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# Pilot-plant scale extraction of phenolic compounds from grape canes: Comprehensive characterization by LC-ESI-LTQ-Orbitrap-MS

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ABSTRACT

Grape canes, also named vine shoots, are well-known viticultural byproducts containing high levels of phenolic compounds, which are associated with a broad range of health benefits. In this work, grape canes (*Vitis vinifera* cv. Pinot noir) were extracted in a 750 L pilot-plant reactor under the following conditions: temperature 80 °C, time 100 min, solid/liquid ratio 1:10. The comprehensive characterization of grape cane phenolic compounds was performed by liquid chromatography coupled to high-resolution/accurate mass measurement LTQ-Orbitrap mass spectrometry. A total of 44 compounds were identified and, 26 of them also quantified, consisting of phenolic acids and aldehydes (17), flavonoids (12), and stilbenoids (15). The most abundant class of phenolics were stilbenoids, among which (*E*)-ε-viniferin predominated. The phenolic profile of grape canes obtained using pilot plant extraction differed significantly from the results of laboratory-scale studies obtained previously. Additionally, we observed a high antioxidant capacity of grape cane pilot-plant extract measured by the radical antioxidant scavenging potential ($ABTS^{\bullet+}$) (2209 ± 125 μmol TE/g DW) and oxygen radical absorbance capacity using fluorescein (ORAC-FL) (4612 ± 155 μmol TE/g DW). Grape cane pilot-plant extract for their phenolic profile may be used as a by-product for the development of novel nutraceutical and pharmaceutical products, improving the value and the sustainability of these residues.

## 1. Introduction

Grape canes from *Vitis vinifera* L. are an abundant viticultural byproduct generated after grapevine pruning. Their production is estimated to range from 2 to 4 tons per hectare of vineyard (Dávila, Gullón, Luis Alonso, Labidi, & Gullón, 2018). According to data from the International Organization of Vine and Wine (OIV), 7.4 million hectares are dedicated to vineyard cultivation in the world (OIV, 2020). Consequently, approximately 14.8–29.6 million tons of grape canes are generated each year worldwide. Grape canes, which are usually burned or incorporated into the vineyard soil, are considered to be an undervalued residue. From the viewpoint of integrated biorefinery and circular economy, they are potentially a high-value resource due to their attractive chemical composition and possible industrial applications. For instance, grape canes can be used as a food ingredient, based on a commercial product called Vineatrol®30, developed and manufactured by Actichem (Montauban, France).

As well as biopolymers such as lignin, cellulose, and hemicellulose, grape canes contain phytochemicals, mainly polyphenols (Riquelme et al., 2019). A procedure to recover the polymeric fraction from vine shoots, including hemicellulosic oligosaccharides, lignin fragments, and cellulosic substrates, has been proposed by Dávila et al. (2017). In the same direction, biopolymers such as cellulose, hemicellulose, lignin, and polyflavonoids from diverse woody resources have been extensively

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studied as bio-based materials with novel applications (Díaz-Galindo et al., 2020; García et al., 2018; García, Gavino, Escobar, & Cancino, 2017; Peredo et al., 2015). Grape canes, however, have been studied principally for the recovery of phenolic compounds (Moreira et al., 2018; Zwingelstein, Draye, Besombes, Piot, & Chatel, 2020).

Grape canes have heterogeneous phenolic profiles, including phenolic acids, flavanols, flavonols, flavanonols, flavanones, and stilbenoids, the most abundant being flavanols and oligomeric stilbenes (Escobar-Avello et al., 2019). Many of these phenolic compounds have promising biological activities. Besides their antimicrobial, antioxidant, anti-cancer, and cardio-protective properties, tannins seem to exert beneficial effects on metabolic disorders and protect against many oxidative stress-related diseases (Smeriglio, Barreca, Bellocco, & Trombetta, 2017). Stilbenes, particularly resveratrol, have a wide range of benefits for human health, such as antioxidant, anti-inflammatory and anti-cancer properties (Ramírez-Garza et al., 2018). Stilbenes can undergo polymerization by oxidative coupling of two to eight units of monomers and these derivatives may exhibit higher activity, stability, and selectivity than resveratrol (Xue et al., 2014).

The stilbene profile of grape canes strongly depends on the cultivar (Lambert et al., 2013), geographic growing area (Vergara et al., 2012), and duration of post-pruning storage (Gorena et al., 2014). Pinot noir canes in particular contain high levels of resveratrol and viniferin (Lambert et al., 2013; Vergara et al., 2012), which can increase five-fold after three months of post-pruning storage (Gorena et al., 2014). The yields of phenolic compounds may also be influenced by extraction methods and specific experimental conditions, such as the particle size of raw material, temperature, extraction time, and solvent composition. A recent review article summarizes the extraction methods and the influence of extraction parameters on polyphenol recovery from grape canes (Zwingelstein et al., 2020).

Over the last decade, the most investigated method to recover polyphenols from grape canes has been conventional extraction using mixtures of aqueous ethanol as the solvent. Emerging techniques, such as ultrasound-assisted (Piñeiro, Marrufo-Curtido, Serrano, & Palma, 2016), microwave-assisted (Moreira et al., 2018), or pressurized low polarity water extraction (Turgut, Feyissa, Baltacıoğlu, Küçüköner, & Karacabey, 2020), are being studied to enhance resveratrol and viniferin recovery from grape canes. However, although less time-consuming, these new methods are not necessarily more efficient than the conventional approaches (Zwingelstein et al., 2020). Their application in the industrial field is still in its early days and much more research needs to be done. On the other hand, few studies have been performed on a bench-, pilot-plant scale, or above for grape canes using mixtures of ethanol and water (Dorosh et al., 2020; Riquelme et al., 2019).

The ultimate objective of the study was to gain new knowledge about scaling up the extraction process of phenolic compounds from grape canes and thus facilitate their industrial application in the fields of food, cosmetics, biomaterials, and other bio-based products. Consequently, a comprehensive identification of polyphenols in grape cane extracts produced in a pilot-plant reactor, using liquid chromatography hybrid linear ion trap quadrupole-Orbitrap-mass spectrometry (LC-LTQ-Orbitrap-MS) analysis was performed. Additionally, the levels of phenolic compounds were quantified, and the reactions involved in the extraction process are discussed.

## 2. Materials and methods

### 2.1. Chemicals

Light exposure was avoided when manipulating the standards. Gallic, 4-hydroxybenzoic, and ellagic acids, catechin, epicatechin, (*E*)-resveratrol, (*E*)-ε-viniferin, eriodictyol, taxifolin, quercetin-3-*O*-glucoside, and quercetin-3-*O*-glucuronide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gallic acid ethyl ester (ethylgallate) and kaempferol-3-*O*-glucoside were acquired from Extrasynthèse (Genay, France).

HPLC grade acetonitrile, formic acid, ethanol, and water were purchased from Merck (Darmstadt, Germany). Ultrapure water was generated by a Milli-Q water purification system (Millipore Bedford, MA, USA). Potable ethanol (96°) from molasses employed for pilot plant scale extraction was purchased from Oxiquim S.A. (Coronel, Chile).

### 2.2. Grape canes: Collection and preparation

Grape canes (*V. vinifera* L. cv. Pinot noir) were collected from vines in an organic vineyard at Viña De Neira, located in Ránquil, Itata Valley, Biobio region, in Southern Chile (36°36′50.33″ S, 72°39′40.63″ W at 279 m of altitude).

After pruning, all samples were cut in 30–50 cm pieces and stored for 3 months at room temperature (19 °C ± 5) and 30–70% relative humidity, in accordance with a Chilean patent (Riquelme et al., 2019). From a total of 500 kg wet sample, 67 kg of dry grape canes were used for the pilot plant extraction. Before extraction, the grape canes were chopped in a Retsch grinder (model SM) at 300–2000 rpm until particle size was below 1 cm.

