmed in SRM mode with reference compound as astilbin (taxifolin-3-*O*-rhamnoside; Figs. S12–S13). This is partially overlapped to a peak at Rt = 9.46, probably corresponding to monoglucuronide ($m/z = 481$). Both significant peaks at Rt = 7.19 and 10.97 gave molecular ion of monohexoside at m/z = 467, while compound eluting at Rt = 8.04 probably corresponds to derivative with pentose moiety (i.e. arabinoside or xyloside, $m/z = 437$). Besides, in the range of diglucosides (m/z = 600.0–660.0, Fig. S14) PIS analysis of dihydroquercetin showed one only main peak at Rt = 9.35 with molecular ion at $m/z = 613$, which is consistent with corresponding rutinoside.

Dihydrokaempferol derivatives were also scanned in PIS mode considering aglicone aromadendrin at m/z = 289 ($[M+H]^+$), and the chromatogram obtained in the range of monoglycosides (m/z = 410–470; Fig. S15) showed different significant peaks but with lower overall intensity compared to scan for dihydroquercetin derivatives. Significant peaks at Rt = 6.22, 8.49 and 10.63 all yielded signals at m/z = 451 corresponding to monohexosides, while main peak at Rt = 10.92 yielded signal at $m/z = 435$ suggesting occurrence of monorhamnoside.

All these results indicate that molecular pattern of dihydroflavonols is also complex and highly expressed in Pinot Blanc grape skin, thus being worth of further investigation.

3.4. Evolution of phenolic composition in Pinot Blanc grape skin during ripening

The amounts of all molecules quantified along berry development are summarized in Table 2. Percentages indicated along the text are calculated from these values.

The evolution of the main classes of phenols and flavonoids is shown in Fig. 1A.

Total phenolic content as the sum of target molecules is retained during ripening. Flavonol glycosides are the most expressed throughout the season and their content is statistically retained, however an accumulation trend can be observed, with minimum average value recorded after veraison and maximum before full ripeness. Phenolic acids, mainly constituted by hydroxycinnamoyl tartaric esters, are second by abundance and their content is clearly diminishing from veraison to harvest. Flavan-3-ols and dihydroflavonol glycosides are last by abundance: the first are more concentrated after veraison and featured by almost constant trend, while the second are less concentrated in early ripening and clearly increase along the season similarly to flavonol glycosides, so that their content at harvest resulted higher than that of flavan-3-ols and similar to total phenolic acids. Compared to cited published data about composition at harvest time (Ferrandino et al., 2012; Liang et al., 2012; Castillo-Muñoz et al., 2010; Masa et al., 2007; Montealegre et al., 2006), and considering underestimation of total content due to lack of standards compounds (respect to chromatograms from PIS scan), dihydroflavonols fraction seem to be considerably abundant; on the contrary, flavan-3-ols fraction seem to be scarce and under-expressed.

3.4.1. Flavonols and dihydroflavonols glycosides profile

Quercetin glycosides clearly represent the largest fraction of total flavonols glycosides at every stage along ripening (between 98.16 % and 85.72 % avg.; Fig. 1B). Total content of quercetin derivatives seems to be almost retained along the whole period. Quercetin-3-*O*-glucuronide had highest content along whole time lapse, followed in order by 3-*O*-glucoside, 3-*O*-rutinoside, 3-*O*-galactoside, 3-*O*-arabinoside, 3-*O*-rhamnoside and 3,4′-*O*-diglucoside (Fig. 2A). All the compounds considered were quantifiable at all time points except for 3,4′-*O*-diglucoside, whose values were below LOQ at veraison and early ripening.

Quercetin glycosides however showed different seasonal trend, since 3-*O*-glucuronide retained constant level along the ripening period and slightly decreased near technological maturity, while all the others significantly increased except for − 3-*O*-rutinoside, which showed nearly constant amount. Average content of quercetin-3-*O*-glucuronide became slightly higher than quercetin-3-*O*-glucoside at harvest. The same seasonal evolution was already reported for different red grape cultivars, for which it allowed statistical differentiation (Castillo-Muñoz et al., 2007). Constant or decreasing trend for quercetin-3-*O*-glucuronide in white grape skin was also reported (Liang et al., 2012). Moreover, highly variable quercetin-3-*O*-glucuronide molar percentage at harvest in white grape skins was already described for international and local varieties: this compound strongly contributed to the separation of cultivars in statistical analysis by the same authors (Zhang et al., 2015; Castillo-Muñoz et al., 2010; Ferrandino et al., 2012; Montealegre et al., 2006). Despite high levels of quercetin-3-*O*-glucuronide at flowering and/or at veraison have already been described, its physiological role in the first phases of fruit development is still unclear respect to general well-known accumulation of flavonol glycosides in response to sunlight exposure. Quercetin-3-*O*-glucuronide was reported to be involved in the resistance to plant diseases, being active against *Botrytis cinerea* through inhibition of the pathogen stilbene oxidase activity and protecting the grape berries between bloom and veraison (Goetz et al., 1999).

It is interesting to note that significant amounts of quercetin-3-*O*-rutinoside (between 6.28 % and 10.09 % avg. on total flavonoids) were not reported in white grapes so far except for Sercial portuguese variety

Table 2
Development of phenolics amount during berry development in 'Pinot Blanc' grape skin from South Tyrol, *DOY: day of year of the corresponding sampling, n: number of samples*.

