DOI: https://doi.org/10.20870/oeno-one.2026.60.3.9887

OPEN ACCESS

ENOLOGY ORIGINAL RESEARCH ARTICLES

Comprehensive secondary metabolite analysis of underexplored Uruguayan red grape varieties: implications for oenological potential

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Article number: 9887

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Associate editor:
Luca Rolle

Received:
03 February 2026

Accepted:
22 June 2026

Published:
23 July 2026

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ABSTRACT

In Uruguayan wines elaborated from *Vitis vinifera* cv Tannat are the most known, being representative of the country wines between international consumers. Nevertheless, work is being carried out to improve quality to obtain premium wines, through blends using less frequent grape varieties being introduced and employed in wine production looking for sensory particularities.

The aim of this study was to determine, by means of HPLC-DAD and HPLC-MS (Orbitrap), the polyphenolic profiles of four red *Vitis vinifera* L. grape varieties cultivated in small vineyards in southern Uruguay (Montevideo and Canelones Provinces) in the 2022–2023 vintages. The selected varieties were: Ancellotta, Aspiran Bouschet (Aramon × Teinturier du Cher × Aspiran, in Uruguay syn. Lacryma Christi), Egiodola (Abouriou × Tinta Negra Mole), and Caladoc (Malbec × Grenache).

To our knowledge, only few previous studies have been focused on the volatile and polyphenolic profiles for these new varieties planted, even when they are cultivated by their color contribution to wines. While the study of free volatile compounds offers valuable insights, directing attention towards glycosylated compounds is imperative for a comprehensive grasp of how aromas evolve in wine and for refining the ultimate sensory characteristics of the product.

KEYWORDS: Ancellotta, Aspiran Bouschet, Egiodola, Caladoc, polyphenols, volatiles, characterization

INTRODUCTION

The grapevine (*Vitis vinifera*) comprises species currently distributed in almost all the continents, usually under subtropical, Mediterranean, and continental-temperate climatic conditions (This *et al.*, 2006). However, and for different reasons (mainly pathogenic problems and climate conditions), the main cultivars used for grape production on a worldwide basis are changing rapidly. Particularly with the preference for labelling wines by cultivar instead of by region of origin, the result implies the existence of numerous cultivars dedicated to the wine industry. However, there are cultivars of grapes that have been specially selected as they perform best for quality wines production.

Wine arrived in Uruguay during the second half of the 19th century, thanks to immigrant families who brought their knowledge from the Mediterranean. Around 1870, two vineyards were established on Uruguayan soil: that of the French Basque Pascual Harriague in Salto Province (Northwest of Uruguay), and the farm of the Catalan Francisco Vidiella in Montevideo Province (South of Uruguay). With the experience of these pioneers as a standard, plus the work of many others who followed them, the wine industry took root in Uruguay and achieved a growth that managed to replace a good part of the foreign production.

At present Uruguay vineyards count almost 5,900 hectares (INAVI, 2024), being fundamentally a country of small producers where a high percentage of the establishments have been family properties for more than three generations. The annual wine production was 66 million liters, produced in 204 wineries (INAVI, 2024). The evolution of the vineyards in the last twenty years shows a marked decrease in surface and number of plants, 42 and 49 % respectively, and an increase of around 40 % in production, because of higher productivity (INAVI, 2024).

Among the Uruguayan wines, those elaborated from *Vitis vinifera* cv Tannat are the most known, being this varietal representative of the country wines between international consumers (Carrau *et al.*, 2011). Nevertheless, work is being carried out to improve quality to obtain premium wines, through blends using less frequent grape varieties being introduced and employed in wine production looking for sensory particularities.

The aim of this study was to determine, by means of HPLC-DAD and HPLC-MS (Orbitrap), the polyphenolic profiles of four red *Vitis vinifera* L. grape varieties cultivated in small vineyards in southern Uruguay (Montevideo and Canelones Provinces) in the 2022–2023 vintages. The selected varieties were: Ancellotta, Aspiran Bouschet (Aramon × Teinturier du Cher × Aspiran, in Uruguay syn. Lacryma Christi), Egiodola (Abouriou × Tinta Negra Mole) and Caladoc (Malbec × Grenache).

