

## Assessment of grapevine stems as source of phenolics with antioxidant properties

### Escobajos de la vid como fuente de compuestos fenólicos con propiedades antioxidantes

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Originales: *Recepción:* 11/07/2019 - *Aceptación:* 12/10/2019

#### ABSTRACT

Winemaking industry generates considerable amounts of bunch stems that are usually wasted despite their potential value as source of bioactive compounds. Phenolic profiles and antioxidant capacity (AC) of bunch stem extracts from eight grape varieties of *Vitis vinifera* L. were determined. Sixteen phenolic compounds (PC) were quantified by high performance-liquid chromatography-diode array detection (HPLC-DAD). The maximum concentrations corresponded to the flavanols (+)-catechin (6462  $\mu\text{g g}^{-1}$  DW) and procyanidin B1 (1987  $\mu\text{g g}^{-1}$  DW), followed by the hydroxycinnamic acid caftaric acid (2967  $\mu\text{g g}^{-1}$  DW). Naringin, myricetin and OH-tyrosol were identified for the first time in grape bunch stems. Total phenolic content (TPC) of extracts, assessed as gallic acid equivalents (GAE), ranged between 47 and 125 mg GAE  $\text{g}^{-1}$  DW. The AC of extracts was appraised by ORAC, ABTS and DPPH assays, with a good correlation between TPC and AC when measured by ABTS and DPPH ( $r \geq 0.92$ ), while for ORAC the correlation was lower ( $r \leq 0.41$ ). Samples of cv. Malbec, the most representative variety of Argentina, presented the highest contents in PC, particularly flavanols. The results showed that grape bunch stems may be an inexpensive, sustainable and high value source of bioactive compounds as functional ingredients.

#### Keywords

antioxidant capacity • bioactive compounds • grape bunch stems • industry by-products.

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## RESUMEN

La industria vitivinícola genera cantidades considerables de escobajo que generalmente se desperdician a pesar de su valor potencial como fuente de compuestos bioactivos. En este trabajo se determinaron los perfiles fenólicos y capacidad antioxidantes (CA) de extractos de escobajo de ocho variedades diferentes de *Vitis vinífera* L. Se cuantificaron 16 compuestos fenólicos (PC) utilizando cromatografía líquida de alta resolución acoplada a detector de arreglo de diodos (HPLC-DAD). Las concentraciones más elevadas obtenidas correspondieron a los flavanoles (+)-catequina (6462  $\mu\text{g g}^{-1}$  peso seco) y procianidina B1 (1987  $\mu\text{g g}^{-1}$  peso seco), seguido del ácido caftárico (2967  $\mu\text{g g}^{-1}$  peso seco). La naringenina, miricetina y OH-tirosol fueron identificados por primera vez en escobajos. El contenido total de compuestos fenólicos (TPC) de los extractos determinado con equivalentes de ácido gálico (GAE) presentó valores entre 47 y 125 mg GAE  $\text{g}^{-1}$  peso seco. La CA de los extractos fue determinada mediante las técnicas ORAC, ABTS y DPPH, evidenciando una buena correlación entre TPC y la CA medida mediante ABTS y DPPH ( $r \geq 0,92$ ), mientras que para ORAC la correlación fue más baja ( $r \leq 0,41$ ). La muestra de variedad más representativa de Argentina, cv. Malbec, presentó los mayores niveles de PC, particularmente flavanoles. Los resultados evidencian que los escobajos pueden ser una fuente económica, sostenible y de alto valor de compuestos bioactivos para su utilización como ingredientes funcionales.

**Palabras clave**

capacidad antioxidante • compuestos bioactivos • raquis de uva • subproductos industriales

## INTRODUCTION

Viticulture is one of the world's most important agro economic activities with an annual production of more than 70 million t of berries coming from a cultivated area of 7.5 million ha (24). Currently, Argentina's vineyards represent around 3% of the grape cultivated area worldwide, indicating the economic importance of this activity in the region (17). About 97.8% of the grape produced in this area is used in the wine and must industry (16), which generates considerable amounts of woody wastes, especially bunch stems. Bunch stems consists of a main axis (rachis) and primary and secondary branches that ended in pedicels supporting the berries. This underused ligno-cellulosic residue, which accumulates during the process of eliminating the stems, constitutes 5% of the processed grape (12).

In the last years the potential valorization of this by-product has been increased, because of the information available regarding their content of purportedly health-promoting phytochemicals. Particularly, there are some reports informing about its richness in phenolic compounds (PC) belonging to the families of flavonoids, phenolic acids and stilbenes (1).