### 2.3. Pilot plant scale extraction

Grape cane extraction was performed on a pilot-plant scale at 80 °C for 100 min, based on a previous bench-scale optimization study (Riquelme et al., 2019). Extractions were carried out in a 750 L stainless-steel reactor using ethanol/water solution (80:20 v/v). Fig. 1 shows the pilot plant extraction process.

After extraction, the solvent was evaporated under vacuum (absolute pressure 0.05 bar) to remove and recover the ethanol through a distillation process. The extract was then spray-dried using a BHS Büttner-Schilde-Haas AG dryer, and stored in the dark at 4 °C.

The whole extraction yield was 5.45 (w/w) calculated as % grams of extract/gram of dry grape canes.

### 2.4. Phenolic profile by LC-LTQ-Orbitrap-MS

Identification and quantification of phenolic compounds in the pilot plant extract was performed by liquid chromatography analysis coupled to high resolution mass spectrometry. An Accela chromatograph (Thermo Scientific, Hemel Hempstead, UK) equipped with a photodiode array detector (PDA), a quaternary pump, and a thermostated autosampler was employed. Chromatographic separation was carried out on an Atlantis T3 Column 2.1 × 100 mm, 3 μm (Waters, Milford, MA, USA). Gradient elution was performed with water/0.1% formic acid (solvent A) and acetonitrile (solvent B) at a constant flow rate of 350 μL/min, and the injection volume was 10 μL. The gradient employed was 0 min, 2% B; 0–2 min, 8% B; 2–12 min, 20% B; 12–13 min, 30% B; 13–14 min, 100% B; 14–17 min, 100% B; and 17–18 min, 2% B. The column was equilibrated for 5 min returning to initial conditions.

For accurate mass measurements, the LC system was coupled to an LTQ-Orbitrap Velos mass spectrometer (Thermo Scientific, Hemel Hempstead, UK), equipped with an electrospray ionization source. The mass spectrometer was operated in the negative mode, and particular parameters were as follows: source voltage, 3 kV; sheath gas, 50 a.u. (arbitrary units); auxiliary gas, 20 a.u.; sweep gas, 2 a.u.; and capillary temperature, 375 °C. Pilot plant extracts were investigated in FTMS mode at a resolving power of 30,000 (FWHM at $m/z$ 400) and data-dependent MS/MS events collected at a resolving power of 15,000. The most intense ions detected in the FTMS spectrum were selected for the data-dependent scan. Parent ions were fragmented by high-energy collisional dissociation with normalized collision energy of 35% and an activation time of 10 ms. The mass range in FTMS mode was from $m/z$ 100 to 1500. LC-LTQ-Orbitrap-MS parameters were adapted from a previous study with some modifications (Escobar-Avello et al., 2019). The data analyses and instrument control were performed with Xcalibur

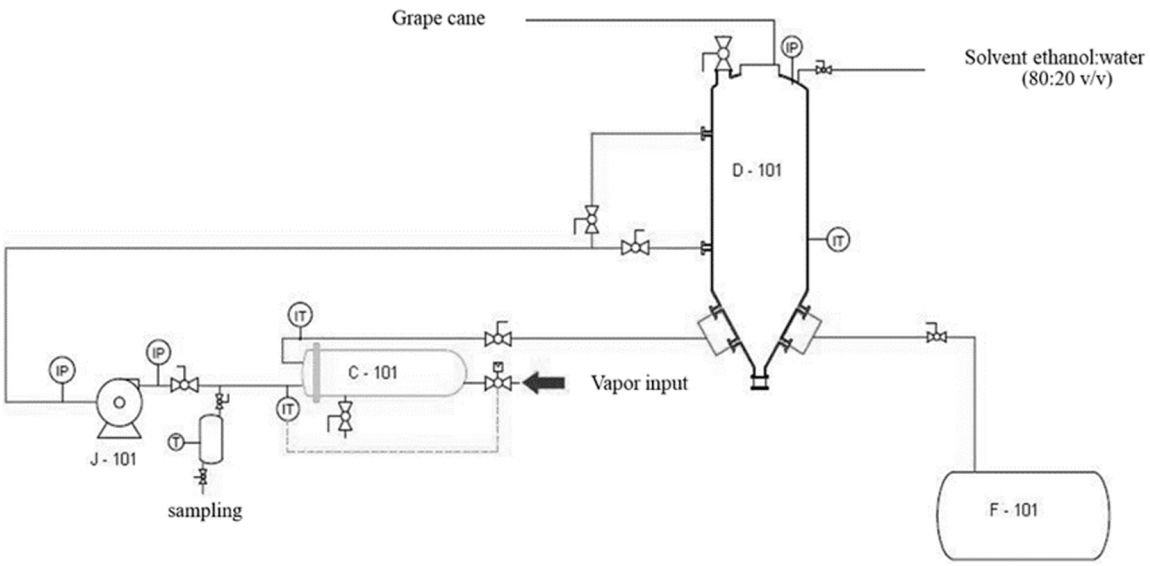


**Fig. 1.** Flowchart of grape cane extraction process in a pilot plant designed by the AutoCAD P&ID (v. 64 bits) program. Principal equipment: D-101 steel reactor (750 L); C-101 heat exchanger; J-101 Pump; F-101 steel tank.

3.0 software (Thermo Fisher Scientific).

Individual compounds were semi-quantified using pure standards when available or the most similar compound. Some analytes, such as glycosylated forms, dimers, or oligomers, were semi-quantified using the aglycon form or the monomer (Heras et al., 2016). The analytical parameters of the calibration curves are shown in Table 1.

### 2.5. Antioxidant capacity assay

Antioxidant capacity of grape cane pilot-plant extract was performed, according to the method reported by Sáez et al. (2018a). The analysis was completed in microplates of 96-well- using a microplate reader (Synergy/HTX multi-mode reader, BioTeK, Winooski, Vermont, USA)-. An $ABTS^{\bullet+}$ (2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) solution (7.5 mM) with $K_2S_2O_8$ (2.5 mM) was diluted with ethanol to obtain an absorbance of 0.7 at 734 nm. Into each well, 190 μL of diluted $ABTS^{\bullet+}$ radical solution was added. After incubation at 5 min and 30 °C, the first read was performed at 734 nm. The sample or Trolox calibration point (10 μL) were added, then the well-plate was incubated for 20 min at 30 °C. The assay of oxygen radical absorbance capacity using fluorescein (ORAC-FL)- was conducted according to the method reported by Ou et al. (2013). The calibration curves were prepared with Trolox, and results reported as μmol Trolox equivalents (TE)/g DW. All assays were performed in triplicate and protected from light.

## 3. Results and discussion

### 3.1. Identification of phenolic compounds

Table 2 shows the **44** phenolic compounds detected in the grape cane extract produced under pilot plant conditions and identified through LC-LTQ-Orbitrap-MS. The main classes of phenolic compounds identified were phenolic acids and aldehydes (**17**), flavonoids (**12**), and stilbenes (**15**). Several of these compounds have been previously identified in grape canes (Escobar-Avello et al., 2019). However, to the best of our knowledge, this is the first time that the phenolic profile of grape canes extracted at pilot plant level has been comprehensively characterized by high-resolution mass spectrometry.