To our knowledge, only few previous studies have been focused on the volatile and polyphenolic profiles for these new varieties planted, even when they are cultivated by their color contribution to wines. In addition, most of the studies were performed on the wines produced (Alcalde-Eon *et al.*, 2006; Landrault *et al.*, 2001; Teissedre & Landrault, 2000). However, to evaluate the color expression on grapes, the study of the corresponding wines, even monovarietal ones, should not substitute the study of the polyphenolic profile in the corresponding grapes (He *et al.*, 2012). In the few studies published about this topic, analysis of phenolic compounds in red grape varieties of *V. vinifera* Caladoc and Marselan were reported but focused to oligomer and polimeric forms and their potential biologic activities (Liu *et al.*, 2010; Liang *et al.*, 2012; Shi *et al.*, 2016; Somkuwar *et al.*, 2018). Besides, grape seeds play a crucial role in winemaking due to the phenolic compounds they contain, impacting the style, characteristics, and aging potential of the resulting wines justifying study their profile for a complete *Vitis* variety characterization (Gutiérrez-Escobar *et al.*, 2021).

Similarly, exploring glycosylated volatile compounds in grapes is essential, as these compounds play a relevant role in shaping the aroma and flavor profile of wines (Ganss *et al.*, 2011).

A nuanced understanding of glycosylated volatile compounds empowers winemakers to unravel the latent aromas within grapes before fermentation. By meticulously identifying and analysing these glycosides, winemakers can anticipate the potential aromas that might emerge during the winemaking and aging processes. This insight enables them to make informed decisions regarding processing techniques, fermentation conditions, and aging methods, all of which contribute to enhancing the final wine's aromatic complexity and overall quality.

In brief, free forms are directly involved in the floral bouquet of grapes, whereas bound forms are non-volatile compounds that do not directly contribute to the aroma. These latter are constituted by an aglycone conjugated to a glycosyl group, like glucose, arabinose, rhamnose, or apiose (Domon & Costello, 1988; Fang *et al.*, 2002). Aglycones are mostly terpenols and terpenic polyols but can also be C6-alcohols, C13-norisoprenoids, benzenic compounds, and others (Ferreira & Lopez, 2019).

While the study of free volatile compounds offers valuable insights, directing attention towards glycosylated compounds is imperative for a comprehensive grasp of how aromas evolve in wine and for refining the ultimate sensory characteristics of the product.

MATERIALS AND METHODS

1. Chemicals and reagents

Methanol anhydrous (MeOH; 99.8 %), perchloric acid (70 %), and malvidin 3-β-D-glucopyranoside (> 90 %) were supplied by Sigma Aldrich. Acetonitrile (ACN; LC-MS grade, 99.9 %) and formic acid (MS grade, 98 %) were purchased from Fluka (St. Louis, MO, USA). Deionised water was produced using an Arium®Pro Lab Water System (Sartorius AG, Goettingen, Germany). Mass calibration

solution (Pierce® ESI Negative Ion Calibration Solution) was supplied by Thermo Fischer Scientific Inc. (Waltham, MA, USA). Standards used for the identification of free targeted phenolic compounds were prepared as reported by Barnaba and colleagues (Barnaba *et al.*, 2015).

2. Grape samples

In this study 4 red grape varieties (*Vitis vinifera* L.) cultivated in Southern Uruguay were considered: Ancellotta, Aspiran Bouschet (Aramon × Teinturier du Cher × Aspiran, in Uruguay syn. Lacryma Christi), Egiodola (Abouriou × Tinta Negra Mole), and Caladoc (Malbec × Grenache).

Grape samples were collected randomly from commercial vineyards in Las Violetas (Canelones Province; 34°31′22″ S 56°16′40″ W, 20 m.a.s.l.) at the final stage of ripening (February–March) in good sanitary conditions during the 2021 and 2022 harvests. According to the INIA (Instituto Nacional de Investigación Agropecuaria) weather station, the average monthly temperature and rainfall registered during the period in 2021 and 2022 was 16.9 ± 2 °C/30–80 mm and 19.8 ± 2.8 °C/50–150 mm respectively. Each sample represents 10 samples by each variety.

Soon after harvesting, grapes samples were transported to the laboratory, and their oenological parameters were determined according to OIV (2023) (Table 1).

TABLE 1. Oenological parameters, mean values ± standard deviation, for grapes collected in 2021 and 2022 vintages.

Grape variety	pH	Total Soluble Solids (° Brix)	Titratable acidity (g/L tartaric acid)
Ancellotta	3.80 ± 0.01	19.24 ± 2.69	6.61 ± 0.08
Lacryma Christi	4.03 ± 0.01	20.30 ± 3.02	3.50 ± 0.03
Egiodola	4.21 ± 0.01	22.70 ± 2.89	3.20 ± 0.02
Caladoc	3.46 ± 0.01	22.30 ± 3.01	5.35 ± 0.06

Besides, for each grape variety grape clusters were quickly frozen at –20 °C and conserved at this temperature until analysed for volatile and phenolic compounds.