	sampling	1	2	3	4	5	6	7
Compound	*DOY*	186	194	208	212	222	228	246
	n	**6**	**6**	**4**	**5**	**5**	**5**	**5**
Quercetin-3-O-glucuronide	p = 0.752	2903.7 ± 1521.4	2323.7 ± 1352.0	2194.4 ± 679.9	2163.3 ± 1359.5	2201.0 ± 710.1	2305.6 ± 386.7	1625.6 ± 723.7
Quercetin-3-glucoside	p = 0.009	363.7 ± 244.4 b	284.5 ± 225.2 b	402.5 ± 200.3 b	709.2 ± 637.4 ab	868.8 ± 293.4 ab	1256.9 ± 226.1 a	1129.2 ± 602.0 a
Quercetin-3-rutinoside	p = 0.978	282.2 ± 160.8	264.5 ± 201.0	322.0 ± 126.2	335.2 ± 286.5	297.8 ± 96.6	341.7 ± 72.1	256.8 ± 137.3
Quercetin-3-galactoside	p = 0.026	83.8 ± 52.1 ab	72.6 ± 61.0 b	95.2 ± 41.0 ab	139.9 ± 128.8 ab	157.1 ± 49.7 ab	231.9 ± 40.2 a	207.7 ± 115.3 ab
Quercetin-3-rhamnoside	p = 0.001	5.0 ± 4.5 b	3.9 ± 4.2 b	11.0 ± 8.0 ab	28.7 ± 25.3 ab	33.9 ± 13.2 ab	43.4 ± 7.3 a	37.3 ± 17.4 ab
Quercetin-3-arabinoside	p < 0.001	8.6 ± 3.6 b	8.4 ± 3.8 b	13.1 ± 5.5 ab	27.6 ± 20.5 ab	32.0 ± 8.8 ab	41.0 ± 9.7 a	42.7 ± 17.9 a
Quercetin-3. 4´-diglucoside	p = 0.013	n.d.	n.d.	n.d.	2.6 ± 1.7 b	3.6 ± 0.9 ab	6.4 ± 1.4 ab	8.9 ± 4.2 a
Quercetin	p = 0.009	30.9 ± 7.7 a	29.4 ± 11.7 ab	16.2 ± 1.8 b	17.8 ± 3.5 ab	20.7 ± 9.5 ab	30.1 ± 11.1 ab	31.2 ± 8.1 ab
total quercetin glycosides	p = 0.868	3647.0 ± 1980.5	2957.5 ± 1836.9	3038.1 ± 1050.7	3406.5 ± 2455.3	3594.3 ± 1142.1	4226.9 ± 711.1	3308.2 ± 1540.1
Kaempferol-3-glucoside	p < 0.001	16.4 ± 12.5 b	11.3 ± 11.2 b	31.3 ± 24.6 ab	176.3 ± 181.8 ab	234.4 ± 95.1 ab	427.2 ± 62.3 a	388.3 ± 229.1 a
Kaempferol-3-galactoside	p = 0.002	11.7 ± 6.4 b	10.1 ± 7.5 b	16.6 ± 8.0 ab	62.1 ± 63.4 ab	79.2 ± 31.9 ab	147.8 ± 25.1 a	131.5 ± 77.5 a
Kaempferol-3-glucuronide	p = 0.032	24.4 ± 21.4 ab	16.8 ± 15.5 b	22.0 ± 12.4 ab	37.8 ± 35.8 ab	45.6 ± 20.4 ab	62.9 ± 12.1 a	40.0 ± 21.2 ab
Kaempferol-3-rutinoside	p = 0.310	14.9 ± 10.6	12.3 ± 12.3	18.4 ± 9.2	29.0 ± 29.6	23.7 ± 9.6	30.5 ± 9.8	22.7 ± 12.6
Kaempferol	p = 0.465	n.d.	n.d.	n.d.	n.d.	n.d.	8.9 ± 2.3	10.2 ± 4.2
total kaempferol glycosides	p = 0.001	67.3 ± 50.7 b	50.6 ± 46.5 b	88.4 ± 52.9 b	305.3 ± 309.5 ab	382.8 ± 153.8 ab	668.4 ± 101.1 a	582.6 ± 336.7 a
Isorhamnetin-3-glucoside	p < 0.001	5.2 ± 0.4 abc	4.5 ± 0.4c	4.6 ± 0.2 BCE	12.2 ± 5.9 abc	15.5 ± 4.2 ab	19.5 ± 5.1 a	19.8 ± 8.2 a
Myricetin-3-glucos	p = 0.301	4.4 ± 1.2	4.4 ± 1.2	5.2 ± 0.6	5.7 ± 1.9	5.8 ± 0.9	5.9 ± 2.3	5.1 ± 1.3
total flavonol glycosides	p = 0.627	3723.9 ± 2030.9	3016.9 ± 1882.4	3136.3 ± 1103.0	3729.7 ± 2766.0	3998.4 ± 1254.4	4920.8 ± 749.8	3915.6 ± 1865.5
Astilbin	p = 0.012	274.9 ± 124.1 a	334.4 ± 186.7 ab	373.0 ± 110.0 ab	605.8 ± 363.5 ab	691.3 ± 241.3 ab	719.1 ± 246.5 ab	754.2 ± 252.4 b
Procyanidin B1	p = 0.484	358.4 ± 157.2	338.9 ± 196.6	393.4 ± 60.3	335.9 ± 117.0	325.4 ± 118.8	257.5 ± 150.8	245.0 ± 72.1
(+)-catechin	p = 0.012	267.5 ± 97.7 a	235.7 ± 110.6 a	172.0 ± 38.6 ab	173.7 ± 79.8 ab	147.6 ± 67.8 ab	117.3 ± 82.9 ab	82.8 ± 35.2 b
(+)-gallocatechin	p = 0.184	6.5 ± 2.4	8.0 ± 3.8	7.9 ± 2.9	12.7 ± 5.7	15.0 ± 6.8	10.8 ± 9.7	8.7 ± 3.8
(-)-epicatechin	p = 0.654	4.5 ± 3.4	2.8 ± 2.9	2.3 ± 2.2	4.9 ± 2.6	5.1 ± 4.4	5.4 ± 5.2	3.1 ± 2.4
total flavan-3-ol	p = 0.257	636.9 ± 254.0	585.5 ± 305.2	575.6 ± 86.0	527.3 ± 203.6	493.0 ± 194.5	390.9 ± 246.3	339.6 ± 110.1
Caftaric acid	p = 0.006	1257.7 ± 279.8 ab	1506.2 ± 567.2 a	994.6 ± 295.3 abc	788.5 ± 387.5 abc	895.6 ± 449.0 abc	572.5 ± 381.2 BCE	503.9 ± 131.0c
***t*-coutaric acid**	p = 0.016	776.6 ± 276.1 a	833.3 ± 368.9 a	500.6 ± 174.5 ab	408.2 ± 240.5 ab	532.1 ± 300.8 ab	349.5 ± 251.4 ab	243.4 ± 92.1 b
***c*-coutaric acid**	p = 0.006	373.6 ± 90.2 a	349.1 ± 87.6 a	245.6 ± 59.8 ab	203.1 ± 84.4 ab	247.5 ± 99.7 ab	184.2 ± 96.1 ab	155.8 ± 34.1 b
Gallic acid	p < 0.001	11.5 ± 1.6 a	9.2 ± 2.5 a	2.8 ± 0.3 ab	2.4 ± 0.4 b	2.4 ± 0.5 b	2.4 ± 0.7 b	2.9 ± 0.5 ab
total phenolic acids	p = 0.01	2419.3 ± 634.8 a	2697.9 ± 1016.4 a	1743.5 ± 527.1 ab	1402.1 ± 708.1 ab	1677.5 ± 844.9 ab	1108.6 ± 728.7 ab	906.0 ± 250.8 b
TOTAL PHENOLICS	p = 0.975	7055.0 ± 2912.2	6634.7 ± 3283.8	5828.3 ± 1170.1	6264.9 ± 3895.0	6860.3 ± 2291.8	7139.5 ± 1844.9	5915.5 ± 2373.9

by means of semiquantitative MS^n determination (Perestrelo et al., 2012), in fact it has long been considered erroneously as a minor compound in white grapes showing molar percentages lower than 1.42 % at harvest (Castillo-Muñoz et al., 2010). The occurrence in red grapes was already well-known from LC-MS phenol screening (Cantos et al., 2002). For these reasons quercetin-3-*O*-rutinoside relative content could be highly discriminatory for Pinot Blanc grape and derived products.

Very low levels of 3,4´-*O*-diglucoside confirm activity for 4'-*O*-hydroxylation at B ring to be negligible and correlated with increasing concentration of corresponding substrate (3-*O*-glucoside). The lack of quercetin-4´-*O*-glucoside verified "a priori" confirms this fact.

Our results about quercetins profile are partially in contrast with published data regarding 'Pinot Blanc' whole berry composition, where the authors reported quercetin-3-*O*-galactoside as the most abundant derivative, with no clues about the presence of quercetin-3-*O*-glucuronide and together with relative low amounts of quercetin-3-*O*-rutinoside (Ferreira et al., 2016). Contradictory results on flavonol composition including major compounds already arised from investigation being conducted with HPLC-DAD respect to HPLC-MS and were highlighted by the authors (Castillo-Muñoz et al., 2010; Masa et al., 2007).