#### 3.1.1. Phenolic acids and aldehydes

Phenolic acids were the most diverse class of phenolic compounds found in the grape cane extracts. Monogalloyl-glucose (*m/z* 331.0668), gallic acid (*m/z* 169.0141), protocatechuic acid-*O*-hexoside isomer 1 and 2 (*m/z* 315.0719, $t_R$. 7.39 min; *m/z* 315.0718, $t_R$. 8.52 min), protocatechuic acid (*m/z* 153.0192), syringic acid hexoside (*m/z* 359.0981), hydroxybenzoyl hexoside (*m/z* 299.0770), 4-hydroxybenzoic acid (*m/z* 137.0243), ellagic acid hexoside (*m/z* 463.0518), and gallic acid ethyl ester (*m/z* 197.0453) were identified by the typical loss of $CO_2$ $[M - H—44]^-$. Phenolic acid hexosides also showed the characteristic cleavage of their hexose moiety $[M - H—162]^-$. Additionally, gallic acid, 4-hydroxybenzoic acid, and gallic acid ethyl ester were identified by comparison with their corresponding pure standards.

Two hydroxycinnamic acids were tentatively identified based on their accurate mass measurements and $MS^2$ fragments. Caftaric acid (*m/z* 311.0406) showed ions at *m/z* 179.0342 (caffeic acid) and 149.0085 (tartaric acid), owing to the loss of a tartaric acid moiety (132 Da) and the presence of a tartaric acid molecule, respectively. Coutaric acid (*m/z* 295.0457) was tentatively identified based on the loss of the tartaric acid (132 Da) and the presence of an ion at *m/z* 163.0391, due to the presence of a coumaric acid molecule.

Ellagic acid (*m/z* 300.9986) and ellagic acid pentoside (*m/z* 433.0410) were also identified. Ellagic acid produced three fragment

**Table 1**
Analytical parameters for phenolic compound quantification using HPLC–MS/MS.

| Compound | $t_R$ (min) | Accurate mass | Calibration curve | $R^2$ | LOD (mg/L) | LOQ (mg/L) |
|---|---|---|---|---|---|---|
| Gallic Acid | 4.29 | 169.0141 | y = 353688 + 717410x | 0.9999 | 0.215 | 0.652 |
| Epicatechin | 13.36 | 289.0714 | y = 1458160 + 1764540x | 0.9880 | 0.864 | 2.620 |
| Taxifolin | 17.03 | 303.0506 | y = 172319 + 259345x | 0.9980 | 0.523 | 1.584 |
| Eriodictyol | 21.07 | 287.0555 | y = 1162620 + 1484100x | 0.9819 | 1.111 | 3.368 |
| Resveratrol | 20.28 | 227.0709 | y = 6536890 + 2896920x | 0.9991 | 0.617 | 1.870 |
| (*E*)-ε-viniferin | 21.69 | 453.1336 | y = 2470030 + 1252110x | 0.9892 | 1.781 | 5.396 |

$t_R$., retention times; $R^2$, correlation coefficients; LOD, limit of detection; LOQ, limit of quantification.

**Table 2**
Identification of phenolic compounds in the grape cane pilot plant extract using LC-ESI-LTQ-Orbitrap-MS in negative mode.

| Compounds | $t_R$ (min) | Accurate mass | Theoretical Mass | Error (ppm) | MS/MS ions (% intensity) | Molecular Formula |
|---|---|---|---|---|---|---|
| **PHENOLIC ACIDS AND ALDEHYDES** | | | | | | |
| Monogalloyl-glucose | 3.63 | 331.0668 | 331.0671 | −0.87 | 169.0134(100), 125.0237(10.12) | $C_{13}H_{16}O_{10}$ |
| Gallic acid* | 4.29 | 169.0141 | 169.0142 | −1.04 | 125.0239(100) | $C_7H_6O_5$ |
| Protocatechuic acid-*O*-hexoside (1) | 7.39 | 315.0719 | 315.0722 | −0.67 | 153.0186(100), 109.0290(11.78) | $C_{13}H_{16}O_9$ |
| Protocatechuic acid | 7.67 | 153.0192 | 153.0193 | −0.97 | 109.0290(100) | $C_7H_6O_4$ |
| Protocatechuic acid-*O*-hexoside (2) | 8.52 | 315.0718 | 315.0722 | −1.01 | 153.0186(100), 109.0290(8.15) | $C_{13}H_{16}O_9$ |
| Syringic acid hexoside | 8.76 | 359.0981 | 359.0984 | −0.63 | 197.0446(100) | $C_{15}H_{20}O_{10}$ |
| Caftaric acid | 9.27 | 311.0406 | 311.0409 | −0.88 | 149.0085(100), 179.0342(41.55) | $C_{13}H_{12}O_9$ |
| Protocatechuic aldehyde | 9.47 | 137.0242 | 137.0244 | −1.33 | 93.0343(100), 109.0291(81.93) | $C_7H_6O_3$ |
| Hydroxybenzoyl hexoside | 9.95 | 299.0770 | 299.0772 | −0.73 | 137.0237(100) | $C_{13}H_{16}O_8$ |
| 4-Hydroxybenzoic acid* | 10.04 | 137.0243 | 137.0244 | −1.03 | 93.0340(100) | $C_7H_6O_3$ |
| Coutaric acid | 11.17 | 295.0457 | 295.0459 | −0.95 | 163.0391(100) | $C_{13}H_{12}O_8$ |
| Hydroxybenzaldehyde | 11.75 | 121.0294 | 121.0295 | −1.13 | 93.0296(100) | $C_7H_6O_2$ |
| Ellagic acid hexoside | 14.00 | 463.0518 | 463.0518 | 0.05 | 300.9975(100) | $C_{20}H_{16}O_{13}$ |
| Gallic acid ethyl ester* | 14.30 | 197.0453 | 197.0455 | −1.00 | 169.0134(100), 125.0239(6.85) | $C_9H_{10}O_5$ |
| Ellagic acid pentoside | 16.03 | 433.0410 | 433.0412 | −0.53 | 300.9976(100), 299.9899(46.54) | $C_{19}H_{14}O_{12}$ |
| Ellagic acid* | 16.95 | 300.9986 | 300.9990 | −1.26 | 257.0077(100), 229.0131(55.46), 185.0235(32.44) | $C_{14}H_6O_8$ |
| Ethyl protocatechuate | 18.58 | 181.0504 | 181.0506 | −1.30 | 153.0187(100), 152.0109(14.47), 109.0290(6.77) | $C_9H_{10}O_4$ |
| **FLAVONOIDS** | | | | | | |
| ***Flavanols*** | | | | | | |
| Catechin* | 11.40 | 289.0715 | 289.0718 | −0.75 | 245.0810(100), 205.0499(40.55), 179.0343(15.63) | $C_{15}H_{14}O_6$ |
| Epicatechin* | 13.36 | 289.0714 | 289.0718 | −1.32 | 245.0807(100), 205.0496(37.72), 179.0340(15.86) | $C_{15}H_{14}O_6$ |
| Procyanidin A-type dimer | 15.80 | 575.1194 | 575.1195 | −0.17 | 449.0856(100), 289.0710(32.12), 539.0912(25.78), 285.0390(21.75) | $C_{30}H_{24}O_{12}$ |
| ***Flavanones*** | | | | | | |
| Eriodictyol-*O*-glucoside (1) | 13.58 | 449.1090 | 449.1089 | 0.23 | 287.0546(100) | $C_{21}H_{22}O_{11}$ |
| Eriodictyol-*O*-glucoside (2) | 19.05 | 449.1089 | 449.1089 | −0.17 | 287.0550(100), 151.0031(6.25) | $C_{21}H_{22}O_{11}$ |
| Eriodictyol* | 21.07 | 287.0555 | 287.0561 | −2.20 | 151.0033(100), 135.0446(4.93) | $C_{15}H_{12}O_6$ |
| ***Flavanonols*** | | | | | | |
| Taxifolin* | 17.03 | 303.0506 | 303.0510 | −1.50 | 285.0391(100), 177.0185(14.36), 125.0239(10.77) | $C_{15}H_{12}O_7$ |
| Astilbin (1) | 17.39 | 449.1090 | 449.1089 | 0.10 | 303.0498(100), 285.0393(93.25), 151.0031(27.06) | $C_{21}H_{22}O_{11}$ |
| Astilbin (2) | 18.23 | 449.1084 | 449.1089 | −1.19 | 303.0494(100), 285.0390(82.97), 151.0029(26.16) | $C_{21}H_{22}O_{11}$ |
| ***Flavonols*** | | | | | | |
| Quercetin-3-*O*-glucoside* | 17.21 | 463.0879 | 463.0882 | −0.72 | 301.0344(100), 300.0268(31.95) | $C_{21}H_{20}O_{12}$ |
| Quercetin-3-*O*-glucuronide* | 17.28 | 477.0670 | 477.0675 | −0.98 | 301.0340(100) | $C_{21}H_{18}O_{13}$ |
| Kaempferol-3-*O*-glucoside* | 18.41 | 447.0930 | 447.0933 | −0.54 | 284.03113(100), 285.0391(73.74), 327.0496(16.43), 255.0287(10.42) | $C_{21}H_{20}O_{11}$ |
| **STILBENES** | | | | | | |
| Resveratrol *C*-hexoside | 13.68 | 389.1238 | 389.1242 | −0.95 | 269.0807(100), 299.0810(2.61), 241.0857(2.42) | $C_{20}H_{22}O_8$ |
| Restrytisol (A or B) | 15.12 | 471.1442 | 471.1449 | −1.48 | 255.0653(100), 377.1018(53.27), 349.1070(43.20) | $C_{28}H_{24}O_7$ |
| Oxyresveratrol | 15.51 | 243.0659 | 243.0663 | −1.48 | 201.0547(100), 109.0290(57.60), 199.0755(28.10), 215.0703(27.08), 157.0651(8.10) | $C_{14}H_{12}O_4$ |
| Oxidized stilbenoid dimer (1) | 16.19 | 471.1449 | 471.1449 | −0.06 | 349.1067(100) | $C_{28}H_{24}O_7$ |
| Stilbenoid dimer 1 (Caraphenol B/C) | 17.81 | 469.1286 | 469.1293 | −1.45 | 451.1183(100), 363.0870(38.83), 375.0867(28.66), 281.0448(1.28) | $C_{28}H_{22}O_7$ |
| Oxidized stilbenoid dimer (2) | 18.90 | 471.1446 | 471.1449 | −0.71 | 349.1070(100), 255.0654(20.66). | $C_{28}H_{24}O_7$ |
| Stilbenoid dimer 2 (heterodimer) | 19.26 | 469.1288 | 469.1293 | −0.92 | 363.0861(100), 375.0860(39.24), 451.1169(6.21) | $C_{28}H_{22}O_7$ |
| Pallidol | 19.52 | 453.1341 | 453.1344 | −0.52 | 359.0918(100), 265.0499(8.70) | $C_{28}H_{22}O_6$ |
| (*E*)-resveratrol* | 20.28 | 227.0709 | 227.0714 | −2.03 | 185.0600(100), 183.0808(37.78), 159.0809(28.91), 157.0653(23.39), 143.0497(16.53) | $C_{14}H_{12}O_3$ |
| Stilbene dimer (resveratrol dimer) | 20.63 | 453.1338 | 453.1344 | −1.26 | 359.0914(100), 289.0859(6.64) | $C_{28}H_{22}O_6$ |
| Stilbenoid trimer | 20.77 | 681.2123 | 681.2130 | −1.07 | 453.1324(100), 359.0906(72.94), 331.0963(62.31), 587.1672(26.06), 227.0702(24.41) | $C_{42}H_{34}O_9$ |
| Resveratrol dimer-*O*-hexoside | 20.85 | 615.1866 | 615.1872 | −1.02 | 453.1323(100) | $C_{34}H_{32}O_{11}$ |
| Stilbenoid dimer 3 (*Scirpusin A*) | 21.31 | 469.1289 | 469.1293 | −2.09 | 375.0861(100), 385.1067(52.43), 359.0913(36.46). 451.1172(22.49), 241.0497(16.56), 347.0912(12.55) | $C_{28}H_{22}O_7$ |
| Stilbenoid tetramer(Hopeaphenol/ Isohopeaphenol) | 21.44 | 905.2580 | 905.2568 | 1.29 | 811.2148(100), 717.1739(71.83), 451.1164(10.45). 359.0908(7.98) | $C_{56}H_{42}O_{12}$ |
| (*E*)-ε-viniferin* | 21.69 | 453.1336 | 453.1344 | −1.58 | 359.0922(90.11), 347.0918(43.73), 435.1234(20.95) | $C_{28}H_{22}O_6$ |