3. Analytical methods

3.1. Identification and quantification of volatile compounds

Extraction of aroma compounds was performed using adsorption and separate elution from an Isolute (Biotage, Charlotte, NC) ENV+ cartridge packed with 1 g highly cross-linked styrene-divinyl benzene (SDVB) polymer following the experimental conditions of Dellacassa *et al.* (2017). In brief, the cartridges were sequentially conditioned with methanol (15 mL) and distilled water (20 mL). A sample of 50 mL of homogenised grape berries diluted with 50 mL of distilled water and containing 0.1 mL of internal standard (1-heptanol at 230 ppm in a 50 % hydroalcoholic solution) was eluted through the cartridge by using a syringe (4–5 mL/min), and the residual washed with 15 mL of distilled water. The "free" aroma components were eluted with 30 mL of dichloromethane, then the solution was dried with Na_2SO_4, concentrated to 1.5 mL on a Vigreux column and stored at −10 °C. Immediately before GC analysis, the solution was further concentrated to 100 µL under a gentle nitrogen stream.

The bound forms were eluted with 30 mL of methanol, and this solution was evaporated to dryness by rotary evaporation, then dissolved in 3 mL of citrate buffer at pH 5, added with Cytolase PCL5 (Gistbrocades, Lille Cedex, France), and reacted at 40 °C for 14 h. After the addition of the same internal standard (1-heptanol), the aglycons were extracted three times with 3 mL of pentane/dichloromethane (2:1, *v/v*). The organic phase was dried with Na_2SO_4, concentrated to 0.5 mL on a small Vigreux column, and further reduced to 100 µL before GC analysis.

The GC-MS analysis was performed as described previously (Giuffrida *et al.*, 2020) using a Shimadzu QP 2020 mass spectrometer equipped with equipped with a MEGA-WAX MS (30 m × 0.25 mm i.d., 0.25 µm film thickness (MEGA) capillary column and commercial and developed libraries (Mondello, 2015; Adams, 2007; McLafferty & Stauffer, 1991). The glycosylated volatile compounds were identified by comparison of their linear retention indices (LRIs; determined in relation to a homologous series of n-alkanes C9-C26) with those of pure standards or reported in the literature. Identities confirmed by comparing mass spectra and retention time with those of authentic standards and comparison of fragmentation patterns in the MS with those from standards or stored on the GC-MS data bases. For quantification purposes internal standard (1-heptanol at 230 ppm in a 50 % hydro alcoholic solution) was used for each analysis (Giuffrida *et al.*, 2020).

3.2. Identification and quantification of phenolic compounds

3.2.1. Sample extraction

To extract anthocyanins and polyphenols compounds, forty grape berries were weighted, the seeds and skins were separated manually, and pulps were discarded following the experimental procedure previously reported (García-Marino *et al.*, 2006; Boido *et al.*, 2011).

3.2.2. Polyphenol extraction from seeds

Extraction was performed following the method of García-Marino *et al.* (2006) with modifications. Briefly, 2 ± 0.1 g of previously lyophilized and ground seeds were macerated for 24 h with 20 mL of a methanol:water solution (75:25, *v/v*). The crude extract was filtered, and the seeds were washed with an additional 20 mL of the same solvent; this procedure was repeated twice. The combined fractions were vacuum filtered to obtain a final volume of 60 mL. The extract was then concentrated under reduced pressure to remove methanol, and the aqueous residue was adjusted to a final volume of 10 mL with ultrapure water (Milli-Q). All extractions were performed in duplicate for each sample.

3.2.3. Polyphenol extraction from skins

A total of 10 ± 0.1 g of grape skins were weighed and macerated for 24 h in the dark at 4 ± 1 °C with 100 mL of methanol acidified with 0.1 % HCl. The crude extract was filtered, and the skins were washed with an additional 100 mL of the same solvent; this procedure was repeated twice. The combined fractions were vacuum-filtered to obtain a final volume of 300 mL. The extract was then concentrated under reduced pressure to remove methanol, and the aqueous residue was adjusted to a final volume of 25 mL with ultrapure water (Milli-Q). All extractions were performed in duplicate for each sample.

3.2.4. Targeted analysis

The targeted analysis allowed identification and quantification anthocyanins in skin extracts. It was performed with an Agilent 1200 Series HPLC (Agilent Technologies, Santa Clara, CA, USA), using a Purospher® LiChroCart® RP18 (250 × 4 mm, 5 µm particle size, Merck Group, Darmstadt, Germany) furnished with a guard column (RP18, 20 × 2 mm, 5 µm) and setting the diode array detector (DAD) at 518 nm, as reported in the OIV method (OIV, 2007). The chromatographic separation was performed with perchloric acid (0.3 %, *v/v*)-MeOH, at a flow rate of 0.450 mL min^{-1} and at a temperature of 40 °C, slightly adapting the method proposed by Castia *et al.* (1992). The organic solvent gradient was set as follows: 0–51 min., linear increase from 17.4 % to 44.5 %; 51–64 min., linear increase to 67.5 %; 64–66 min., linear increase to 100 %; 66–69 min., isocratic elution at 100 %; 69.1–73 min., equilibration at 17.4 %. Ten µL of samples were injected.