Kaempferol derivatives are second by abundance among flavonols (between 1.45 % and 13.76 %), with clear increase from pre-veraison to technological maturity. The 3-*O*-glucoside was the most abundant and 3-*O*-galactoside, 3-*O*-glucuronide and 3-*O*-rutinoside followed in order. They all were quantifiable during the whole period of investigation (Fig. 2B). Kaempferol-3-*O*-galactoside was unexpectedly higher than 3-*O*-glucuronide, considering reference literature on most common white grapes flavonol composition, where 3-*O*-glucoside and 3-*O*-glucuronide were always reported as the most abundant. It is evident how sugar moieties (e.g glucose or rhamnose) have different relative abundance among the different flavonol backbones quercetin and kaempferol, indicating their different affinity for the existing glycosyl-transferase isoforms.

Isorhamnetins were minor compounds along the period considered (0.16–0.55 % avg.). Only 3-*O*-glucoside resulted quantifiable at all time points: in fact, 3-*O*-rutinoside signals lacked correct ion ratio confirmation for most samples analysed and was thus excluded from calculation (Fig. 3). Putative isorhamnetin-3-*O*-galactoside was also detectable at all stages.

Isorhamnetin-type flavonols, especially the 3-*O*-glucoside derivative, allowed an additional differentiation of some white grape cultivars with PCA, explaining 18.23 % of the total variance (Castillo-Muñoz et al., 2010). The determination of minor compounds was aimed by the authors and its importance was discussed. Occurrence of molecules at low levels can be highly discriminatory, especially if regarding few cultivars.

Myricetin-3-*O*-glucoside was quantifiable in all samples along ripening and was then calculated, while the content of – 3-*O*-galactoside, despite higher than LOD, was close to or below LOQ in all samples and therefore excluded from calculation. Occurrence of – 3-*O*-glucuronide also emerged from PIS experiments with NL scan. Myricetin-3-*O*-glucoside amount was in the range of isorhamnetin-3-O-glucoside (0.12–0.20 %; Fig. 3). It is also worth noticing that it occurred at low levels at veraison, conversely to single flavonol glycosides that are quantifiable only in correspondence of enzymatic expression towards full ripeness and harvest (like quercetin-3,4'-*O*-diglucoside). This means that myricetin biosynthesis is a constitutive segment of polyphenolic metabolome and not induced along ripening. Significance of these data questioned the authors. The biosynthesis of myricetin derivates appeared to be specific of red cultivars from first comprehensive screenings of white grapes except when referred to as "occurrence at trace levels" (Ferrandino et al., 2012; Castillo-Muñoz et al., 2010; Mattivi et al., 2006). This latter aspect evocates exact quantitative threshold limits for trace (i.e. non-significant) levels to be stated respect to real lack or existence of a particular correlated enzymatic activity, also considering the performances of the methodologies adopted. In fact, more recent evidences about presence of myricetin and minor

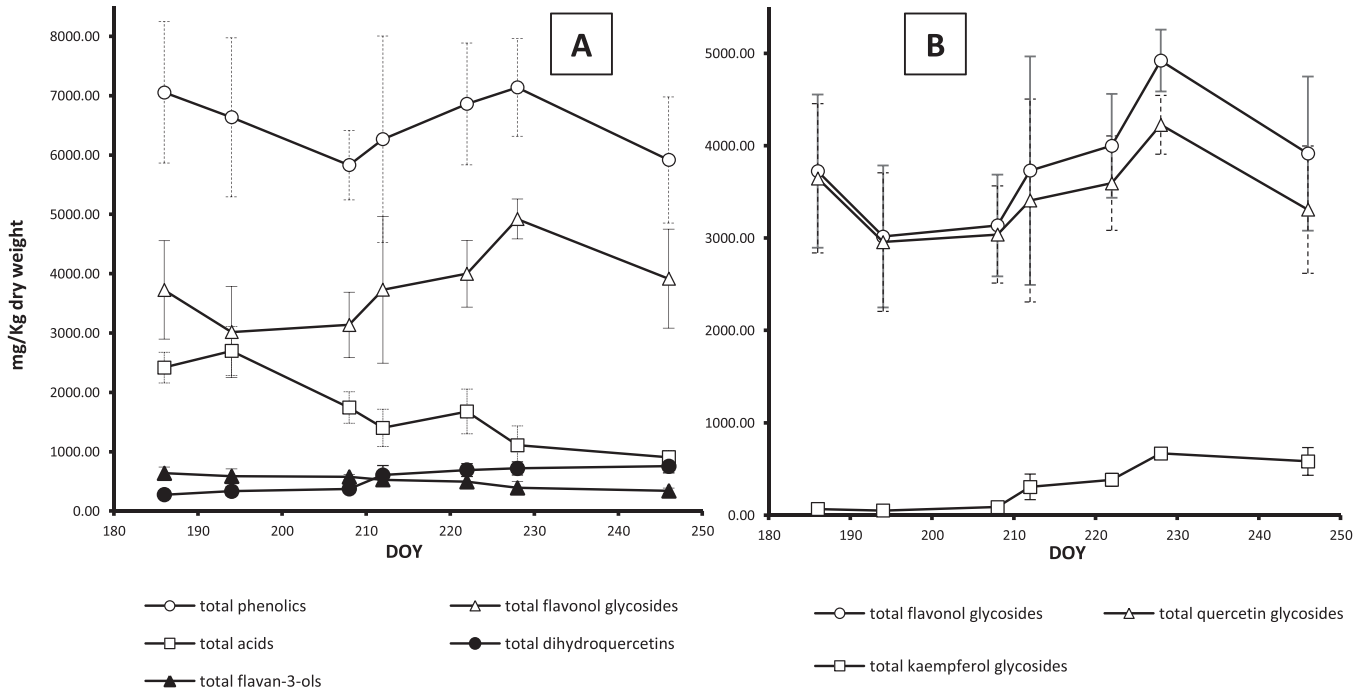

Fig. 1. Development of the phenolic groups composition (A) and flavonols glycosides composition (B) in 'Pinot Blanc' grapes skin during ripening, Each data point represents the mean ± standard error (mg/kg dry weight) of corresponding samples pool (number of samples as reported in Table 2). The time-lapse covers berry development period from bunch-closure (day of the year, DOY, 185) to ripeness (DOY 246).