* Comparison with standard. $t_R$, retention times; Isomers are shown in the bracket.

ions at *m/z* 257.0077 (loss of $CO_2$), *m/z* 229.0131 (loss of $CO_2$ and CO), and *m/z* 185.0235 (loss of two $CO_2$ and one CO) (Yan, Yin, Ma, & Liu, 2014), and was further confirmed by comparison with its pure standard. Ellagic acid pentoside showed fragment ions at *m/z* 300.9976, due to the loss of a pentosyl unit (132 Da) (Gasperotti, Masuero, Vrhovsek, Guella, & Mattivi, 2010). The presence of gallic and ellagic acids indicated that grape canes contain hydrolyzable tannins (Luque-Rodríguez, Pérez-Juan, & Luque De Castro, 2006), which were hydrolyzed under the superheating conditions of pilot-plant extraction.

Two phenolic aldehydes were detected, hydroxybenzaldehyde (*m/z* 121.0294) and protocatechuic aldehyde (*m/z* 137.0242), which both gave the similar fragments at *m/z* 93.0296 and *m/z* 93.0343, arising from the losses of CO (28 Da) and $CO_2$ (44 Da) moieties, respectively. Furthermore, protocatechuic aldehyde showed a fragment at *m/z* 109.0291 corresponding to the loss of a carbon monoxide molecule. Phenolic aldehydes were probably generated by heat-induced lignin degradation (Luque-Rodríguez et al., 2006). A high percentage of lignin (38.7%) was observed in grape canes in a previous study (Riquelme et al., 2019).

Ethyl protocatechuate (*m/z* 181.0504) was also tentatively

identified. $MS^2$ of *m/z* 181.0504 produced ions at *m/z* 153.0187[M − $CH_2CH_3^+$]$^-$ and *m/z* 109.0290[M − $CH_2CH_3^+$−$CO_2$]$^-$ (Baderschneider & Winterhalter, 2001). The presence of this compound could be attributed to the condensation of the protocatechuic acid with ethanol.

### 3.1.2. Flavonoids

This phenolic class is subdivided into subclasses of flavanols, flavanones, flavanonols, and flavonol derivatives.

*Flavanols.* Proanthocyanidins, also referred to as condensed tannins, have been extensively reported in grape canes (Cebrián-Tarancón et al., 2018a; Escobar-Avello et al., 2019; Montero, Sáez, von Baer, Cifuentes, & Herrero, 2018; Sáez et al., 2018b). However, in the grape cane pilot plant extract only three flavanols were detected in $MS^2$ mode.

The identification of catechin (*m/z* 289.0715) and epicatechin (*m/z* 289.0714) was confirmed by comparison with standards. These compounds have been previously reported in laboratory-scale grape cane extracts (Escobar-Avello et al., 2019).

