Bioactive compounds and total antioxidant capacity of cane residues from different grape varieties

Running title: Bioactives and antioxidant potential of grape canes

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Abstract

BACKGROUND: Every year the viticulture activity generates considerable amounts of underused lignocellulosic residues as grape cane that generally are composted or burned despite their potential value as a source of bioactive compounds. To know their phytochemical composition and total antioxidant capacity (TAC) may be a useful way of exploiting different high-added value applications.

RESULTS: 21 phenolic compounds (PC) and two carotenoids (Car) were quantified by high performance-liquid chromatography-diode array detection (HPLC-DAD) in 8 grape varieties from different locations in Mendoza, Argentina. The maximum concentrations corresponded to the stilbene ε-viniferin (10552 μg g^{-1} DW), followed by the flavanols (+)-catechin (3718 μg g^{-1} DW) and (-)-epicatechin (2486 μg g^{-1} DW). As well, lutein and *β*-carotene were quantified at levels ranged between 350 and 2400 ng g^{-1} DW. The TAC of the extracts was assessed by ORAC, ABTS and DPPH assays, with a good correlation between TAC and total PC for each sample ($r \geq 0.82$).

CONCLUSION: Samples of cv. Malbec, the most representative variety of Argentina's winemaking industry, presented high contents of PC, particularly ε-viniferin, (+)-catechin and (-)-epicatechin. Quercetin-3-galactoside, OH-tyrosol

and Car were reported for the first time in grape canes of the 8 varieties. The results add knowledge related to this inexpensive source of high value bioactive compounds which could be used as functional ingredients.

Keywords: grapevine canes; phenolic profiling; stilbenes; carotenoids, bioactive phytochemicals; industry by-products.

Introduction

Grape is the world's largest fruit crop, with an annual production of 75 million tons, where about 50% is allocated in winemaking [1]. Currently, Argentina's vineyards represent around 3% of the global grape cultivated area [2], indicating the importance of viticulture activity in the economy of the region. This activity implies management practices such as pruning to enhance high-quality production. As result, considerable amounts of underused or poorly valorized lignocellulosic residues are generated every year [3]. Grape cane residues represent about an average volume of 2.5 tons per ha per year that generally are composted or burned [4–6], despite their potential value as a source of bioactive compounds.

Nowadays, the majority of studies performed on grape canes are focused in bioactive compounds such as PC, more specifically on characterization of stilbenes. These compounds are synthesized by plants in response to different stresses (pathogen infection, traumatic damage, ultraviolet irradiation, etc.) [7,8]. Different authors have found high concentrations of monomeric stilbenes, i.e.

trans-resveratrol [3,5,9], although oligomeric stilbenes such as *ε*-viniferin have also been reported [6,10]. The stilbenes level and composition of this by-product depends on plant characteristics (genus, variety, age, etc.), as well as growth environmental conditions and processing conditions after pruning [6,11,12]. In turn, different stilbene profiles (qualitative and quantitative) can result due to chemical changes before (biosynthesis), during (mechanical wounding) or after (storage time and conditions) pruning of grape plants [7]. Billet *et al*. [13] informed that the metabolism of stilbenes continues in wood after pruning, synthesizing trans-resveratrol and trans-piceatannol. Nevertheless, little or nothing is known about other phytochemicals in such by-products, including PC of other phenolic's families (flavanols, flavonols and phenolic acids) or compounds such as carotenoids (Car), which also have bioactive properties as to be used as functional food ingredients. Carotenoids are natural pigments widely distributed in many plant materials. Some authors have reported studies of Car in grapevine leaves and berries [14–16] with *β*-carotene and lutein representing 85% of the total composition. But no reports of these compounds has been informed for grape canes. Having this in mind, the identification and quantification of new compounds will provide information that may explain other extract properties, like total antioxidant capacity (TAC) [17]. Additionally, it could help to find new sources of phytochemicals with different applications as functional ingredients.

The concept of TAC encompasses the individual antioxidant actions of different compounds and their additive, synergistic or antagonistic interactions in foods

and biological fluids samples [17]. The *in vitro* assays most commonly used to measure TAC in food and biological samples have been 2,20-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), oxygen radical absorbance capacity (ORAC) and ferric reducing ability of plasma (FRAP). These tests are classified according to the reaction mechanism in two groups: ABTS, DPPH and FRAP are based on electron transfer, while ORAC is based on hydrogen atom transfer [18]. Thus, the information given by PC profiles and TAC may be helpful to explain possible synergic effects among compounds associated with the overall bioactivity of the extract, providing information for samples characterization so increasing the product value.