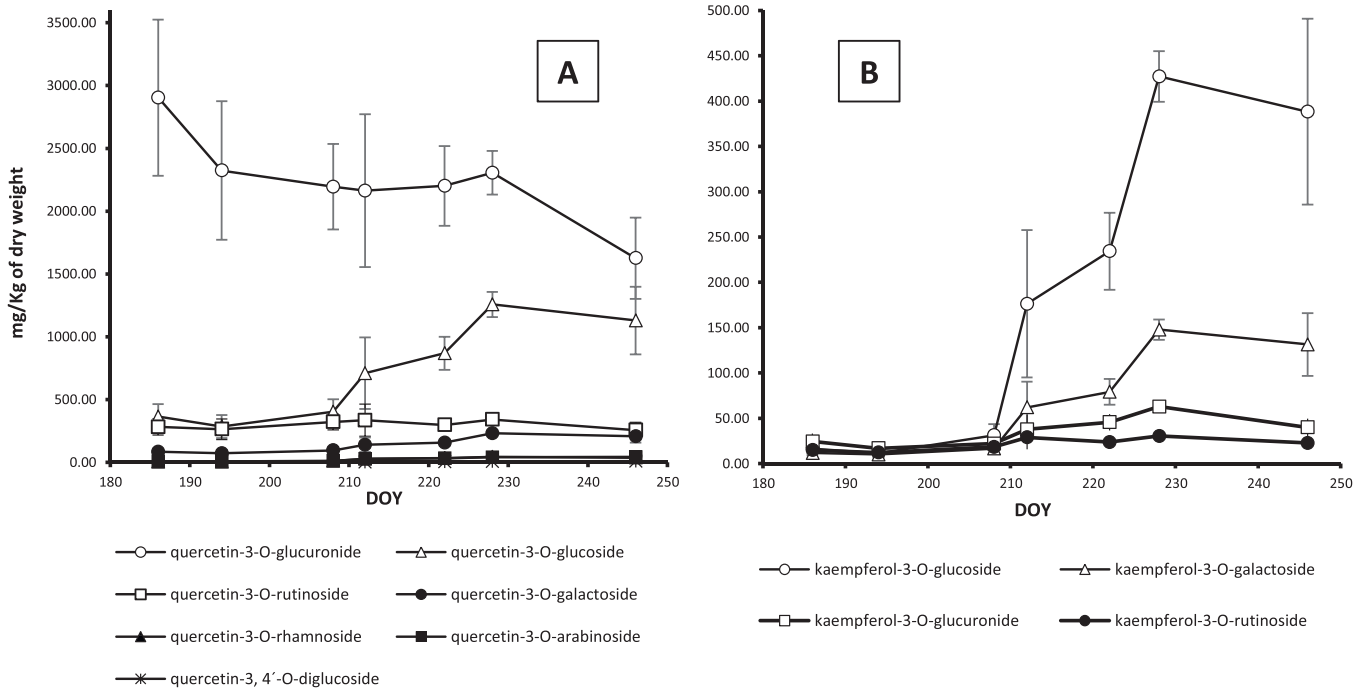

Fig. 2. Development of quercetin glycosides composition (A) and kaempferol glycosides composition (B) in 'Pinot Blanc' grapes skin during ripening, Each data point represents the mean ± standard error (mg/kg dry weight) of corresponding samples pool (number of samples as reported in Table 2). The time-lapse covers berry development period from bunch-closure (day of the year, DOY, 185) to ripeness (DOY 246).

dihydroflavonol glycosides in white grapes using UHPLC-MS/MS with both targeted and untargeted approaches (Dal Santo et al., 2016; present work).

Recently, the transcriptome analysis of key genes involved in the flavonoid biosynthetic pathway has provided new insights into flavonoid pattern variation in grape berries. Higher expression levels of key enzymes, including F3′5′H, anthocyanin O-methyltransferase and acyltransferase, occurred in red- and pink- compared to white-skinned varieties, indeed their expression largely varied among cultivars (Lu et al., 2021). This explains the high accumulation of anthocyanins and other non-anthocyanin flavonoids, such as delphinidin-like flavonols, in the skin of coloured varieties. Nevertheless, in the skin of Shine Muscat cv.

(*Vitis labruscana* × *V. vinifera*) colorless table grape the expression of F3′5′H was recently reported, even if at low levels (Lu et al., 2021). This agrees with the study of Bogs et al. (2006), who reported the expression of F3′5′H and VvCytoB5 to be extremely low in white grapes during ripening, even though both genes were expressed in Chardonnay prior to veraison. Consequently, the general statement that withe grape varieties are not able to produce delphinidin-like flavonols shall be revised according to the integrative analyses of metabolome and transcriptome profiles specific for each grape cultivar.

By looking at the parentage atlas of grapevine variety, the Pinot Noir is genetically connected to the red-skinned variety Dureza (D'Onofrio et al., 2021; Vouillamoz and Grando, 2006). Dureza represents an ancient ancestor recurring in several pedigrees of central Europe varieties. In this regards, the sibship cluster of the white cultivar Chasselas is also related to Dureza, hence a common ancestor could be the possible explanation for the myricetin production recorded in both Chasselas (Ferrandino et al., 2012) and Pinot Blanc (present study). Interestingly, a compound putatively identified as myricetin derivate was reported in the Italian founder white skinned Garganega variety (Dal Santo et al., 2016), suggesting the hypothesis that ancient varieties subjected to a lower selective pression retain the production of delphinidin-like flavonols. It has to be considered that Pinot Blanc directly arose from Pinot Noir, red-skinned variety, by the deletion of a region encompassing both the VvMybA1 and VvMybA2 functional genes, encoding transcriptional factors regulating the anthocyanin biosynthesis in *V. vinifera* grapes (Vezzulli et al., 2012; Azuma, 2018). Interestingly, a direct correlation between the MYB haplotype (combination of functional or not functional Myb alleles) and the F3'5'H/F3'H expression ratio has been recently reported (Azuma, 2018). Hence, the specific flavonoid pattern of Pinot blanc can be explained by taking in consideration the somatic mutation together with a corresponding differentially modulated expression of key genes regulating the flavanol biosynthetic pathway, such as a possible down regulation in the expression of F3′5′H gene.

Dihydroflavonols (that is astilbin) showed total amount between total quercetins and kaempferols (Fig. 1). Some authors previously reported relatively considerable amounts of dihydroquercetins in the skin of particular white grape cultivar compared to others, and used this as a tool for differentiation, with astilbin being the most expressed compound of this class (Masa et al., 2007). Our efforts unveiled the presence of several derivatives beside astilbin, which was apparently the most abundant and the only available as reference compound in our investigation for quantitative assay, but further investigation is needed to elucidate their structure and content. The complexity of dihydroquercetin derivatives composition and their high relative content, compared to the other flavonoid classes, suggested that astilbin and other components could be highly effective as marker compounds for Pinot Blanc characterization and identification.

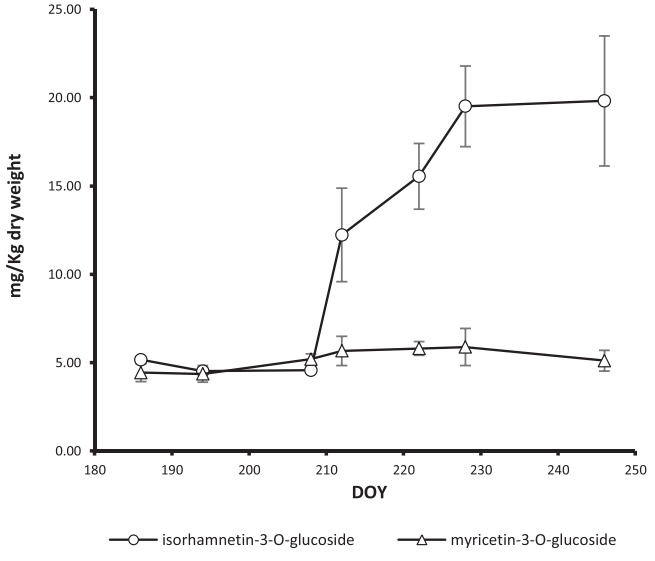

Fig. 3. Development of isorhamnetin and myricetin glycosides composition in 'Pinot Blanc' grapes skin during ripening, Each data point represents the mean ± standard error (mg/kg dry weight) of corresponding samples pool (number of samples as reported in Table 2). The time-lapse covers berry development period from bunch-closure (day of the year, DOY, 185) to ripeness (DOY 246).

3.4.2. Hydroxycinnamoil-tartrate and phenolic acids profile

Both *trans* and *cis* isomers of hydroxycinnamoil-tartrate have been described in red and white grapes, with *trans* isomers being majoritary over corresponding *cis* isomer. *Cis* isomers of both caftaric and coutaric acid were not available as reference compounds in our investigation but were determined on the basis of their retention time, since they had already been reported to possess slightly lower Rt compared to their *trans* isomers (Lu et al., 1999). Sufficient separation was obtained only for coutaric acid isomers, so that *cis*- isomer content was calculated as equivalents of its *trans*- isomers and reported separately, while caftaric acid appeared as a double ("shouldered") peak in chromatograms and its content is thus reported as sum of isomers. Only *p*-hydroxycinnamoil derivatives are considered.