3.2.5. Untargeted analysis

The untargeted analysis aimed at the tentative identification of free and bound phenols in both skin and seed extracts. The chromatographic separation was performed with a Thermo Ultimate™ 3000 HPLC (Thermo Scientific, Sunnyvale, CA, USA) on an AccucoreTM Polar Premium LC column (150 mm × 3 mm, 2.6 µm particle size; Thermo Fischer Scientific, Waltham, MA, USA) with H_2O-ACN, at a flow rate of 0.3 mL min^{-1} and at a temperature of 40 °C. A slow gradient was set for the organic solvent in order to maximize the chromatographic separation of potential isobaric compounds: 0–4 min, isocratic elution at 6 %; 4–8 min, linear increase to 7 %; 8–10 min, to 10 %; 10–14 min, at 10 %; 14–16 min, to 15 %; 16–24 min, at 15 %; 24–26 min, to 20 %; 26–30 min, to 25 %; 30–32 min, at 25 %; 32–36 min, to 35 %; 36–38 min, at 35 %; 38–42 min, to 50 %; 42–44 min, at 50 %; 44–48 min, to 100 %; 48–50 min, at 100 %; 50–51 min, to 6 %. The column was equilibrated at 6:94 (*v/v*) ACN-aqueous formic acid (0.1 %, *v/v*) from 51 to 55 min. Ten µL of samples were injected, helding the autosampler at 5 °C.

The Ultimate™ 3000 HPLC was coupled with a tandem mass spectrometer (Q-ExactiveTM; Thermo Scientific, Bremen, Germany) furnished with a heated electrospray source (HESI-II). The mass analysis was performed in negative ion mode, adapting the method proposed by Barnaba *et al.* (2017). Spectra were acquired through full MS-data dependent MS/MS experiment (full MS–dd MS/MS) and the mass resolving power was set at 140,000 full width at half-maximum (FWHM, calculated for *m/z* 200, 1.5 Hz) for full MS spectra and at 17,500 FWHM (12 Hz) for dd-MS2. Ions with an ionization response more intense than 1.1·105 were fragmented with stepped normalised collision energy (NCE: 25, 35, and 45 arbitrary units), repeating the dd-MS2 experiments only after 6 s from the previous one. The HESI source was set as follows: spray voltage, 2.80 kV; sheath gas flow rate at 30 arbitrary units; auxiliary gas flow rate at 20 arbitrary units; capillary temperature, at 310 °C; capillary gas heater temperature, 280 °C.

Thermo Scientific™ Dionex™ Chromeleon™ 7.2 Chromatography Data System (CDS) software was used for timing of chromatography and mass acquisition. Thermo Fisher Scientific Xcalibur and TraceFinderTM software (Thermo Scientific, Waltham, MA, USA) were used for data processing and evaluation.

4. Compound identification

As regards targeted analysis, identification of anthocyanins was based on their UV-VIS spectra (230–600 nm) as reported by Castia *et al.* (1992), while quantification was based on their response at 518 nm (OIV, 2007) expressing all compounds as malvidin malvidin-3-glucoside.

As regards untargeted analysis, free phenolic compounds were tentatively identified through the potential match of experimental high-resolution accurate mass (mass tolerance < 5 ppm, (SANCO, 2013)) and MS/MS fragmentation profile with those reported in literature (Barnara *et al.*, 2015), and of experimental isotope patterns with the theoretical one based on chemical formula. Phenolic compounds bound to sugar residues were tentatively identified through the potential matching of accurate mass (mass tolerance < 5 ppm (SANCO, 2013)) and experimental MS/MS spectra with those of glycosides just described in the literature for different matrices (Fang *et al.*, 2002; Kečkeš *et al.*, 2013; Qiu *et al.*, 2013, Alakolanga *et al.*, 2014; Amessis-Ouchemoukh *et al.*, 2014; Benayad *et al.*, 2014; Beelders *et al.*, 2014; Hjelmeland *et al.*, 2015). Furthermore, by detecting neutral loss of the sugar residue in the MS/MS spectrum (Aisporna *et al.*, 2022) and by comparing experimental isotope spacing and relative abundance to theoretical values based on chemical formulae, further identification evidence was obtained.