In recent years, the demand of stilbenes increased due to their potential in commercial applications and health-promoting properties [19]. Antioxidant, cardioprotective, anti-inflammatory, antimicrobial, antifungal, antiaging and anticancer properties have been reported for stilbenes [20]. As well, different applications of cane extracts have been reported as food additives [21], raw material for activated carbon or paper pulp [22], natural fungicides, and in the extraction of bio-compounds starting from alcoholic distillates [19]. On the other hand, the TAC properties of Car can help to reduce the risks of degenerative illness, as well as cancer, cardiovascular and ophthalmological diseases [23]. As well, some Car have pro-vitamin A activity (*β*-carotene) and protect against age-related macular degeneration (lutein). The mentioned Car have also been

associated with important functional properties, especially TAC and prevention of cardiovascular diseases. Given the variety of their benefits, are widely used by the nutraceutical (i.e. as natural colorants) and cosmetic industries [24]. The beneficial effects of some bioactive substances found at high levels in this by-product (i.e. ε-viniferin) have not been deeply studied, particularly those aspects concerning on their bioavailability. In this context, more studies related to digestive stability, bioaccessibility, and the efficiency of transepithelial passage of compounds need to be explored deeply in the future [25].

The objective of this study was to determine by HPLC-DAD individual PC of different families (flavanols, flavonols, stilbenes and phenolic acids) and Car present in 16 cane extracts of 8 grape varieties at different locations. Moreover, the *in vitro* TAC (ABTS, DPPH and ORAC) and total phenolic content (TPC) were determined to perform the correlation amongst qualitative and quantitative profiles of individual PC.

Materials and methods

Standards and chemicals

Standards of 3-hydroxytyrosol (≥99.5%), (-)-gallocatechin (≥98%), (-)-gallocatechin gallate (≥99%), (-)-epicatechin gallate (≥98%), (-)- epigallocatechin gallate (≥95%), (+)-catechin (≥99%), procyanidin B2 (≥90%), (-)-epicatechin (≥95%), astilbin (≥98%), syringic acid (≥95%), cinnamic acid (99%), caftaric acid (≥97%), p-coumaric acid (98%), trans-resveratrol (≥99%), (+)-ε-viniferin (≥95%),

quercetin 3-β-D-glucoside (≥90%), quercetin 3-β-D-galactoside (≥97%), kaempferol-3-glucoside (≥99%), kaempferol (≥90%), naringin (≥95%), naringenin (≥95%), lutein (≥96%) and ß-carotene (≥93%) were purchased from Sigma-Aldrich (Steinheim, Germany). Stock solutions of PC were prepared in methanol at the concentration levels of 1000 mg mL^{-1}. For stock solutions of Car, the standards were weighed and dissolved in ethanol (lutein) and n-hexane (ß-carotene). Stock solutions of 40 and 200 mg L^{-1} of lutein and ß-carotene were prepared.

The standards used for calibration were prepared in the initial mobile phase of each chromatographic method.

Trolox reagent (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), $NaH_2PO_4.2H_2O$, $Na_2HPO_4.12H_2O$, fluorescein, ABTS, $K_2S_2O_8$, DPPH and AAPH were from Sigma-Aldrich (Steinheim, Germany). HPLC-grade acetonitrile (MeCN), formic acid (FA), acetone, methanol, ethanol and methyl tert-butyl ether (MTBE) were from Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA). Ethanol and Folin-Ciocalteu reagent were from Merck (São Paulo, Brazil). Hexane was from Tedia (Fairfield, CA, USA). Other reagents used were of analytical grade. Ultrapure water was obtained from a Milli-Q system (Millipore, Billerica, MA, USA).

Sample preparation

This study was conducted with different cane samples of *Vitis vinifera* L. of the cultivars Malbec (MB), Cabernet Sauvignon (CS), Cabernet Franc (CF), Chardonnay (CH), Sauvignon Blanc (SB), Pinot Noir (PN), Merlot (ML) and Viognier (VG), collected during the pruning season (2017) from vineyards of different locations of Mendoza's region, Argentina. Immediately after pruning (1 day), each sample was cut into small pieces (2-4 cm long) with a pruning scissor and dried at 60 °C in an air-circulation oven until a constant weight (5 days). Then, the dried canes were powdered in an analytical mill (A 11 basic; IKA, Staufen, Germany) and stored at room temperature in hermetic plastic bags until extraction.

PC extraction

Extraction of PC from samples was performed by solid-liquid method according to previous reports [10], with slight modifications. Briefly, 1 g of powdered sample was extracted with 50 mL acetone/water (50:50 v/v) in ultrasonic washer at 50 Hz and 60 °C during 60 min. The mixture was centrifuged 10 min at 806 x g and filtered through filter paper. Then, the extract was stored in sealed dark-glass bottles at -20 °C prior to analysis. Extractions were performed in triplicate. Finally, an aliquot of extract was filtered through a 0.2 µm PTFE vial filter and analyzed by HPLC-DAD.

In order to select the optimum extraction conditions, a previous optimization under different variables was performed (data not shown). The efficiency of each

condition used (type of solvent, extraction temperature, extraction time and ultrasound application) was evaluated by measurement of TPC of extracts. After doing this optimization, acetone was selected as extraction solvent and the other conditions presented before were also the best in terms of TPC of extracts. The final condition is in agreement with previous papers that report that the use of acetone as a component of extraction mixture has better efficiency for stilbenoids extraction [8,10,26,27].