A procyanidin A-type dimer (*m/z* 575.1194) was tentatively identified. The $MS^2$ spectrum of *m/z* 575.1194 showed ions at *m/z* 449.0856, 289.0710, 539.0912, and 285.0390. The ion at *m/z* 449.0856 corresponded to the elimination of a phloroglucinol (126 Da) molecule from the A-type dimer. The ions at *m/z* 289.0710 and 285.0390 arose from quinone methide cleavage of the A-type linkage (Li et al., 2012). The ion at *m/z* 539.0912 was attributed to the loss of two water molecules (36 Da). A procyanidin A-type dimer has not been previously detected in laboratory-scale grape cane extracts, although five type-B dimers (at *m/z* 577) were successfully identified (Escobar-Avello et al., 2019). A likely explanation is that B-type procyanidin dimers can be converted into A-type dimers by radical oxidation (Kondo et al., 2000; Osman & Wong, 2007). In addition, other studies have determined that the conversion is significantly affected by temperature and pH (Chen, Yuan, Chen, Jia, & Li, 2014). Therefore, the presence of a procyanidin A-type dimer in the extract was attributed to the oxidative environment in the pilot plant reactor.

The minor presence of oligomeric procyanidins and prodelphinidins contrasts with the results previously obtained on a laboratory scale (Escobar-Avello et al., 2019), and could be explained by the epimerization, hydrolysis, and oxidation/condensation reactions of the tannins caused by the temperature (80 °C) and oxygen in the pilot plant reactor. Similarly, flavan-3-ols could undergo oligo/polymerization under heat treatment (Fan et al., 2016). Polymerization and degradation processes of these compound classes have been previously studied by UHPLC-LTQ-Orbitrap-HRMS (Vallverdú-Queralt et al., 2017).

*Flavanones.* Eriodictyol and two of its glycoside isomers were detected. Eriodictyol (*m/z* 287.0555) revealed product ions at *m/z* 151.0033 and *m/z* 135.0446 corresponding to *retro*-Diels-Alder fragmentation in the C-ring, and was confirmed by comparison with the standard. Two eriodictyol-*O*-glucoside (*m/z* 449.1090, $t_{R.}$ 13.58 min; *m/z* 449.1089, $t_{R.}$ 19.05 min) generated ions at *m/z* 287.0546 and *m/z* 287.0550, respectively, and were tentatively identified based on accurate mass measures and cleavage of the glycoside moieties (162 Da).

*Flavanonols.* Taxifolin (*m/z* 303.0506) and two of its glycoside isomers, astilbin 1–2 (*m/z* 449.1090, $t_{R.}$ 17.39 min; *m/z* 449.1084, $t_{R.}$ 18.23 min), were identified. Both astilbin isomers exhibited cleavage of the rhamnosyl moiety (146 Da), generating ions at *m/z* 303.0498 and *m/z* 303.0494 (taxifolin), and ions at *m/z* 285.0393 and *m/z* 285.0390 due to the consecutive loss of a water molecule (18 Da). Taxifolin (or dihydroquercetin) was confirmed by comparison with its pure standard. These compounds was recently reported in grape canes (Escobar-Avello et al., 2019).

*Flavonols.* Quercetin-3-*O*-glucoside (*m/z* 463.0879), quercetin-3-*O*-glucuronide (*m/z* 477.0670), and kaempferol-3-*O*-glucoside (*m/z* 447.0930) were identified by comparison with pure standards. All have been previously reported in grape cane extracts (Escobar-Avello et al., 2019; Moreira et al., 2018). In a study of six onion varieties submitted to different temperatures, Sharma et al. (2015) reported that quercetin and its glucoside derivatives increased at 120 °C, subsequently decreasing at 150 °C. Our results are consistent with these findings, as the flavonol glycosides were not affected by pilot plant extraction conditions.

### 3.1.3. Stilbenes

Stilbenoids, probably the most investigated phenolic group in grape canes, exhibit many patterns of oligomerization and glycosylation. Oligomeric stilbenes are generated by oxidative coupling of *trans*-resveratrol, isorhapontigenin, oxyresveratrol, or piceatannol (Rivière, Pawlus, & Mérillon, 2012).

(*E*)-resveratrol (*m/z* 227.0709) showed product ions at *m/z* 185.0600, 183.0808, 159.0809, 157.0653 and 143.0497 and was confirmed by comparing the retention time and $MS^2$ data with an authentic standard. Oxidation of resveratrol generates some well-known dimeric compounds and also other complex oxidized derivatives. Thus, although some compounds were tentatively identified by comparing accurate mass and fragmentation patterns with those in the literature, their precise structures should be elucidated by NMR spectroscopy techniques.

Oxyresveratrol (*m/z* 243.0659), two oxidized stilbenoid dimers (*m/z* 471.1449, $t_{R.}$ 16.19 min; *m/z* 471.1446, $t_{R.}$ 18.90 min), and restrytisol A or B (*m/z* 471.1442) were tentatively identified in the grape cane pilot plant extract. Oxyresveratrol was identified by comparison with the mass spectra reported by Huang et al. (2010). The precursor ion at *m/z* 471.1442 of restrytisol (A or B) showed product ions at *m/z* 377.1018, 349.1070, and 255.0653. The $MS^2$ of both oxidized stilbenoid dimers (*m/z* 471) showed an intense product ion at *m/z* 349.1067 and *m/z* 349.1070, while stilbenoid dimer 2 also gave a product ion at *m/z* 255.0654. Restrytisol (A or B) and oxidized stilbenoid dimers have been previously identified in grape canes (Escobar-Avello et al., 2019).

(*E*)-ε-viniferin (*m/z* 453.1336) was verified by matching with a pure standard. The $MS^2$ of (*E*)-ε-viniferin showed ions at *m/z* 435.1234, 359.0922, and 347.0918. Pallidol (*m/z* 453.1341) was also tentatively identified by comparing the fragments with literature data (Flamini & De Rosso, 2018). The ion at *m/z* 265.0499 is characteristic of pallidol and could be essential for identification by mass spectrometry. An undefined stilbenoid dimer (*m/z* 453.1338) produced ions at *m/z* 359.0914, corresponding to the loss of a phenol (94 Da), and at *m/z* 289.0859, associated with the sequential losses of CO (28 Da) and $H_2C$ = C = O (42 Da). The latter fragment has been observed for different regioisomers, such as parthenocissin A, quadrangularin A, and ampelopsin D (Flamini & De Rosso, 2018; Moss, Mao, Taylor, & Saucier, 2013).

Three dimers with [M − H]$^-$ ion signals at *m/z* 469 were tentatively identified. Stilbenoid dimer 1 (*m/z* 469.1286) produced $MS^2$ ions at *m/z* 451.1183, 363.0870, 375.0867, and 281.0448, the latter reported by Püssa et al. (2006) for caraphenol. Caraphenol B and C were initially isolated from the roots of *Caragana sinica*, and their structures were elucidated through spectroscopic methods (Luo, Zhang, & Hu, 2001), only to be revised and then confirmed by synthesis (Snyder & Brill, 2011). Therefore, using our spectrometry approach, it was not plausible to assign the name of the structure with precision. Stilbenoid dimer 2 (*m/z* 469.1288) showed a similar fragmentation pattern, although with the absence of the ion at *m/z* 281.0448. Stilbenoid dimer 3 (*m/z* 469.1289) gave product ions at *m/z* 451.1172, 385.1067, 375.0861, 359.0913, 347.0912, and 241.0497. The product ion at *m/z* 241.0497 is characteristic of scirpusin A, as verified previously by NMR analysis (Sáez et al., 2018).