5. Statistical analysis

Statistical analysis was performed with Statistica 7.1 Software (StatSoft, 2005), using only normally distributed data and separately considering targeted and untargeted results for both skin and seed. In case of untargeted analysis, the test was performed using ionisation intensity expressed as the peak area, as adopted in a similar metabolome approach (Cuadros-Inostroza *et al.*, 2010), normalising the peak areas as relative areas (%) in comparison to the total ion ionisation intensity. Samples were randomly processed in a single analytical batch and one of them was repeated every 3 samples, confirming the narrow repeatability of the analytes' normalised area (R.S.D. always < 11 %).

RESULTS

1. Skin phenolic profiles characteristic for each variety

The targeted approach (Table 2) made it possible to quantify 9 anthocyanidin derivatives, 5 of which were anthocyanins (delphinidin-3-glucoside, DP; cyanidin-3-glucoside, CN; petunidin-3-glucoside, PT; peonidin-3-glucoside, N; malvidin-3-glucoside, MV) and 4 coumarylated anthocyanins (coumarylated delphinidin-3-glucoside, coum-DP; coumarylated cyanidin-3-glucoside, coum-CN; coumarylated peonidin-3-glucoside, coum-PN; coumarylated malvidin-3-glucoside, coum-MV), allowing to define the skin characteristic profile for each variety.

As regards quantification, external solvent calibration curve for malvidin-3-glucoside was used, with the R2 value higher than 0.99, the linearity range of 3 order of magnitude (0.2–200 mg mL^{-1}) and the acceptable recovery ranging from 80 % to 120 % for each variety.

Table 2 summarised the content of each compound for all grape varieties under study. Ancellota and Lachryma Christi were the varieties with the highest content of anthocyanidin derivatives and, among these, malvidin-3-glucoside was the most abundant, followed by delphinidin-3-glucoside, petunidin-3-glucoside, and coumarylated malvidin-3-glucoside.

The untargeted approach (Table S1) allowed the detection of 66 phenolic compounds, of which 41 were tentatively identified in the form of hexose (32), deoxyhexose (7), hexose-pentose (1), and hexose-deoxyhexose (1), based on the neutral loss and the characteristic sugar ring fragmentations (Domon & Costello, 1988).

The compounds detected were classified as flavonols and their glycosylated derivatives (21), hydroxybenzoic acids and their glycosylated derivatives (11), hydroxycinnamic acids and their glycosylated derivatives (8), glycosylated flavanonols (7), flavan-3-ols and their glycosylated derivatives (5), alkylphenyl alcohols and their glycosylated derivatives (4), glycosylated simple phenol (1), glycosylated flavanones (2), hydroxybenzoketones and derivatives (2), hydroxybenzaldehyde (1), hydroxycoumarin (1), glycosylated hydroxyphenylacetic acid (1), glycosylated flavone (1), and glycosylated hydroxybenzoether (1). Table 3 summarises accurate masses, retention times and fragments of phenolic compounds tentatively identified.

Compounds 1-10, 12-20, 22-25, 28, 30-33, 35-49, and 52-66 were found in at least one variety of skin and seed. Compounds 11, 13, 27, 29, 34, 50, and 51 were not found in skin extracts, while compounds 11, 21, and 26 were not detected in seed extracts.

2. Grape volatile profiles

Glycosylated volatile compounds are often precursors to more complex and diverse aromas which, during winemaking processes such as fermentation and aging, these compounds break down into their aromatic constituents, contributing to a broader spectrum of aromas and flavors in the resulting wine. In addition, glycosylated compounds can play a crucial role in the aging process of red wines by their gradual hydrolysis releasing components able to add complexity to the wine's aroma.

While both glycosylated and free volatiles contribute to the aromatic profile of red *Vitis* varieties, the delayed and nuanced expression of glycosidated compounds can be considered more important for the long-term sensory development and overall quality perception of these wines. Accordingly, this study aimed to analyse the profile of glycosylated volatile components found in the grapes of selected varieties. The goal was to assess their potential significance in winemaking, whether for varietal wines production or for blending with traditional *Vitis* varieties.

The lack of previous studies addressing the aromatic profile of the *Vitis vinifera* L. varieties investigated in this work provides additional academic interest and originality to the present study. Table 3 presents the most relevant volatile aglycones extracted and identified in grapes from samples collected during the 2022–2023 harvest, together with their concentration ranges (µg/kg).

TABLE 2. Targeted analysis for the identification and quantification of anthocyanins in skin extracts.