Carotenoid extraction

For the extraction of Car, 250 mg dry powder of cane were slurred with 1 mL ultrapure water plus 1 mL ethanol/hexane at 40:60 v/v (as extracting solution) in a ceramic mortar. The mixture was poured into a glass tube. Then, 2 mL of extracting solution were added into the tube. Sample was extracted in an ultrasonic bath at 50 Hz in darkness during 15 min and centrifuged for 15 min at 1227 x g. The supernatant was collected in a glass Khan tube and evaporated to dryness under vacuum (SpeedVac concentrator). Two additional extractions were carried out using 3 mL of hexane and repeating the steps described below to achieve the final dried extract. Extractions were performed in triplicate. Finally, the dried extract was re-suspended in 1 mL of methanol/MTBE solution (1:1 v/v) and 5 µL were injected in the HPLC-DAD.

Chromatographic Methods

Analysis of PC were done using a Dionex Ultimate 3000 HPLC-DAD system (Dionex Softron GmbH, Thermo Fisher Scientific Inc., Germering, Germany) and a reversed phase Kinetex C_{18} core shell column (3.0 mm x 100 mm, 2.6 µm) (Phenomenex, Torrance, CA, USA). As mobile phases ultrapure water with 0.1% FA (A) and MeCN (B) were used. Analytes were separated using a previously reported method [28] with the following gradient: 0–1.7 min, 5% B; 1.7–11 min, 30% B; 11–14 min, 95% B; 14–15.5 min, 95% B; 15.5–17 min, 5% B; 17–20, 5% B. The mobile phase flow was 0.8 mL min^{-1}. The column temperature was 35 °C, and the injection volume was 1 µL. The quantification wavelengths for different families of analytes were 254 nm for quercetin 3-β-D-glucoside, quercetin 3-β-D-galactoside, kaempferol-3-glucoside) and (-)-gallocatechin, 280 nm for (+)-catechin, procyanidin B2, (-)-epicatechin, (-)-gallocatechin gallate, (-)-epicatechin gallate, (-)- epigallocatechin gallate, astilbin, naringin, naringenin, syringic acid and OH-tyrosol, 320 nm for cinnamic acid, caftaric acid, p-coumaric acid, *trans*-resveratrol and (+)-ε-viniferin, and 370 nm for kaempferol.

For Car, an Accucore C_{30} column (3.0 mm x 150 mm, 2.6 µm) (Thermo Fisher Scientific Inc, Bellefonte, PA, USA) was used. The mobile phases were methanol (A), MTBE (B) and ultrapure water (C). The percent of C was maintained at a constant 4% throughout the total chromatographic run. Analytes were separated using the following gradient: 0 min, 26% B; 0–10 min, 76% B; 10–14 min, 90% B; 14–16 min, 26% B; 16–20, 26% B. The mobile phase flow was 0.4 mL min^{-1}. The column temperature was 10°C and the injection volume was 5 µL. The

quantification wavelengths for lutein and ß-carotene were 445 nm and 450 nm, respectively.

The identity of analytes was attributed by comparison of the retention times (tR) and absorbance values of detected peaks in samples with those obtained by injection of each pure standard. To verify the peak identification and the absence of interferences at the analytes tR, some samples were also added with known concentrations of compounds. Quantification of analytes was performed using an external calibration with pure standards of each compound. Linear ranges between 0.5 to 40 µg mL^{-1} were obtained with the exception of naringin, naringenin and kaempferol (0.5-20 µg mL^{-1}) with coefficient of determination (r^2) higher than 0.991 for all the studied analytes.

For Car, linear ranges between 0.0625-20 µg mL^{-1}, with r^2 values higher than 0.9972 were obtained. Results were expressed as µg g^{-1} (PC) and ng g^{-1} (Car) of cane dry weight (DW). The software used to control the HPLC-DAD system and to process data was Chromeleon™ 7.1.

Total phenolic content

The TPC was spectrophotometrically measured with an UV–vis spectrophotometer Cary-50 (Varian Inc., Mulgrave, Australia) from an aliquot of the extract, from which the solvent has been removed and re-suspended with ethanol/water (50:50 v/v). To quantify TPC Folin–Ciocalteu assay (FC) as reported by Antoniolli *et al.* [2] at 765 nm and the direct reading of the absorbance

at 280 nm of the sample diluted 1:100 v/v was used. Results were expressed as mg of gallic acid equivalents per g of cane dry weight (mg GAE g^{-1} DW) from calibration curves made with the standard solutions (three replicates) in the range between 20 and 200 mg L^{-1} (R^2 = 0.998 and R^2 = 0.999, respectively for each method).