Two glycosylated stilbenes were tentatively identified by comparing their mass spectra with the literature (Escobar-Avello et al., 2019). Resveratrol *C*-hexoside (*m/z* 389.1238) showed fragment ions at *m/z* 269.0807, 299.0810, and 241.0857 corresponding to the losses of 120 Da and 90 Da, characteristic of *C*-hexosides. Resveratrol dimer-*O*-hexoside (*m/z* 615.1866) was also detected and gave an intense product ion at *m/z* 453.1323 arising from the loss of a sugar moiety (162 Da). Various resveratrol glycoside dimers have been experimentally

demonstrated by NMR in Riesling wine, including ε-viniferin diglucosides, and pallidol mono- and diglucosides (Baderschneider & Winterhalter, 2000). Likewise, a resveratrol *trans*-dehydrodimer (δ-viniferin) and its 11- and 11'-β-*O*-glucosides have been reported in cell suspension cultures of *V. vinifera* (Waffo-Teguo et al., 2001). The glycosylation of resveratrol may help to protect compounds from enzymatic oxidation and extend their half-life in cell tissues (Regev-Shoshani, Shoseyov, Bilkis, & Kerem, 2003).

A stilbenoid trimer (*m/z* 681.2123) was also tentatively identified, showing product ions at *m/z* 587.1672, 453.1324, 359.0906, 331.0963, and 227.0702, which were generated by the typical stilbenoid losses of a phenol (94 Da), a unit of resveratrol (228 Da), and a phenol group (94 Da) from the ion at *m/z* 453. The product ion at *m/z* 331.0963 was probably due to the loss of $C_7H_6O_2$ (122 Da) from *m/z* 453.1324 or a loss of CO (28 Da) from the ion at *m/z* 359.0906. Additionally, the presence of a deprotonated resveratrol unit was detected at *m/z* 227.0702. The proposed fragmentation pathway for the stilbenoid trimer (*m/z* 681.2123) is illustrated in Fig. 2.

A tentatively identified stilbenoid tetramer (*m/z* 905.2580) gave a mixed spectrum, apparently due to the co-elution of hopeaphenol and isohopeaphenol, which have been previously identified in grape canes (Escobar-Avello et al., 2019). The main product ions detected at *m/z* 811.2148, 717.1739, 451.1164, and 359.0908 were produced following the characteristic fragmentation pathway of stilbene oligomers (Moss et al., 2013).

The higher-order oligomers are often the product of either oxidative coupling to resveratrol/ε-viniferin or an intermolecular Friedel – Crafts reaction (Keylor, Matsuura, & Stephenson, 2015). These routes can generate highly diverse and complex structures, entailing additional studies for the elucidation of the oligomers. Furthermore, several uncontrolled reactions could occur in the oxidative environment of the pilot-plant reactor leading to new and unknown compounds.

### 3.2. Concentrations of individual phenolic compounds in the grape cane pilot-plant extract.

The semi-quantification of phenolic compounds was performed by external calibration curves using six reference compounds. The linearity of the method obtained with a regression coefficient ($R^2$) was higher than 0.9819. The LOD values range from 0.215 to 1.781 mg/L, whereas the LOQ values range from 0.652 to 5.396 mg/L (Table 1).

The main phenolic classes obtained in the grape cane pilot-plant extract were stilbenes (55%), phenolic acids and aldehydes (28%), and flavonoids (17%), as shown in Fig. 3 A. The individual semi-quantification of phenolic compounds in the grape cane extract obtained on a pilot-scale is presented in Fig. 3 C-E.

#### 3.2.1. Phenolic acids and aldehydes

Phenolic acids found abundantly in agro-food waste have application in the cosmetic, pharmaceutical, and polymer industries (Tinikul, Chenprakhon, Maenpuen, & Chaiyen, 2018). They are also used in the food and beverage industry for their organoleptic, color, sensory, nutritional, and antioxidant properties (Valanciene et al., 2020).

The total amount of phenolic acids and aldehydes in the grape cane pilot-plant extract was 4117 ± 530 mg/kg DW (Fig. 3A). The predominant phenolic aldehyde was hydroxybenzaldehyde (1295 ± 131 mg/kg DW) (Fig. 3C), and the high amount of hydroxybenzaldehyde acid was likely due to the thermal degradation of lignin. Also detected in high levels was protocatechuic aldehyde (711 ± 100 mg/kg DW). Protocatechuic acid was previously quantified in grape canes at concentrations of 3 to 379 mg/kg (Luque-Rodríguez et al., 2006; Sánchez-Gómez et al., 2014). Protocatechuic aldehyde has been studied for its regulatory effects on myocardial fibrosis, which is associated with cardiovascular disease (Wan et al., 2019). Additionally, an isomer of protocatechuic acid-*O*-hexoside was quantified (388 ± 53 mg/kg DW).

The presence of hydrolyzable tannins in grape canes could be explained by the high levels of ellagic acid and gallic acid (Luque-Rodríguez et al., 2006), which were hydrolyzed by the pilot plant extraction conditions. Ellagic acid was observed in high levels (284 ± 7 mg/kg DW), representing a recovery up to 5-fold higher than values reported in previous studies (0.01 to 53.25 mg/kg) (Goufo et al., 2020). In contrast, the ellagic acid pentoside (164 ± 16 mg/kg DW) was detected in minor levels.

The concentration of gallic acid (279 ± 58 mg/kg DW) was in agreement with levels reported for different vine-shoot cultivars using superheated liquid extraction (Delgado-Torre, Ferreiro-Vera, Priego-Capote, Pérez-Juan, & Luque De Castro, 2012). A derivative of gallic acid identified as gallic acid ethyl ester (ethyl gallate) was also detected