Caftaric acid was the most abundant phenolic acid in Pinot Blanc grape skin at all time points, followed by *trans*-coutaric and *cis*-coutaric in order (Fig. 4A). These showed similar seasonal trend, with highest levels after veraison and lowest at harvest.

Caftaric/coutaric ratio (both as sum) ranged between 1.09 and 1.33, while *trans/cis* isomers ratio for coutaric acid ranged between 1.56 and 2.39 along ripening. This result is coherent with what previously reported for 'Pinot Blanc' whole berry extracts (Vrovsek et al., 1998). Caftaric acid was already reported to be the most abundant phenolic acid constituent in 'Pinot Blanc' grape juice (Singleton et al., 1986).

When berry skins from different cultivars were screened for their hydroxycinnamoil tartate content at technological maturity (Ferrandino et al., 2012), the ratio between the sum of coutaric acids and trans-caftaric acid was > 1 for most coloured varieties (23 over 27) but not for most white cultivars (only 2 over 7). Moreover, *trans/cis* ratio for coumaroyl derivatives ranged between 3.6 and 9.4 approximately. Montealegre et al. (2006) also reported higher amount for caftaric acids in most white grape skins examined (4 over 6), these having total coumaric/total caftaric ratio > 2 and average *trans/cis* ratio for coutaric acids comprised between 2.0 and 3.1. From another investigation, most red grapes skins examined showed higher content of coutaric acids than caftaric acid, with higher ratio for trans isomers (Falchi et al., 2006). White varieties showed higher differentiation than coloured ones in their tartaric acid esters composition after PCA analysis (Ferrandino et al., 2012).

In this work 'Pinot Blanc' showed higher amount for total caftarics compared to total coutaric, in line with most other white cultivars reported so far, but their ratio at harvest resulted lower than values in the cited literature and the same is for *trans/cis* isomers ratio for coutaric acid. These indicate a peculiar hydroxycinnamoil tartaric esters composition for Pinot Blanc grape and possible use of their ratios as molecular markers and for differentiation.

Except for gallic acid, whose levels were above LOQ in all samples yet lower than indicated for most other varieties, peaks from all the other benzoic acids were not quantitable at all along the whole ripening period investigated. The same thing is also valid for free hydroxycinnamic acids considered (p-coumaric, caffeic and ferulic). Despite mass interface conditions were purposely not optimized for detection of phenolic acid derivatives, as evinced by LOQ values obtained for different phenolic groups (Table 1), free phenolic acid amount seem to be negligible in this cultivar.

3.4.3. Catechins and proanthocyanidins

Procyanidin B1 resulted the compound with the far highest content

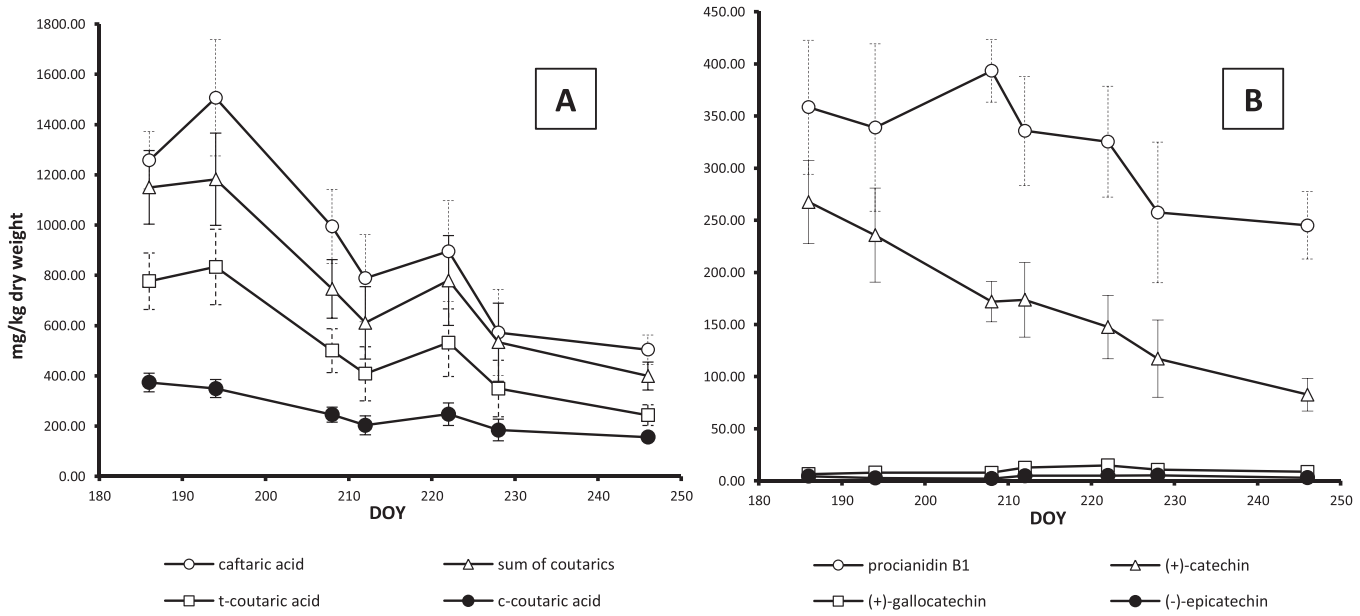

Fig. 4. Development of hydroxycinnamoil-tartrate composition (A) and flavan-3-ols composition (B) in 'Pinot Blanc' grapes skin during ripening, Each data point represents the mean ± standard error (mg/kg dry weight) of corresponding samples pool (number of samples as reported in Table 2). The time-lapse covers berry development period from bunch-closure (day of the year, DOY, 185) to ripeness (DOY 246).

among flavan-3-ols along whole ripening, retained constant level along the ripening period and slightly decreased near technological maturity (Fig. 4B). It was followed by (+)-catechin, showing a clear diminishing trend along berry development. (+)-gallocatechin and (-)-epicatechin have much lower content with almost constant value along ripening. This order of abundance is coherent with flavan-3-ols monomers and oligomers content reported so far: dimers are often reported as major constituents, and (+)-catechin is usually more expressed than its isomer (-)-epicatechin (3- to 10- fold approx.) (Montealegre et al., 2006; Escribano-Bailón et al., 1995). Ratio between (+)-catechin and (-)-epicatechin content appears particularly low from our results.

Procyanidin B2 amount resulted between LOD and LOQ in most samples. Previous investigations on flavan-3-ols monomeric/oligomeric composition in white grapes described occurrence of different dimeric structures belonging to B-type (single 4–8 or 4–6 interflavanic bond), with B3 and B4 among the most abundant (Montealegre et al., 2006; Jara-Palacios et al., 2014). Analyses were conducted in parallel with SRM transition for B-type procyanidins ($m/z = 579.1$) active throughout the run time, and no signals other than those belonging to procyanidin B1-B2 appeared in the chromatogram (data not shown).

As mentioned for quercetin-3-*O*-glucuronide, (+)-catechin also exhibited activity against *Botrytis Cynerea* through inhibition of the pathogen stilbene oxidase activity from the same investigation (Goetz et al., 1999). The diminishing trend appreciated for both phenolic groups would be coherent with conjugated strategy for protecting the grape berries between bloom and veraison.