Total antioxidant capacity

The TAC of cane extracts were evaluated by ORAC, ABTS and DPPH assays. These methods were selected because they are based on different reaction mechanisms, as explained above. Trolox was employed as standard and results expressed as µmol of Trolox equivalents per gram of cane dry weight (µmol TE g^{-1} DW) as mean ± standard deviation (SD).

The ORAC assay was performed as previously reported [2], with some modifications. Cane extract solutions prepared as per TPC determination were diluted to 1 : 500 v/v in 75 mmol L^{-1} NaH_2PO_4-Na_2HPO_4 (pH 7.0). Later, 50 µL aliquots of diluted samples and Trolox standards (0 – 50 µmol L^{-1}) were added to a 96-well plate. Then, 100 µL of fluorescein solution were added and the mixture incubated 7 min at 37 °C before addition of 50 µL of 140 mmol L^{-1} peroxyl radical generator AAPH. Fluorescence was monitored at 485 nm excitation and 538 nm emission with 1 min intervals for 90 min using a microplate fluorometer (Fluoroskan Ascent FL, Thermo Fisher Scientific Inc, Wilmington, DE). The area below the curve of the fluorescence decay during 90 min was calculated for each sample by integrating the relative fluorescence curve.

For ABTS assay the method described by Re *et al.* [29] with some modifications was employed. ABTS radical cation ($ABTS^{\bullet+}$) was produced by mixing 2.5 mL of 7 mM ABTS stock solution and 44 µL of 140 mM $K_2S_2O_8$, both diluted with ultrapure water. This mixture was stored 12-16 h in darkness and then diluted with 80% methanol solution to an absorbance of 0.70 ± 0.02 at 734 nm. After addition of 10 µL of Trolox (0 – 2000 µmol L^{-1}) or the diluted sample to 2.5 mL of diluted $ABTS^{\bullet+}$ solution, absorbance readings were taken after 7 min of the initial mixing with an UV–vis spectrophotometer Cary-50.

The DPPH assay was based on Ruiz-Moreno *et al.* [30] with modifications. A stock solution of 61 µM $DPPH^{\bullet}$ was prepared in methanol and then stored at room temperature in darkness before use. The stock solution was diluted with methanol to an absorbance of 1.0 ± 0.1 at 515 nm. An aliquot of each diluted extract sample (10 µL) was added to 2.5 mL of fresh $DPPH^{\bullet}$ solution, shaken and incubated in darkness. Decrease in mixture absorbance was recorded after 30 min with an UV – vis spectrophotometer Cary-50.

Values of TPC and TAC were analyzed by Pearson Correlation test with Statgraphics® Centurion XVI v.16.0.7 (Statpoint Technologies Inc., Warrenton, VA, USA) statistical software. Pearson value (r) and p-values were computed.

Results and discussion

Total antioxidant capacity and TPC

Table 1 presents the results for the cane extracts. Samples showed levels ranging from 36 to 20 mg GAE g^{-1} DW and 32 to 22 mg GAE g^{-1} DW of TPC both by FC and 280 nm lecture, respectively. Gharwalová *et al.* [31] reported lower values (between 6 and 20 mg GAE g^{-1} DW) and Çetin *et al.* [4] obtained similar values of TPC (between 25 and 36 mg GAE g^{-1} DW) but with different cultivars than those presented here.

Several *in vitro* methodologies have been proposed to estimate the capacity of total antioxidants to neutralize the reactive oxygen and nitrogen species (ROS/RNS). An appropriate use of TAC measurement both, in the food and in vivo experiments, can give justification for understanding the complex phenomena. Additionally, comprehend a tool for initial sample screening when a quick decision about composition is needed [17]. In this work three assays, ORAC, DPPH and ABTS were used to provide a reliable assessment of AC. The TAC are generally associated with PC, so this relationship was analyzed. For the ORAC assay, the range of TAC was between 347 (MB3) and 660 (MB5) μmol TE g^{-1} DW. Karacabey *et al.* [32] found values ranging from 310 to 1300 μmol TE g^{-1} DW for Pinot Noir. The samples MB3, CS2 and CS1 showed the lowest values, while MB1, MB5 and ML had the highest. The ABTS and DPPH assays are based on relative ability of antioxidants to reduce a colored oxidant (blue/green $ABTS^{\bullet+}$ and purple $DPPH^{\bullet}$). The TAC determined by ABTS and DPPH varied from 108 to 221 μmol TE g^{-1} DW and from 88 to 188 μmol TE g^{-1} DW, respectively. Gullón *et al.* [33] analyzed the TAC of the ethyl acetate extracts from vine shoots liquors by

ABTS, with values in the range of 63 – 178 μmol TE g^{-1} DW and for DPPH were in the range of 23 – 42 μmol TE g^{-1} DW. Previous studies reported values of TAC from 86 to 239 μmol TE g^{-1} DW and 33 – 141 μmol TE g^{-1} DW by ABTS and DPPH methods, respectively [32,34]. The values for ABTS registered in the present work are similar to those reported by Karacabey *et al.* [32] and slightly superior than those presented by Gullón *et al.* [33], but the DPPH are higher than those presented by this author and Moreira *et al.* [34]. In the present study the TPC were measured by two methods, FC and 280 nm lecture expressed as mg of GAE, was positively correlated with ORAC, ABTS and DPPH values for the same samples with $r \geq 0.83$ (see Table 2). This is in agreement with reports that showed high correlation between TPC and TAC in cane samples [31,32]. Additionally, there is positive correlation amongst TAC assays ($p \leq 0.05$, $r \geq 0.82$), having the DPPH assay higher correlation with ORAC than ABTS.