Anthocyanins	Ancellotta	Egiodola	Lacryma Christi	Caladoc
		Skin (mg/kg)		
DP	740,0 ± 74,0	18,5 ± 4,6	171,1 ± 42,7	34,4 ± 8,3
CN	142,0 ± 19,8	5,1 ± 0,5	23,5 ± 2,3	1,59 ± 0,3
PT	627,0 ± 150,4	29,2 ± 2,92	196,4 ± 19,6	53,7 ± 12,7
PN	385,0 ± 61,6	41,0 ± 4,1	251,0 ± 25,1	20,5 ± 5,4
MV	1389,0 ± 375,0	200,0 ± 20,3	1104,2 ± 110,4	563,0 ± 143.2
coum-DP	50,2 ± 9,0	5,2 ± 0,5	9,1 ± 0,9	12,5 ± 3,4
coum-CN	11,3 ± 2,2	3,1 ± 0,3	3,5 ± 0,3	< 0,2
coum-PN	39,3 ± 3,9	10,2 ± 1,0	16,9 ± 1,6	10,7 ± 2,7
coum-MV	143,0 ± 24,3	65,0 ± 13,5	101,1 ± 20,2	239,0 ± 56,4
	3526,8	**377,5**	**1875,5**	**935,4**

*DP, delphinidin; CN, cyanidin; PT, peonidin; MV, malvidin; *coum*-DP, Delphinidin-3-O-(6'-p-coumaroyl)glucoside; *coum*-CN, Cyanidin-3-O-(6'-p-coumaroyl)glucoside; *coum*-PN, Peonidin-3-O-(6'-p-coumaroyl)glucoside; *coum*-MV, Malvidin-3-O-(6'-p-coumaroyl)glucoside.

TABLE 3. Concentrations (micrograms per Liter equivalents of 1-heptanol) of glycosidically bound compounds liberated by enzymatic hydrolysis from the Vitis varieties grapes from 2022 and 2023 vintages [mean of three repetitions and standard deviation (SD) for 10 samples each year]. Part 1/2.

Compound	LRI[a]	Identity assignment[b]	Ancellota	Caladoc	Egiodola	Lacrima Christi
			Average content (µg/kg) ± SD			
C6 compounds						
Hexanal	1080	A	25,3 ± 2,5	12,3 ± 1,2	25.4 ± 2,3	11,3 ± 1,1
trans-2-Hexenal	1220	A	1,0 ± 0,1	2,6 ± 0,2	1,1 ± 0,1	2,0 ± 0,2
1-Hexanol	1368	A	163,4 ± 49,0	89,2 ±26,7	47,0 ± 14,1	85,6 ±25,7
cis-3-Hexen-1-ol	1382	A	5,8 ± 11,5	29,8 ± 59,6	16,6 ± 33,2	16,0 ± 32,1
trans-2-Hexen-1-ol	1410	B (1)	29,7 ± 14,8	5,2 ± 2,6	14,2 ± 7,1	33,6 ± 16,8
			225,31	**139,21**	**104,38**	**148,78**
Terpenes						
trans-Linalool oxide (furanoid)	1450	A	1,9 ± 0,3	1,8 ± 0,1	1,4 ± 0,1	0
cis-Linalool oxide (furanoid)	1473	A	4,2 ± 0,8	1,7 ± 0,3	1,3 ± 0,3	0
Citronellol	1773	A				2,4 ± 0,2
Nerol	0	2,5 ± 0,3	4,3 ± 0,4	12,2 ± 3,6	4,5 ± 1,3	16,6 ± 4,9
Geraniol	1855	A	32,9 ± 9,8	21,4 ± 6,4	28,8 ± 8,6	70,4 ± 21,1
α-Terpineol	1721	A	3,1 ± 0,4	3,4 ± 0,5	0	3,9 ± 0,5
trans-8-Hydroxylinalool	2272	B (1)	16,1 ± 3,2	7,8 ± 1,5	7,3 ± 1,5	16,0 ± 3,2
cis-8-Hydroxylinalool	2311	B (1)	64,4 ± 3,2	10,9 ± 1,1	8,6 ± 0,9	14,4 ± 1,4
			130,7	**62,1**	**56,6**	**124,0**
Norisoprenoids						
3-Hydroxy-β-damascone	2535	B (3)	14,2 ± 4,2	14,6 ± 4,3	11,4 ± 3,4	14,5 ± 4,3
3-Oxo-α-ionol	2653	B (4)	50,1 ± 5,0	13,3 ± 1,3	40,0 ± 4,0	27,5 ± 2,7
4-Oxo-β-ionol	2658	B (4)	70,8 ± 7,0	16,1 ± 1,6	17,0 ± 1,7	3,8 ± 0,3
3-Oxo-7,8-dihydro-α-ionol	2726	B (3)	18,8 ± 3,7	0	15,7 ± 3,1	0
Vomifoliol	3167	B (2)	74,2 ± 22,2	110,9 ± 33,2	115,6 ± 34,7	40,2 ± 12,0
7,8-Dihydrovomifoliol	3263	B (4)	2,5 ± 0,2	1,2 ± 0,1	4,2 ± 0,4	0
			230,6	**156,21**	**204,09**	**86,1**
Shikimate derivates						
Benzaldehyde	1534	A	0,7 ± 0,1	1,7 ± 0,2	0,9 ± 0,1	0
Methyl salicylate	1770	A	1,3 ± 0,1	3,6 ± 0,3	3,6 ± 0,3	0
Guaiacol	1873	A	14,7 ± 5,8	2,6 ± 1,0	4,6 ± 1,8	24,7 ± 9,8
Benzyl alcohol	1882	A	177,4 ± 53,2	225,6 ± 67,6	158,1 ± 47,5	140,7 ± 42,2
2-Phenylethanol	1918	A	108,8 ± 21,7	97,0 ± 19,4	132,1 ± 26,4	135,0 ± 27,0
p-Cresol	2089	A	2,07 ± 0,2	0,9 ± 0,1	1,7 ± 0,1	1,6 ± 0,1
m-Cresol	2140	A	0,67 ± 0,1	0	1,1 ± 0,1	0,7 ± 0,1
4-Vinylguaiacol	2180	A	171,6 ± 34,3	29,9 ± 5,9	55,6 ± 11,1	74,1 ± 14,8
2,6-Dimethoxyphenol	2273	A	108,4 ± 13,0	23,6 ± 2,8	49,2 ± 5,9	151,6 ± 18,1
4-Vinyphenol	2377					
Vanillin	2560	A	0	2,4 ± 0,2	5,3 ± 0,5	23,3 ± 2,3
Methyl vanillate	**2601**	**B (1)**	**3,8 ± 0,3**	**7,6 ± 0,7**	**4,0 ± 0,4**	**34,6 ± 3,4**