4. Conclusions

A reversed-phase rapid-separation UHPLC-MS method was developed for the characterization of the phenolic fraction of dried grape skin, with particular emphasis on flavonols glycosides. This method was validated and allowed the quantitation of 16 flavonols/dihydroflavonols (with full resolution of structural −3-*O*-hexosides isomers), 4 flavan-3-ols and 4 phenolic acids in dried grape skin extracts of 'Pinot Blanc' from South Tyrol along the berry development period, from pre-veraison to ripeness. Together with precursor ion scan (PIS) and neutral loss scan (NLS) for unavailable reference compounds, the method also provided putative implications of enzymatic activities underlying corresponding biosynthesis. To our knowledge this is the first comprehensive phenolic profile characterization of 'Pinot Blanc' grape skin.

Interesting aspects aroused from our investigation. Flavonol glycosides were the most abundant group of polyphenolics at all stages. Quercetin glycosides were majoritarian and showed high ratio of quercetin-3-*O*-rutinoside and different other minor derivatives. The occurrence of isorhamnetin glycosides as minor compounds at every stage and a large expression of dihydroquercetins were reported. Besides, myricetin derivatives were screened and their presence featuring different sugar moieties was confirmed. Such evidence prevent from strict assessment that F3',5'H is not expressed in white grape varieties. Expression of 3-*O*-rutinoside moiety was common to all four flavonol backbones investigated and to dihydroquercetin. Moreover, ratio for hydroxycinnamoil tartrates and flavan-3-ols content at harvest could be a further distinctive trait to be used for cultivar differentiation as reported for other cultivars.

Thanks to these evidences, a certain grade of peculiarity of 'Pinot Blanc' grape skin phenolic composition in comparison to other white grape varieties can be prefigured. This could help the definition of a molecular fingerprint for taxonomical identification and authenticity of products susceptible of adulteration. Moreover, the need for quali-quantitative analysis of both major and minor compounds from different phenolic structures seem to be mandatory for the correct assessment of phenolic profiles of grape cultivars, the establishment of exact metabolic activity and to enhance statistical differentiation between cultivars. However, further investigations through direct comparison with other cultivars and after different sample preparation procedures would be helpful for confirmation of data trueness and real statistical differentiation.

CRediT authorship contribution statement

Enrico Serni: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Selena Tomada:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing – review & editing. **Peter Robatscher:** Funding acquisition, Project administration, Resources, Methodology, Supervision, Writing – review & editing. **Florian Haas:** Funding

acquisition, Project administration, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2022.104731.

References

Adams, D.O., 2006. Phenolics and ripening in grape berries. Am. J. Enol. Vitic. 57 (3), 249–256.

Agati, G., D'Onofrio, C., Ducci, E., Cuzzola, A., Remorini, D., Tuccio, L., Lazzini, F., Mattii, G., 2013. Potential of a multiparametric optical sensor for determining in situ the maturity components of red and white Vitis vinifera wine grapes. J. Agric. Food Chem. 61 (50), 12211–12218.

Anonymous, 2020. Vini Alto Adige. Pinot Bianco (2020). ⟨https://www.vinialtoadige.com/it/vitigni/pinotbianco/22–12228.html⟩ (Accessed 29 November 2020).

(G) arcia-Beneytez, E., Revilla, E., Cabello, F., 2002. Anthocyanin pattern of several red grape cultivars and wines made from them. Eur. Food Res. Technol. 1 (215), 32–37.

Azuma, A., 2018. Genetic and environmental impacts on the biosynthesis of anthocyanins in grapes. Hortic. J. 87, 1–17.

B. Magnusson , U. Örnemark (Eds.), 2014. Eurachem Guide: the Fitness for Purpose of Analytical Methods – a Laboratory Guide to Method Validation and Related Topics, second ed., ISBN 978-91-87461-59-0. ⟨http://www.eurachem.org⟩.

Bachteler, K., Riedel, M., Tisch, C., Merkt, N., Amann, R., Wünsche, J.N., 2013. Influence of berry shrivel on concentrations of resveratrol, catechin, epicatechin and ε-viniferin in berries of Vitis vinifera L. cultivars' Zweigelt','Pinot Blanc' and 'Pinot Gris'. Eur. J. Hortic. Sci. 78 (2), 49–55.

Bogs, J., Ebadi, A., McDavid, D., Robinson, S.P., 2006. Identification of the flavonoid hydroxylases from grapevine and their regulation during fruit development. Plant Physiol. 140 (1), 279–291.

Boss, P.K., Davies, C., Robinson, S.P., 1996. Expression of anthocyanin biosynthesis pathway genes in red and white grapes. Plant Mol. Biol. 32 (3), 565–569.

Boulton, R., 2001. The copigmentation of anthocyanins and its role in the color of red wine: a critical review. Am. J. Enol. Vitic. 52 (2), 67–87.

Braidot, E., Zancani, M., Petrussa, E., Peresson, C., Bertolini, A., Patui, S., Vianello, A., 2008. Transport and accumulation of flavonoids in grapevine (Vitis vinifera L.). Plant Signal. Behav. 3 (9), 626–632.

Cantos, E., Espin, J.C., Tomás-Barberán, F.A., 2002. Varietal differences among the polyphenol profiles of seven table grape cultivars studied by LC−DAD−MS−MS. J. Agric. Food Chem. 50 (20), 5691–5696.

Carreño, J., Almela, L., Martínez, A., Fernández-López, J.A., 1997. Chemotaxonomical classification of red table grapes based on anthocyanin profile and external colour. LWT-Food Sci. Technol. 30 (3), 259–265.

Castillo-Muñoz, N., Gómez-Alonso, S., García-Romero, E., Hermosín-Gutiérrez, I., 2007. Flavonol profiles of Vitis vinifera red grapes and their single-cultivar wines. J. Agric. Food Chem. 55 (3), 992–1002.

Castillo-Muñoz, N., Gómez-Alonso, S., García-Romero, E., Hermosín-Gutiérrez, I., 2010. Flavonol profiles of Vitis vinifera white grape cultivars. J. Food Compos. Anal. 23 (7), 699–705.

Ceci, A.T., Bassi, M., Guerra, W., Oberhuber, M., Robatscher, P., Mattivi, F., Franceschi, P., 2021. Metabolomic characterization of commercial, old, and red-fleshed apple varieties. Metabolites 11 (6), 378. https://doi.org/10.3390/metabo11060378.

Cerovic, Z.G., Moise, N., Agati, G., Latouche, G., Ghozlen, N.B., Meyer, S., 2008. New portable optical sensors for the assessment of winegrape phenolic maturity based on berry fluorescence. J. Food Compos. Anal. 21 (8), 650–654.

Cheynier, V., Comte, G., Davies, K.M., Lattanzio, V., Martens, S., 2013. Plant phenolics: recent advances on their biosynthesis, genetics, and ecophysiology. Plant Physiol. Biochem. 72, 1–20.

Colombo, R.C., Roberto, S.R., da Cruz, M.A., de Carvalho, D.U., Yamamoto, L.Y., Nixdorf, S.L., Hermosín-Gutiérrez, I., 2021. Characterization of the phenolic ripening development of 'BRS Vitoria'seedless table grapes using HPLC–DAD–ESI-MS/MS. J. Food Compos. Anal. 95, 103693.