Identification and quantification of bioactive compounds

By HPLC-DAD, 21 PC belonging to different chemical families including non-flavonoids (hydroxybenzoic and hydroxycinnamic acids, stilbenes and phenyl ethanol analogs) and flavonoids (flavanols, flavanones and flavonols) were determined and quantified. The PC were successfully separated and their identity attributed by comparing their elution times and UV-vis spectra with those obtained for pure standards. Figure 1 shows that MB1 has the largest number of PC. In terms of total concentration, expressed as the sum of quantified compounds, samples MB1 and SB1 exhibited the highest amounts.

For all the analyzed samples, the family of stilbenes presented the highest levels, with concentrations ranging between 2673 and 10634 µg g^{-1} DW (data not shown). The cultivars that presented highest amounts were Sauvignon Blanc (SB1), followed by Malbec (MB1) and Viognier (VG). For the last cultivar, this is the first report of the presence of stilbene compounds. Previous studies of canes showed values ranging from 4000 to 7000 µg g^{-1} DW in cv. Pinot Noir [3,6], which is in agreement with our data for samples of the same variety (PN, 5637 µg g^{-1} DW). Gabaston *et al.* [35] found 4700 µg g^{-1} DW in Merlot, 3400 µg g^{-1} DW in Chardonnay and 4000 µg g^{-1} DW in Sauvignon Blanc, while we found 4629 µg g^{-1} DW in Merlot, 4110 µg g^{-1} DW in Chardonnay and between 7585 and 10634 µg g^{-1} DW in Sauvignon Blanc. Some authors [9,35] reported higher values ranging from 6500 to 8500 µg g^{-1} DW in Cabernet Sauvignon, but Piñeiro *et al.* [8] informed lower concentrations in Malbec (4700 µg g^{-1} DW), as compared with the data presented here. The major stilbenoid compound was ε-viniferin with the highest level in sample SB1 (10552 µg g^{-1} DW), representing about 60% of total assessed PC. Other authors have reported lower values with respect to our data for ε-viniferin, ranging from 2600 to 3300 µg g^{-1} DW in Sauvignon Blanc canes [5,35], 2200 µg g^{-1} DW in Cabernet Sauvignon, 1900 µg g^{-1} DW in Merlot, 1300 µg g^{-1} DW in Chardonnay, 2600 µg g^{-1} DW in Malbec and 3700 µg g^{-1} DW in Pinot Noir [8,10,35]. Zwingelstein *et al.* [11] informed values of ε-viniferin ranging from 1620 to 6667 µg g^{-1} DW in French grape varieties. The resveratrol dimer ε-viniferin has been reported as having several beneficial health properties [36]. This fact

highlights the relevance of the high concentrations found in this work. It is also interesting because there are evidence that it is more effective than *trans*-resveratrol in improving some functions of vascular and heart system [37]. As well, there are reports proving that all oligomers of trans-resveratrol (i.e. ε-viniferin) exhibit remarkably higher TAC than *trans*-resveratrol [20]. The stilbene *trans*-resveratrol was the second most abundant compound detected after the *ε*-viniferin. It was found at levels between 6 (CH) and 250 μg g^{-1} (CS2), in accordance with published data [4,31,38]. However, our results for *trans*-resveratrol were lower than in other reports, with values ranging from 122 to 7306 μg g^{-1} [3,5,8–11,13,19,26,35]. Higher levels of *trans*-resveratrol than ε-viniferin may be due to the influence of storage conditions (time, temperature, sample treatment) of canes [5,7,11,13,20,39]. In fact, some authors found that the post-pruning storage period significantly increase resveratrol levels, but not those of ε-viniferin [11,46]. On the contrary, this changes are not observed if grape canes are frozen or ground immediately after collection [7,40]. At the industrial level is important to decide which are the ideal storage conditions for optimizing phytochemical contents, as well as to make an efficient processing of this by-product [11,13]. The data reported up to now suggest that exist the possibility of manage the chemical composition of stilbenes in canes, particularly searching strategies to increase the level of a given compound according to a specific necessity of bioactivity. Besides of that, there are necessity of more studies related to the bioaccesibility and bioavailability of compounds. In the present work, samples were immediately