[a] Linear retention index based on a series of n-hydrocarbons reported according to their elution order on Carbowax 20M. [b] A, identities confirmed by comparing mass spectra and retention time with those of authentic standards supplied by Aldrich (Milwaukee, WI) and Fluka (Buchs, Switzerland). B, identities tentatively assigned by comparing mass spectra with those obtained from the literature [(1) Mondello, 2015; (2) Adams, 2001; (3) McLafferty & Stauffer, 1991. (4) Strauss *et al.*, 1987; (3) Winterhalter, 1990; (4) Marais *et al.*, 1992].

TABLE 3. Concentrations (micrograms per Liter equivalents of 1-heptanol) of glycosidically bound compounds liberated by enzymatic hydrolysis from the Vitis varieties grapes from 2022 and 2023 vintages [mean of three repetitions and standard deviation (SD) for 10 samples each year]. Part 2/2.

Compound	LRI[a]	Identity assignment[b]	Ancellota	Caladoc	Egiodola	Lacrima Christi
			Average content (µg/kg) ± SD			
Acetovanillone	2635	A	28,9 ± 3,8	7,9 ± 0,8	26,8 ± 2,6	20,2 ± 2,0
3,4-Dimethoxyphenol	2738	A	41,7 ± 4,1	26,3 ± 2,6	41,3 ± 4,1	32,1 ± 3,2
Zingerone	2786	A	21,5 ± 2,1	12,0 ± 1,2	25,1 ± 2,5	12,9 ± 1,2
3,4,5-Trimethoxybenzyl alcohol	2879	A	14,5 ± 1,4	6,3 ± 0,6	11,0 ± 1,1	20,8 ± 2,1
3,4,5-Trimethoxyphenol	3060	B (1)	84,6 ± 21,1	49,0 ± 12,2	88,5 ± 22,1	251,2 ±62,8
Homovanillic acid	3099	A	78,6 ± 15,7	79,1 ± 15,8	190,4 ± 38,1	47,0 ± 9,4
			134,8	**114,3**	**256,1**	**138,0**

[a] Linear retention index based on a series of n-hydrocarbons reported according to their elution order on Carbowax 20M. [b] A, identities confirmed by comparing mass spectra and retention time with those of authentic standards supplied by Aldrich (Milwaukee, WI) and Fluka (Buchs, Switzerland). B, identities tentatively assigned by comparing mass spectra with those obtained from the literature [(1) Mondello, 2015; (2) Adams, 2001; (3) McLafferty & Stauffer, 1991. (4) Strauss *et al.*, 1987; (3) Winterhalter, 1990; (4) Marais *et al.*, 1992].

The presence of monoterpenols was highlighted, particularly the furanic and pyranic forms of linalool oxides, as well as α-terpineol, nerol, and geraniol, all of which are key contributors to grape aroma. A marked presence of C13-norisoprenoids was also observed, especially 3-hydroxy-β-damascenone, 3-oxo-α-ionol, 4-oxo-β-ionol, 3-oxo-7,8-dihydro-α-ionol, and vomifoliol. Among shikimate-derived compounds, the most relevant components – although present at low concentrations – were benzaldehyde, methyl salicylate, and vanillin. Within the group of volatile phenols, the presence of benzyl alcohol and β-phenylethyl alcohol was particularly noteworthy.