Corte-Real, J., Archaimbault, A., Schleeh, T., Cocco, E., Herrmann, M., Guignard, C., Hausman, J.-F., Iken, M., Legay, S., 2021. Handling wine pomace: the importance of drying to preserve phenolic profile and antioxidant capacity for product valorization. J. Food Sci. 86 (3), 892–900.

D'Onofrio, C., Tumino, G., Gardiman, M., Crespan, M., Bignami, C., De Palma, L., Barbagallo, M.G., Muganu, M., Morcia, C., Novello, V., Schneider, A., Terzi, V., 2021. Parentage atlas of italian grapevine varieties as inferred from SNP genotyping. Front. Plant Sci. 11, 2265.

Dal Santo, S., Fasoli, M., Negri, S., D'Incà, E., Vicenzi, N., Guzzo, F., Tornielli, G.B., Pezzotti, M., Zenoni, S., 2016. Plasticity of the berry ripening program in a white grape variety. Front. Plant Sci. 7, 970.

Dimitrovska, M., Bocevska, M., Dimitrovski, D., Murkovic, M., 2011. Anthocyanin composition of Vranec, Cabernet Sauvignon, Merlot and Pinot Noir grapes as indicator of their varietal differentiation. Eur. Food Res. Technol. 232 (4), 591–600.

Downey, M.O., Dokoozlian, N.K., Krstic, M.P., 2006. Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. Am. J. Enol. Vitic. 57 (3), 257–268.

Duthie, G.G., Gardner, P.T., Kyle, J.A., 2003. Plant polyphenols: are they the new magic bullet? Proc. Nutr. Soc. 62 (3), 599–603.

Escribano-Bailón, M.T., Guerra, M.T., Rivas-Gonzalo, J.C., Santos-Buelga, C., 1995. Proanthocyanidins in skins from different grape varieties. Z. Lebensm. Unters. Forsch. 200 (3), 221–224.

Falchi, M., Bertelli, A., Lo Scalzo, R., Morassut, M., Morelli, R., Das, S., Cui, J., Das, D.K., 2006. Comparison of cardioprotective abilities between the flesh and skin of grapes. J. Agric. Food Chem. 54 (18), 6613–6622.

Ferrandino, A., Carra, A., Rolle, L., Schneider, A., Schubert, A., 2012. Profiling of hydroxycinnamoyl tartrates and acylated anthocyanins in the skin of 34 Vitis vinifera genotypes. J. Agric. Food Chem. 60 (19), 4931–4945.

Ferrandino, A., Pagliarani, C., Carlomagno, A., Novello, V., Schubert, A., Agati, G., 2017. Improved fluorescence-based evaluation of flavonoid in red and white winegrape cultivars. Aust. J. Grape Wine Res. 23 (2), 207–214.

Ferreira, V., Fernandes, F., Pinto-Carnide, O., Valentão, P., Falco, V., Martín, J.P., Ortiz, J.M., Arroyo-Garcia, R., Andrade, P.B., Castro, I., 2016. Identification of Vitis vinifera L. grape berry skin color mutants and polyphenolic profile. Food Chem. 194, 117–127.

Ferreira, V., Fernandes, F., Carrasco, D., Hernandez, M. G., Pinto-Carnide, O., Arroyo-García, R., . & Castro, I. (2017). Spontaneous variation regarding grape berry skin color: A comprehensive study of berry development by means of biochemical and molecular markers. Food Research International, 97, 149-161.

Ferrer-Gallego, R., Brás, N.F., García-Estévez, I., Mateus, N., Rivas-Gonzalo, J.C., de Freitas, V., Escribano-Bailón, M.T., 2016. Effect of flavonols on wine astringency and their interaction with human saliva. Food Chem. 209, 358–364.

Figueiredo-González, M., Martínez-Carballo, E., Cancho-Grande, B., Santiago, J.L., Martínez, M.C., Simal-Gándara, J., 2012. Pattern recognition of three Vitis vinifera L. red grapes varieties based on anthocyanin and flavonol profiles, with correlations between their biosynthesis pathways. Food Chem. 130 (1), 9–19.

Flamini, R., Mattivi, F., Rosso, M.D., Arapitsas, P., Bavaresco, L., 2013. Advanced knowledge of three important classes of grape phenolics: anthocyanins, stilbenes and flavonols. Int. J. Mol. Sci. 14 (10), 19651–19669.

Fontana, A.R., Antoniolli, A., Bottini, R., 2013. Grape pomace as a sustainable source of bioactive compounds: extraction, characterization, and biotechnological applications of phenolics. J. Agric. Food Chem. 61 (38), 8987–9003.

Fournier-Level, A., Lacombe, T., Le Cunff, L., Boursiquot, J.M., This, P., 2010. Evolution of the VvMybA gene family, the major determinant of berry colour in cultivated grapevine (Vitis vinifera L.). Heredity 104 (4), 351–362.

Friedel, M., Stoll, M., Patz, C.D., Will, F., Dietrich, H., 2015. Impact of light exposure on fruit composition of white 'Riesling' grape berries (Vitis vinifera L.). VITIS J. Grapevine Res. 54 (3), 107–116.

Garrido, J., Borges, F., 2013. Wine and grape polyphenols—a chemical perspective. Food Res. Int. 54 (2), 1844–1858.

Goetz, G., Fkyerat, A., Métais, N., Kunz, M., Tabacchi, R., Pezet, R., Pont, V., 1999. Resistance factors to grey mould in grape berries: identification of some phenolics inhibitors of Botrytis cinerea stilbene oxidase. Phytochemistry 52 (5), 759–767.

Heras-Roger, J., Díaz-Romero, C., Darias-Martín, J., 2016. What gives a wine its strong red color? Main correlations affecting copigmentation. J. Agric. Food Chem. 64 (34), 6567–6574.

Jaitz, L., Siegl, K., Eder, R., Rak, G., Abranko, L., Koellensperger, G., Hann, S., 2010. LC–MS/MS analysis of phenols for classification of red wine according to geographic origin, grape variety and vintage. Food Chem. 122 (1), 366–372.

Jara-Palacios, M.J., Hernanz, D., González-Manzano, S., Santos-Buelga, C., Escudero-Gilete, M.L., Heredia, F.J., 2014. Detailed phenolic composition of white grape by-products by RRLC/MS and measurement of the antioxidant activity. Talanta 125, 51–57.

Joslyn, M.A., Dittmar, H.F.K., 1967. The proanthocyanidins of Pinot blanc grapes. Am. J. Enol. Vitic. 18 (1), 1–10.

Kallithraka, S., Bakker, J., Clifford, M.N., 1997. Evaluation of bitterness and astringency of (+)-catechin and (-)-epicatechin in red wine and in model solution. J. Sens. Stud. 12 (1), 25–37.

Lee, C.Y., Jaworski, A., 1989. Major phenolic compounds in ripening white grapes. Am. J. Enol. Vitic. 40 (1), 43–46.