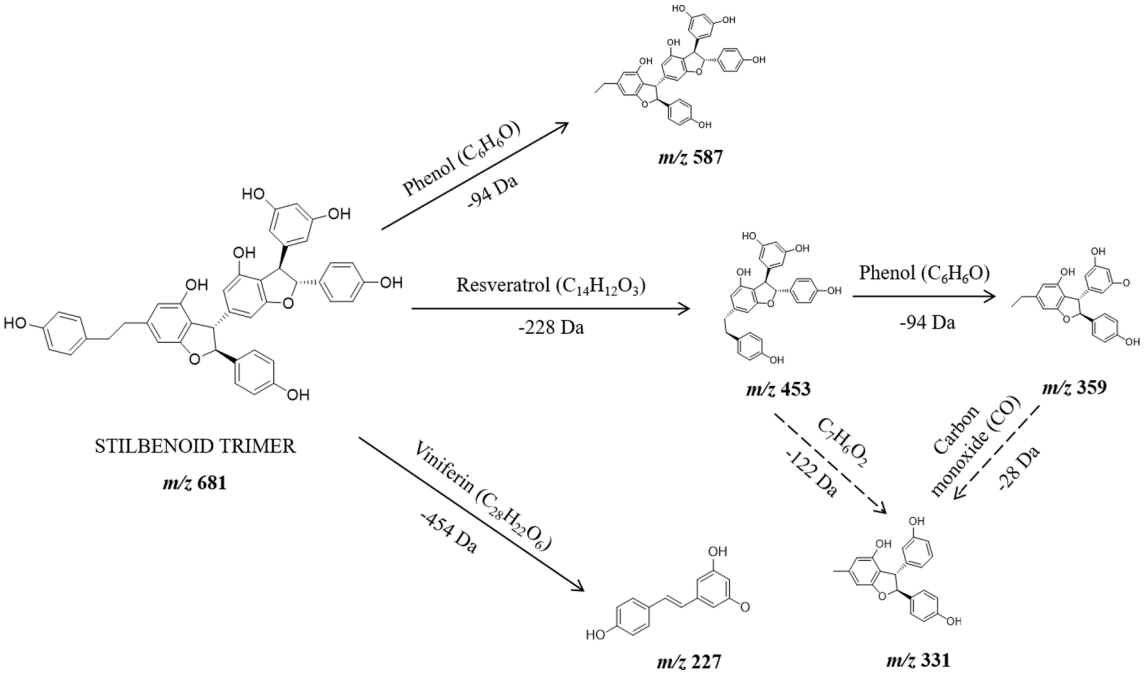


**Fig. 2.** Proposed fragmentation pathway for a stilbenoid trimer (*m/z* 681.2123).

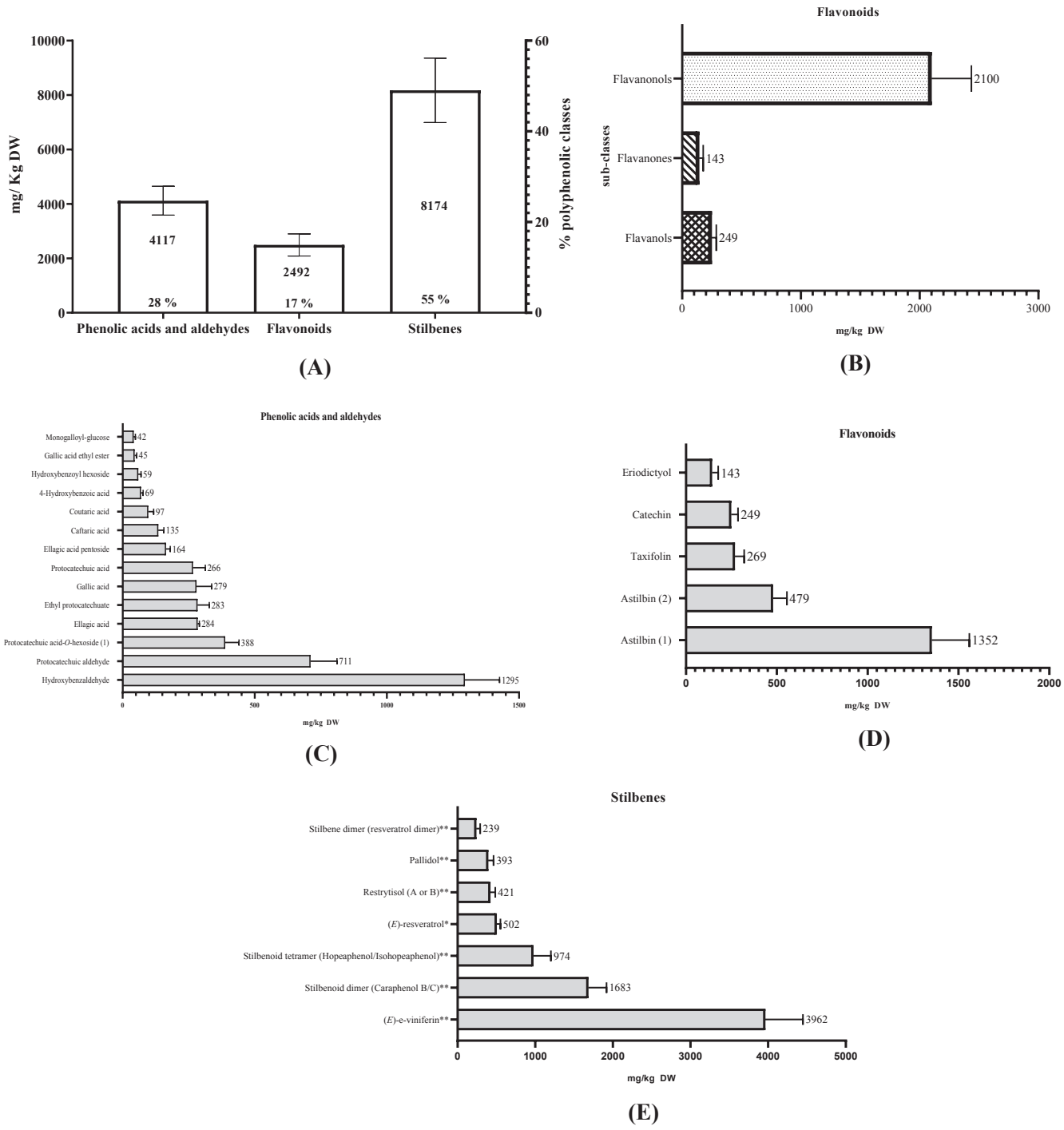


**Fig. 3.** Semi-quantification of phenolic compounds in a grape cane pilot-plant extract (expressed in mg/kg DW). (A) Concentration and percentage levels of each phenolic class in the extract. (B) Cumulative levels of flavonoid subclasses: flavanonols (expressed in equivalents of taxifolin), flavanones (expressed in equivalents of eriodictyol), and flavanols (expressed in equivalents of epicatechin). (C) Levels of individual phenolic acids and aldehydes (expressed in equivalents of gallic acid). (D) Levels of individual flavonoids. (E) Levels of individual stilbenes (expressed as: *(*E*)-resveratrol equivalents and **(*E*)-ε-viniferin equivalents).

(45 ± 8 mg/kg DW). This metabolite is reported in plants, but as it can also be formed by gallic acid condensation with ethanol, its origin in the extract is difficult to establish.

Monogalloyl-glucose was found at low levels (42 ± 6 mg/kg DW), although its detection provided evidence for the presence of gallotannins in the grape cane pilot plant extract.

The concentration of protocatechuic acid (266 ± 47 mg/kg DW) was in accordance with the results reported for vine shoots of grape cultivars such as Tempranillo, Cabernet Franc, Malbec, and Mazuelo (Delgado-Torre et al., 2012). This compound has very promising inhibitory properties against neurodegenerative diseases like Alzheimer's and Parkinson's (Krzysztoforska, Mirowska-Guzel, & Widy-Tyszkiewicz, 2019). The high level of ethyl protocatechuate (283 ± 45 mg/kg DW) is provisionally attributed to the condensation of protocatechuic acid with ethanol.

Two hydroxycinnamic acids, caftaric (135 ± 21 mg/kg DW) and coutaric (97 ± 20 mg/kg DW) acids, were semi-quantified at higher levels than a previous reported (77.60 and 19.39 mg/kg, respectively)

(Goufo et al., 2020). Caftaric acid has shown anti-oxidant and anti-inflammatory effects in indomethacin-induced gastric ulcers (Tanyeli et al., 2019).

The concentration of 4-hydroxybenzoic acid (69 ± 8 mg/kg DW) is similar to levels reported in Sauvignon Blanc vine-shoot extracts using superheated liquid extraction (Delgado-Torre et al., 2012). 4-hydroxybenzoic acid has recently emerged as a promising intermediate for diverse value-added bioproducts with potential biotechnological applications in food, pharmacy, cosmetics, and fungicides (Wang, Bilal, Hu, Wang, & Zhang, 2018). We also detected a low concentration of hydroxybenzoyl hexoside (59 ± 10 mg/kg DW).

#### 3.2.2. Flavonoids

Flavonoids have a wide variety of cosmetic, nutraceutical, and pharmaceutical applications, due to their anti-inflammatory and anti-oxidative properties (Panche, Diwan, & Chandra, 2016).

The cumulative concentration of flavonoids recovered from the pilot plant grape cane extract was 2492 ± 408 mg/kg DW (Fig. 3A). The highest level was observed for flavanonols (2100 ± 336 mg/kg DW), followed by flavanols (249 ± 38 mg/kg DW), and flavanones (143 ± 34 mg/kg DW) (Fig. 3B), while three flavonols were detected but not quantified.

*Flavanonols*. High levels of two isomers of astilbin 1 (1352 ± 208 mg/kg DW) and astilbin 2 (479 ± 76 mg/kg DW) were observed (Fig. 3D). High levels of astilbin have been reported in Sauvignon grapes during noble rot stages, suggesting it is used by the plant to fight against botrytis (Landrault et al., 2002). Taxifolin was also detected in a significant concentration (269 ± 52 mg/kg DW), similar to a study using ohmic heating to extract bioactive compounds from Loureiro vine pruning residue collected in Minho-Portugal (Jesus et al., 2020). Taxifolin has shown a wide range of pharmacological properties, such as an antimicrobial, antioxidant, anti-Alzheimer, hepatoprotective, and cardioprotective (Sunil & Xu, 2019), and is therefore of interest for the development of new natural drugs for the control of various illnesses.