DISCUSSION

The present study provides a comprehensive characterization of the phenolic and glycosylated volatile profiles of four underexplored red grape varieties cultivated in Uruguay, contributing novel information to a field where data are still scarce. In contrast to previous studies, which have mainly focused on wines rather than grapes (Alcalde-Eon *et al.*, 2006; Landrault *et al.*, 2001), the present work allows a direct evaluation of varietal potential at the grape level.

The markedly higher anthocyanin content observed in Ancellotta and Lacryma Christi confirms their well-known role as teinturier or high-color-contributing varieties. In particular, the dominance of malvidin-3-glucoside, followed by delphinidin and petunidin derivatives, is consistent with previous reports for *Vitis vinifera* red cultivars (He *et al.*, 2012), where these compounds are associated with color stability and bluish-red hues in wines.

Compared to international varieties, the total anthocyanin content observed in Ancellotta is notably high, supporting its technological use in blends to enhance color intensity. The relatively high proportion of coumarylated anthocyanins, especially in Caladoc, is also relevant, as these forms are known to increase pigment stability through intramolecular co-pigmentation, potentially improving color persistence during aging. In contrast, Egiodola exhibited significantly lower anthocyanin concentrations, suggesting a more limited contribution to color but potentially different enological applications, such as blending for complexity rather than intensity.

The untargeted analysis revealed a wide diversity of phenolic compounds, particularly glycosylated flavonols and phenolic acids. This diversity is consistent with metabolomic studies in other grape varieties (Cuadros-Inostroza *et al.*, 2010), where phenolic complexity is associated with both antioxidant properties and sensory attributes such as bitterness and astringency. The differences observed between skin and seed profiles highlight the importance of extraction management during vinification, especially in varieties with distinct seed phenolic compositions.

One of the most relevant findings of this study is the clear varietal differentiation based on glycosylated volatile compounds. Although their concentrations were generally lower than those reported for Tannat, the relative distribution of compound classes (monoterpenes, norisoprenoids, and benzenoids) differed significantly among varieties. Monoterpenes such as geraniol, nerol, and linalool derivatives are known contributors to floral and citrus aromas, and their presence – particularly in Lacryma Christi – suggests a potential for enhanced aromatic expression compared to more neutral varieties. This is consistent with previous studies indicating that glycosidically bound terpenes represent an important reservoir of aroma compounds that can be released during fermentation (Ferreira & Lopez, 2019). The detection of C13-norisoprenoids, including vomifoliol and ionol derivatives, is also noteworthy, as these compounds are associated with complex aroma notes such as dried fruit, honey, and tobacco. Their presence across all varieties suggests a shared potential for contributing to wine aging bouquet, although at lower levels than Tannat. In contrast, the relatively high levels of benzenoid derivatives, such as benzyl alcohol and 2-phenylethanol, may contribute to sweet and floral nuances, particularly in Egiodola and Lacryma Christi.

The PCA results confirm that anthocyanin composition is a major driver of varietal differentiation, particularly through the contribution of delphinidin, petunidin, and cyanidin derivatives. However, when considering the broader phenolic and volatile datasets, a more complex separation emerges, reflecting the multifactorial nature of grape composition. Importantly, glycosylated volatile compounds appear as effective markers for distinguishing these varieties from Tannat, reinforcing their potential role in varietal fingerprinting and authenticity studies.

These results have oenological implications, among which we can highlight the following: Ancellotta and Lacryma Christi with high anthocyanin content makes them suitable for improving color intensity and stability in blends; Caladoc have balanced phenolic profile and relatively high proportion of acylated anthocyanins may contribute to both color stability and structural complexity; Egiodola have lower color intensity but distinctive volatile profile may enhance aromatic complexity in blends. The presence of diverse glycosylated aroma precursors across all varieties highlights their potential to contribute to wine aroma development during fermentation and aging, particularly when appropriate enzymatic or fermentation strategies are applied.

Overall, these findings support the idea that minor or underutilized grape varieties represent a valuable resource for diversifying wine styles in Uruguay. Their distinct phenolic and aromatic profiles may be strategically exploited to complement traditional varieties such as Tannat, contributing to innovation and differentiation in a highly competitive wine market.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the assistance Mega, Legnano, Italy, which made their work possible. We are grateful for the assistance of the winegrowers in the Las Violetas area, who provided us with samples of the grape varieties under study which made this work possible. The authors would also like to thank the CSIC and ANII agencies for their financial support.

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