Lenk, S., Buschmann, C., Pfündel, E.E., 2007. In vivo assessing flavonols in white grape berries (Vitis vinifera L. cv. Pinot Blanc) of different degrees of ripeness using chlorophyll fluorescence imaging. Funct. Plant Biol. 34 (12), 1092–1104.
Liang, N.N., He, F., Bi, H.Q., Duan, C.Q., Reeves, M.J., Wang, J., 2012. Evolution of flavonols in berry skins of different grape cultivars during ripening and a comparison of two vintages. Eur. Food Res. Technol. 235 (6), 1187–1197.
Lu, S., Wang, J., Zhuge, Y., Zhang, M., Liu, C., Jia, H., Fang, J., 2021. Integrative analyses of metabolomes and transcriptomes provide insights into flavonoid variation in grape berries. J. Agric. Food Chem. 69 (41), 12354–12367.
Lu, Y., Foo, L.Y., 1999. The polyphenol constituents of grape pomace. Food Chem. 65 (1), 1–8.
Ma, W., Guo, A., Zhang, Y., Wang, H., Liu, Y., Li, H., 2014. A review on astringency and bitterness perception of tannins in wine. Trends Food Sci. Technol. 40 (1), 6–19.
Masa, A., Vilanova, M., Pomar, F., 2007. Varietal differences among the flavonoid profiles of white grape cultivars studied by high-performance liquid chromatography. J. Chromatogr. A 1164 (1–2), 291–297.
Mattivi, F., Guzzon, R., Vrhovsek, U., Stefanini, M., Velasco, R., 2006. Metabolite profiling of grape: flavonols and anthocyanins. J. Agric. Food Chem. 54 (20), 7692–7702.
Maul, E., Töpfer, R. , 2015. Vitis International Variety Catalogue (VIVC): a cultivar database referenced by genetic profiles and morphology. In BIO Web Conferences 5, EDP Sciences.
Montealegre, R.R., Peces, R.R., Vozmediano, J.C., Gascueña, J.M., Romero, E.G., 2006. Phenolic compounds in skins and seeds of ten grape Vitis vinifera varieties grown in a warm climate. J. Food Compos. Anal. 19 (6–7), 687–693.
Niu, S., Hao, F., Mo, H., Jiang, J., Wang, H., Liu, C., Zhang, Y., 2017. Phenol profiles and antioxidant properties of white skinned grapes and their coloured genotypes during growth. Biotechnol. Biotechnol. Equip. 31 (1), 58–67.
OIV , 2017. OIV FOCUS 2017 - Distribution of the World's Grapevine Varieties, International Organization of Vine and Wine, Paris, France.
Pandey, K.B., Rizvi, S.I., 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxid. Med. Cell. Longev. 2 (5), 270–278.
Pedri, U., Pertoll, G., 2013. Influence of different locations on grape and wine quality with the grapevine variety 'Pinot blanc'. *Mitteilungen Klosterneuburg, Rebe und Wein.* Obstbau Früchteverwertung 63 (4), 173–186.
Perestrelo, R., Lu, Y., Santos, S.A., Silvestre, A.J., Neto, C.P., Câmara, J.S., Rocha, S.M., 2012. Phenolic profile of Sercial and Tinta Negra Vitis vinifera L. grape skins by HPLC–DAD–ESI-MS^n: Novel phenolic compounds in Vitis vinifera L.grape. Food Chem. 135 (1), 94–104.
Philipp, C., Eder, P., Brandes, W., Regner, F., Patzl-Fischerleitner, E., Eder, R. , 2017. Characterization of the pear-aroma profile and its impact on the quality and typicity of Austrian Pinot blanc wines, In: Proceedings of the BIO Web of Conferences 9, EDP Sciences.
Philipp, C., Sari, S., Eder, P., Patzl-Fischerleitner, E., Eder, R. , 2019. Austrian Pinot blanc wines: Typicity, wine styles and the influence of different oenological decisions on the volatile profile of wines. In: Proceedings of the BIO Web of Conferences 15, EDP Sciences.
Robinson, J., Harding, J., Vouillamoz, J. , 2013. Wine Grapes: a Complete Guide to 1,368 Vine Varieties, Including their Origins and Flavours, Penguin, UK.
Schultz, H.R., Stoll, M. , 2015. Deutsches Weinbaujahrbuch 2016, Verlag Eugen Ulmer.
Serni, E., Venir, E., Romano, G., Guerra, W., Robatscher, P., 2020a. Determination of major phenolics content in dried apples from three new cultivars (Malus domestica Borkh.) using HPLC-UV-FL with pentafluorophenyl stationary phase. Food Anal. Methods 1–9.
Serni, E., Pedri, U., Valls, J., Sanoll, C., Dordevic, N., Überegger, E., Robatscher, P., 2020b. Chemical description and organoleptic evaluation of Pinot noir wines from different parts of Italy: a three-year investigation. OENO One 54 (2), 393–410.
Shalaby, S., Horwitz, B.A., 2015. Plant phenolic compounds and oxidative stress: integrated signals in fungal–plant interactions. Curr. Genet. 61 (3), 347–357.
Sharma, A., Shahzad, B., Rehman, A., Bhardwaj, R., Landi, M., Zheng, B., 2019. Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. Molecules 24 (13), 2452.
Singleton, V.L., 1988. Wine phenols. In: Linskens, H.F., Jackson, J.F. (Eds.), Wine Analysis. Springer-Verlag, Berlin, Germany, pp. 173–218.
Singleton, V.L., Noble, A.C., 1976. Wine flavor and phenolic substances. In: Charalambous, G., Katz, I. (Eds.), Phenolic, Sulfur, and Nitrogen Compounds in Food Flavors. American Chemical Society, Chicago, pp. 47–70.
Singleton, V.L., Zaya, J., Trousdale, E.K., 1986. Caftaric and coutaric acids in fruit of Vitis. Phytochemistry 25 (9), 2127–2133.
Somers, T.C., Verétte, E., 1988. Phenolic composition of natural wine types. In: Linskens, H.F., Jackson, J.F. (Eds.), Wine Analysis. Springer-Verlag, Berlin, Germany, pp. 219–257.
Teixeira, A., Eiras-Dias, J., Castellarin, S.D., Gerós, H., 2013. Berry phenolics of grapevine under challenging environments. Int. J. Mol. Sci. 14 (9), 18711–18739.
Terrier, N., Poncet-Legrand, C., Cheynier, V., 2009. Flavanols, flavonols and dihydroflavonols. In: Wine Chemistry and Biochemistry. Springer, New York, NY, pp. 463–507.
Tomaz, I., Huzanić, N., Preiner, D., Stupić, D., Andabaka, Ž., Maletić, E., Kontic, J.K., Ašperger, D., 2019. Extraction methods of polyphenol from grapes: extractions of grape polyphenols. In: Polyphenols in Plants. Academic Press, pp. 151–167.
Treutter, D., 2005. Significance of flavonoids in plant resistance and enhancement of their biosynthesis. Plant Biol. 7 (6), 581–591.
Valls, J., Agnolet, S., Haas, F., Struffi, I., Ciesa, F., Robatscher, P., Oberhuber, M., 2017. Valorization of Lagrein grape pomace as a source of phenolic compounds: analysis of the contents of anthocyanins, flavanols and antioxidant activity. Eur. Food Res. Technol. 243 (12), 2211–2224.
Vezzulli, S., Leonardelli, L., Malossini, U., Stefanini, M., Velasco, R., Moser, C., 2012. Pinot blanc and Pinot gris arose as independent somatic mutations of Pinot noir. J. Exp. Bot. 63 (18), 6359–6369.
Vouillamoz, J.F., Grando, M.S., 2006. Genealogy of wine grape cultivars: 'Pinot' is related to 'Syrah'. Heredity 97 (2), 102–110.
Vrhovšek, U., 1998. Extraction of hydroxycinnamoyltartaric acids from berries of different grape varieties. J. Agric. Food Chem. 46 (10), 4203–4208.
Zhang, M.X., Liu, C.H., Nan, H.J., Li, Z., 2015. Phenolic compound profiles in skins of white wine and table grape cultivars grown in the national grape germplasm resource nursery of China. South Afr. J. Enol. Vitic. 36 (1), 154–164.