*Flavanols*. Highly variable levels of the extensively studied catechin were recently reported in grape canes (65 to 6735 mg/kg) (Goufo et al., 2020). The concentration in our study (249 ± 38 mg/kg DW) (Fig. 3D) was similar to the results obtained with toasted vine-shoot chips of Airén and Cencibel varieties extracted at 180 °C for 45 min (Cebrián-Tarancón et al., 2018b). Epicatechin and procyanidin A-type dimer were detected but not quantified (<LOQ). Procyanidin A-type dimer may be the result of thermal conversion from B-type procyanidin dimers, which seem sensitive to thermal-oxidative processes.

*Flavanones*. As well as the parent compound eriodictyol (143 ± 34 mg/kg DW), two glycosides, eriodictyol-*O*-glucoside isomers 2 and 1, were detected but not quantified (<LOQ). These compounds are not commonly found in grape canes, although they were also identified in our previous work (Escobar-Avello et al., 2019). Eriodictyol defends against oxidative stress and could have application in nutraceuticals for the prevention of cardiovascular disease (Lee et al., 2015).

*Flavonols*. Three flavonols: quercetin-3-*O*-glucuronide, quercetin-3-*O*-glucoside, and kaempferol-3-*O*-glucoside were also only detected but not quantified (<LOQ). Although predominant in grapevine leaves (Goufo et al., 2020), these flavonols are not usually reported in grape canes. Notwithstanding, all these compounds have been previously quantified in whole Bois Noir-infected and also healthy Chardonnay canes (Rusjan & Mikulic-Petkovsek, 2015). Glycosylated quercetin and kaempferol have potential as natural drugs because of their anti-influenza, anti-leishmania, and anti-inflammatory activities (Xiao, 2017).

#### 3.2.3. Stilbenes

The most studied stilbene, resveratrol, is associated with activity against pathologies related to oxidative stress, inflammatory biomarkers, type 2 diabetes, and cardiovascular and neurological diseases (Ramírez-Garza et al., 2018).

Stilbenoids were the predominant polyphenol class detected in the grape cane extract (8174 ± 1185 mg/kg DW in total) (Fig. 3A). The most abundant was (*E*)-ε-viniferin (3962 ± 485 mg/kg DW) (Fig. 3E), its concentration almost 3-fold higher than in a previous study of a Pinot noir grape cane extract obtained using ultrasound under laboratory conditions (Sáez et al., 2018). A stilbenoid (resveratrol) dimer (239 ± 51 mg/kg DW) and pallidol (393 ± 71 mg/kg DW) were semi-quantified, the concentration of the latter falling within the range previously reported in grape canes (4 to 1276 mg/kg) (Goufo et al., 2020).

Three stilbenoid heterodimers (at *m/z* 469) were observed, the most abundant being heterodimer 1 (Caraphenol B/C) (1683 ± 236 mg/kg DW), while heterodimers 2 and 3 were only detected but not quantified (<LOQ). An isomer at *m/z* 469, namely ampelopsin A, has been previously quantified in grape canes (204–207 mg/kg) (Sáez, Gayoso, et al., 2018).

High levels of a stilbenoid tetramer (hopeaphenol/isohopeaphenol) (974 ± 227 mg/kg DW) were also found. Both hopeaphenol and isohopeaphenol have been detected in grapevine canes at higher concentrations (1439 and 3521 mg/kg, respectively) (Goufo et al., 2020), whereas a comparable level of hopeaphenol (1126 ± 294 mg/kg DW) was detected in woody canes of Pinot noir collected in France (Lambert et al., 2013).

The concentration of (*E*)-resveratrol (502 ± 50 mg/kg DW), although significant, was lower than the levels obtained by Vergara et al. (2012) (723 to 5590 mg/kg) in extracts obtained using ultrasound under laboratory conditions from eleven samples of Pinot noir collected in different locations of Southern Chile. The lower levels of resveratrol in our study could be due to oxidation, degradation, and epimerization reactions during the pilot plant extraction. The oxidation of resveratrol is simple and can be mediated by plant constituents such as peroxidases, exogenous fungal laccases, and also reactive oxygen species generated by UV irradiation (Keylor et al., 2015).

The oxidized dimer with the highest concentration was restrytisol (A or B) (421 ± 65 mg/kg DW). Oxidized stilbenoid dimers have been previously identified in grape canes of the Pinot noir variety, one at notably high concentrations (up to 868 ± 14 mg/kg DW) (Sáez et al., 2018).

Finally, we also detected a low amount of oxyresveratrol, resveratrol *C*-glucoside, resveratrol dimer-*O*-hexoside, and an unidentified stilbenoid trimer. However, all of them were below the quantification limit (<LOQ).

### 3.3. Antioxidant capacity of grape cane pilot-plant extract

The antioxidant capacity of grape canes has been studied by several authors (Dorosh et al., 2020; Jesus et al., 2019; Karacabey & Mazza, 2010; Moreira et al., 2018) and through diverse methods of antioxidant capacity. High variability in the antioxidant activity has been found in the literature, which could be partially explained by grape canes variety, extractions conditions, or analytical protocols used in each study.

The antioxidant capacity of grape cane pilot-plant extract measured by the radical antioxidant scavenging potential ($ABTS^{\bullet+}$), and by assay of oxygen radical absorbance capacity using fluorescein (ORAC-FL) were 2209 ± 125 μmol TE/g DW and 4612 ± 155 μmol TE/g DW, respectively. Those concentrations were very similar to a previous study of a Pinot noir grape cane extraction at a bench-scale of 7L where the values found were 1954 ± 264 μmol TE/g and 6895 ± 996 μmol TE/g, respectively and under the same extraction conditions (Riquelme et al., 2019).

## 4. Conclusion

Pilot-plant scale extraction of grape canes allowed the recovery of 44 phenolic compounds. Grape cane pilot-plant extract showed a high antioxidant capacity measured by $ABTS^{\bullet+}$ (2209 ± 125 μmol TE/g DW) and ORAC-FL (4612 ± 155 μmol TE/g DW). The most diverse classes

were phenolic acids and aldehydes (17), while the most predominant were the stilbenes (8174 ± 1185 mg/kg DW), (*E*)-ε-viniferin being the most abundant (3962 ± 485 mg/kg DW).

The phenolic composition of grape cane extracts is highly dependent on the extraction conditions. The phenolic profile obtained on a pilot scale showed some differences compared with a previous laboratory-scale extraction (Escobar-Avello et al., 2019). The most relevant differences were the minor presence of oligomeric procyanidins and prodelphinidins. Moreover, phenolic aldehydes were only present in pilot grape cane extract. These differences highlight the importance of carrying out a comprehensive characterization of phenolic profiles in the scale-up process before industrial exploitation. Their unique mixture of phenolic compounds makes grape cane pilot-plant extracts a promising by-product for the development of novel nutraceutical and pharmaceutical products.

## CRediT authorship contribution statement

**Danilo Escobar-Avello:** Writing - original draft, Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Visualization. **Claudia Mardones:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Vania Saéz:** Conceptualization, Investigation, Writing - review & editing. **Sebastián Riquelme:** Methodology, Formal analysis. **Dietrich Baer:** Conceptualization, Investigation, Writing - review & editing. **Rosa M. Lamuela-Raventós:** Investigation, Resources, Writing - review & editing, Supervision, Funding acquisition. **Anna Vallverdú-Queralt:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Supervision, Funding acquisition.

## Declaration of Competing Interest

None.

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