dried and processed after pruning, so the storage time of canes was minimal before its analysis. Therefore, the high levels of ε-viniferin found in the present work could be explained by the way that vegetal material was processed soon after pruning. As well, it is noteworthy that the higher levels of stilbenes found in our data may be related with the vineyards exposition to UV-B irradiance, since in Mendoza's region the high altitude vineyards (1000 to 1450 m a.s.l.) receive more UV-B and consequently produce more secondary metabolites than others located in lower altitudes [11,41–43]. In terms of bioavailability of compounds found, there are reports showing that tra*ns*-resveratrol has high stability after the gastrointestinal simulation, which turned it into a compound with great bioactive potential [44]. Willenberg *et al*. [45] reported that ε-viniferin is not able to pass the intestinal barrier, while *trans*-resveratrol do it and can be metabolized. Besides of that, this oligomer may act locally on the intestinal epithelium. Other study informed that the *trans*-resveratrol and ε-viniferin were able to reduce intestinal glucose uptake from isolated porcine jejunum and ileum *in vitro*. In this case, ε-viniferin exhibited the strongest inhibitory potential, which may be useful to prevent diseases as arteriosclerosis and diabetes [46].

For the flavonoid family, the more abundant compounds were (+)-catechin and (-)-epicatechin, with maximum content in MB1 and minimum in MB3. For all samples, (+)-catechin concentrations were higher than those of (-)-epicatechin (between 74% and 50 % of total flavonols content), whereas the sample MB3 showed an opposed tendency. These flavanols have been also reported in

previous studies, with maximum values found for (+)-catechin and (-)-epicatechin of 1100 and 500 μg g^{-1} DW, respectively [38,47]. It is interesting to point out that the concentrations found for (+)-catechin and (-)-epicatechin in the present work are similar to those reported for grape pomace extracts (GPE) [2,48]. This fact may be helpful to obtain a rich extract in those compounds, with the possibility of different applications than GPE since cane extracts have negligible content of anthocyanins (red color).

In all the analyzed samples syringic, cinnamic and caftaric acids were found at quantifiable levels. The last compound was the most abundant of this family in all samples, with maximum concentration in sample SB1 (1755 μg g^{-1} DW). Liu *et al.* [49] showed that caftaric acid has ability to inhibit the oxidative damage of free radicals in vitro on biological macromolecules as proteins, lipids and DNA. The reported levels for this phenolic acid in grape canes are relevant for exploring the by-product as a new source of the compound for biotechnological applications as functional ingredient, as well as natural antioxidant in food systems. Gonthier *et al.* [50] studied the microbial metabolism of caftaric acid, showing that their metabolites might exert local effects on the colon and also in a systemic way. Otherwise, there is little information about bioaccesibility and bioavailability of caftaric acid, thus requiring new studies to better understand it potentiality as bioactive ingredient.

OH-tyrosol was detected in all samples at levels higher than 270 μg g^{-1} DW, with a maximum concentration of 367 μg g^{-1} for VG. OH-tyrosol was reported here for

the first time in grape canes, whereas few studies had reported its presence in wine derivatives as pomace, where it was found at lower levels (9 to 39 μg g^{-1} DW) [48]. Its high antioxidant power has been demonstrated *in vitro*, as well as its preventive capacity in several pathologies [51], thus highlighting the potentiality of the results reported here. In terms of bioavailability, OH-tyrosol is absorbed in the small bowel and colon by passive transport with an efficiency that oscillates from 75% up to 100%. This fact underlines its potential role as a nutraceutical and as a therapeutic agent [52].

In the present work, we also found different flavonols, being quercetin-3-galactoside the most abundant in all samples, with concentrations ranged between 6 and 988 μg g^{-1} DW. This flavonoid has been never reported in canes. Studies indicated that quercetin glucosides are absorbed in the upper parts of the gastrointestinal tract, probably at duodenum [53]. Naringin and naringenin were determined in most samples with a maximum concentration of 247 and 139 μg g^{-1} DW, respectively. These flavonols have been previously reported in vine shoot extracts for Moreira *et al.* [34], with levels ranging from 2 to 50 μg g^{-1} DW of extract. These compounds have protective effects against metabolic diseases, thus being the cane extracts a potential ingredient or additive for the prevention and management of these type of diseases. Despite their enormous health benefits, naringin and naringenin have low bioavailability so, some authors have proposed their transport by using nanoparticles or nanosuspensions to increase their availability in a biological system [54,55].

Two Car were determined and quantified by comparing their elution times and UV-vis spectra with pure standards by HPLC-DAD (Fig. S1). Table 3 present the results obtained for Car quantification. The compounds lutein and *β*-carotene were quantified at levels ranged between 350 and 2400 ng g^{-1} DW in all samples, being the first report for these bioactive compounds in grape canes. The levels of *β*-carotene were similar in all samples. Only few studies had reported Car in wine derivatives as leaf and berry [14–16]. Chitarrini *et al.* [15] found that β-carotene concentrations (84 – 146 mg Kg^{-1} fresh leaves) did not vary significantly over the time course after mechanical wounding, while lutein decreased during the first 6 h post procedure (160 – 251 mg Kg^{-1} fresh leaves). Gutiérrez-Gamboa *et al.* [16] informed levels ranging from 1336 and 364 to 3003 and 704 µg g^{-1} DW in grape extracts for β-carotene and lutein, respectively. Bunea *et al.* [14] studied the Car concentration in grape skin extracts cultivated in organic and conventional systems, obtaining values in the range of 0.23 – 0.59 µg g^{-1} FW and between 0.47 – 0.85 µg g^{-1} FW for lutein and *β*-carotene, respectively. Lutein and *β*-carotene are natural pigments, which are currently allowed as food additives due to their bioactive properties [24], so the identification of new sources of economic access are interesting from a technological point of view. Like other carotenoids, the bioavailability of these compounds are relatively low due to their hydrophobicity, so these compounds need a carrier to improve their absorption and further distribution [56].

In light of the results reported, we established that canes from the MB, ML, CS, CF, PN, VG, SB and CH grape varieties had different phytochemical compositions, showing diverse qualitative and quantitative profiles and varying AC. The production of secondary metabolites by vine plants depends not only on genetic characteristics, but also on growth environmental conditions. The content of phenolic compounds varied between the different cultivars and within the same variety, likely due differences in the availability of certain compounds among varieties, vintages and terroir-related effects. Furthermore, factors as storage condition, extraction temperature, extraction time and solvent composition can influence the phytochemical profile obtained. Taking into account the grape varieties from where canes were obtained, the sample MB1 of Malbec showed up from the other cultivars in terms of its high content of most PC, especially of flavonoid and stilbene families. This sample comes from Gualtallary location, which is characterized by vineyards at high altitudes ca. 1500 m a.s.l.) with cooler areas than the other locations. This fact is associated with previous reports where high altitude vineyards have high PC concentrations since a superior UV-B exposure of plants [41] and literature cited therein. The canes of Viognier variety were characterized for the first time, standing out from the other varieties for its highest content of OH-tyrosol. The particularly high PC contents in some samples was well correlated with their AC, as shown in Table 2. Note that Malbec is the main cultivar in Argentina, representing 37% of the red grape area, being considered as the emblematic wine for the country. Accordingly, Malbec is also

the variety producing most pruning residues so application of these by-products may be of interest.

Conclusions

Contents of PC in cane extracts obtained from different grape varieties implanted in Argentina and their correlation with TAC were presented. This report also allowed the determination of compounds different than stilbenes, some of them that had not been reported before such as quercetin-3-galactoside and OH-tyrosol. The Car compounds lutein and ß-carotene were also reported for the first time in grape canes. The new information is important to justify the properties of extracts and to establish possible synergic effects between compounds for a given TAC obtained. Knowledge of the profiles and properties of the samples will increase the value of possible functional ingredients, helping to make a decision when selecting of the most convenient material for scale extraction.

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Figure legends

Figure 1: Heat-map of individual PC for each sample. Green boxes indicate that a compound occurred at higher concentration than the mean level in a sample, while red boxes means the compound was at a lower level. The medium colors represent intermediate concentrations between higher and lower values. Average contents (µg g^{-1} DW cane) with their SD, n = 3 replicates. Samples identification as Table 1. n.d., not detected.

Figure 1: Heat-map of individual PC for each sample. Green boxes indicate that a compound occurred at higher concentration than the mean level in a sample, while red boxes means the compound was at a lower level. The medium colors represent intermediate concentrations between higher and lower values. Average contents (μg g^{-1} DW cane) with their SD, n = 3 replicates. Samples identification as Table 1. n.d., not detected.

	MB					CS			CF		ML	V	PN	SB		CH
	MB1	MB2	MB3	MB4	MB5	CS1	CS2	CS3	CF1	CF2	ML			SB1	SB2	
HYDROXYBENZOIC ACIDS																
Syringic acid	52 ± 2	34 ± 3	77 ± 4	18 ± 1	23 ± 1	22 ± 3	23 ± 1	24 ± 1	25 ± 3	30 ± 1	35 ± 2	42 ± 3	33 ± 5	31 ± 2	28 ± 3	29 ± 3
HYDROXYCINNAMIC																
Cinamic acid	24 ± 2	12 ± 1	11 ± 1	15 ± 2	19 ± 3	15 ± 1	20 ± 2	16 ± 1	25 ± 2	15 ± 1	11 ± 1	19 ± 2	19 ± 2	17 ± 1	20 ± 1	9 ± 1
Caftaric acid	1017 ± 39	803 ± 120	704 ± 76	609 ± 24	923 ± 79	671 ± 47	587 ± 29	790 ± 50	925 ± 88	789 ± 10	1211 ± 72	916 ± 14	561 ± 69	1755 ± 10	1235 ± 22	644 ± 24
p-coumaric acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	10 ± 8	n.d.	n.d.	n.d.	n.d.
STILBENES																
Trans-resveratrol	30 ± 3	n.d.	n.d.	n.d.	n.d.	n.d.	250 ± 36	96 ±14	n.d.	50 ± 7	75 ± 9	209 ± 25	159 ± 6	82 ± 8	n.d.	6 ± 1
E-viniferin	10001 ± 568	5566 ± 570	3137 ± 350	3835 ±298	5052 ± 681	3884 ± 627	2924 ± 447	2577 ± 290	2777 ± 467	6318 ± 288	4554 ± 496	8948 ±106	5478 ± 841	10552 ± 147	7585 ± 105	4104 ± 383
FLAVANOLS																
(+)-catechin	3718 ± 169	2546 ± 239	53 ± 3	2219 ± 132	2100 ± 274	1538 ± 122	1061 ± 177	1834 ± 39	907 ±120	1641 ± 80	1516 ± 185	2173 ± 229	1095 ± 175	2859 ± 151	2294 ± 17	739 ± 87
Procyanidin B2	107 ± 4	75 ± 13	52 ± 6	42 ± 1	44 ± 2	42 ± 2	37± 1	46 ± 1	49 ± 3	65 ± 3	52 ± 2	84 ± 2	46 ± 4	71 ± 5	80 ± 1	43 ± 3
(-)-epicatechin	2486 ± 40	820 ± 122	125 ±12	650 ± 52	716 ± 32	807 ± 57	289 ± 3	229 ± 16	294 ± 27	1205 ± 55	970 ± 134	1051 ± 105	390 ± 48	1049 ±24	680 ± 35	566 ± 100
(-)-gallocatechin	465 ± 35	151 ± 1	50 ± 3	339 ± 29	124 ± 11	80 ± 10	70 ± 8	104 ± 3	180 ± 21	119 ± 16	196 ± 27	156 ±18	89 ± 6	263 ± 28	52 ± 5	138 ± 10
(-)-epigallocatechin gallate	106 ± 1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	27 ± 5	n.d.	n.d.	n.d.	n.d.	n.d.
(-)-gallocatechin gallate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3 ± 0.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
(-)-epicatechin gallate	157 ± 14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FLAVANONES																
Naringin	74 ± 6	37 ± 2	22 ± 1	12 ± 1	28 ± 0.2	1 ± 0.1	7 ± 0.5	n.d.	39 ± 2	59 ± 0.1	60 ± 4	92 ± 7	200 ± 11	94 ± 0.1	62 ± 0.3	247 ± 18
Naringenin	61 ± 2	63 ± 5	42 ± 1	35 ± 0.4	44 ± 3	109 ± 9	139 ± 10	82 ± 1	45 ± 3	30 ± 0.1	51 ± 2	51 ± 3	69 ± 3	67 ± 1	75 ± 0.4	57 ± 2
FLAVONOLS																
Astilbin	24 ± 4	29 ± 5	15 ± 1	18 ± 1	57 ± 5	n.d.	n.d.	n.d.	n.d.	40 ± 9	26 ± 2	n.d.	n.d.	n.d.	n.d.	n.d.
Quercetin-3-galactoside	522 ± 5	988 ± 100	179 ± 14	252 ± 1	321 ± 28	54 ± 6	113 ± 19	102 ± 12	26 ± 2	6 ± 0.1	82 ± 3	317 ± 4	157 ± 19	202 ± 26	51 ± 5	134 ± 10
Quercetin-3-glucoside	292 ± 28	338 ± 59	212 ± 20	349 ± 31	167 ± 24	73 ± 4	n.d.	35 ± 1	172 ± 18	n.d.	87 ± 4	84 ± 10	82 ± 11	n.d.	107 ± 2	n.d.
Kaempferol-3-glucoside	63 ± 3	54 ± 2	40 ± 1	43 ± 1	40 ± 3	29 ± 1	27 ± 1	40 ± 1	58 ± 4	38 ± 1	117 ± 15	51 ± 3	29 ± 3	92 ± 10	45 ± 2	41 ± 2
Kaempferol	64 ± 4	n.d.	n.d.	n.d.	1 ± 0.01	n.d.	n.d.	n.d.	n.d.	n.d.	3 ± 0.1	5 ± 0.1	1 ± 0.01	92 ± 6	29 ± 1	n.d.
OTHER COMPOUNDS																
OH-tyrosol	350 ± 12	346 ± 11	270 ± 5	289 ±4	296 ± 11	285 ± 6	296 ± 4	296 ± 6	308 ± 4	310 ± 7	309 ± 11	367 ± 34	291 ± 3	326 ± 3	358 ± 4	315 ± 5
TOTAL LMW-PPs	19413	11763	4921	8678	9880	7499	5697	6189	5748	10625	9268	14427	8429	17299	12536	6768

Lower to higher concentration levels