Furthermore, this raw material has also been showed as a rich source of dietary fiber (13). Normally, bunch stems are used for animal feeding and as organic fertilizer (compost) (1, 3), which limited their potential value as a source of bioactive compounds. Hence, the use of this raw material as source of PC would increase its economic value and reduce the environmental impact of winery activity (19).

Several studies have shown different biological properties for bunch stem extracts, mainly because their antioxidant capacity (AC) (31). The *in vitro* assays most commonly used to measure AC in food and biological samples have been oxygen radical absorbance capacity (ORAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,20-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS). The ORAC reaction mechanism is based on hydrogen atom transfer, while DPPH and ABTS methods are based on electron transfer (10). Thus, the information given by PC profiles and the material AC may help to understand which compounds, either acting individually or in interaction with others, are responsible for the antioxidant effects shown by the extracts, so providing an additional value of the extract.

For all the above, this raw material could be a rich potential source of PC with antioxidant biological properties that would provide added value and reduce the environmental impact of viticulture activity. This work presents the characterization of PC in bunch stems of different grapevine varieties cultivated in Argentina. The objective was to identify and quantify by HPLC-DAD, the individual PC of different families (flavanols, flavonols, stilbenes and phenolic acids) in bunch stem extracts of eight grape varieties cultivated in the region. Moreover, the *in vitro* AC (by ABTS, DPPH and ORAC) and TPC were measured to study the correlation amongst qualitative and quantitative profiles of individual PC.

## MATERIALS AND METHODS

### Chemicals

HPLC-grade standards of PCs (purity  $\geq$  95%), Trolox reagent (6-hydroxy-2,5,7,8-tetra-methylchroman-2-carboxylic acid), NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O, Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O, and fluorescein were from Sigma-Aldrich (Steinheim, Germany). HPLC-grade acetonitrile (MeCN), formic acid (FA) and methanol were from Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA). Ethanol and Folin-Ciocalteu reagent were from Merck (São Paulo, Brazil). 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 bunch stem samples from vineyards of *V. vinifera* of the cultivars Malbec (MB), Cabernet Sauvignon (CS), Cabernet Franc (CF), Chardonnay (CH), Sauvignon Blanc (SB), Pinot Noir (PN), Petit Verdot (PV) and Syrah (SY). Samples were obtained after the separation of bunch stems from berries at the beginning of the winemaking process during season (2018) from vineyards of different locations of Mendoza's region, Argentina. Each sample was freeze-dried for 5 days. The dried bunch stems were powdered in an analytical mill (A 11 basic; IKA, Staufen, Germany) and stored protected from light at room temperature in plastic tubes until extraction.

### Extract preparation

Extraction of PC from samples was performed by solid-liquid method according to previous reports (9), with slight modifications. Briefly, 1 g of powdered sample was extracted with 50 mL ethanol/water (50:50 v/v) in ultrasonic washer at 50 Hz and 60°C during 60 min. The mixture was centrifuged 10 min at 3000 rpm 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 each extract was filtered through a 0.2  $\mu$ m PTFE vial filter and analyzed by HPLC-DAD.

### Chromatography

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 C18 column (3.0 mm x 100 mm, 2.6  $\mu$ m) (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 (11) 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<sup>-1</sup>. The column temperature was 35°C, and the injection volume was 1  $\mu$ L. The quantification wavelengths for different families of analytes were 254 nm, 280 nm, 320 nm, and 370 nm. Analytes present in the samples were quantified using an external calibration with pure standards also used to achieve identification. Results were expressed as  $\mu$ g g<sup>-1</sup> PC of dry weight (DW) of bunch stem. 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. To quantify total PC Folin-Ciocalteu assay (FC) as reported by Antonioli *et al.* (2015) 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 GAE g<sup>-1</sup> DW from calibration curves made with the standard solutions (three replicates) in the range between 20 and 200 mg L<sup>-1</sup> (R<sup>2</sup> = 0.998 and R<sup>2</sup> = 0.999, respectively for each method).

### Antioxidant capacity

The AC of bunch stem extracts were evaluated by ORAC, ABTS and DPPH assays (10). 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 bunch stem dry weight (μmol TE g<sup>-1</sup> DW) as mean ± standard deviation (SD).

The ORAC assay was performed as previously reported (2), with some modifications. Stem extract solutions were diluted ranging from 1: 750 to 1: 1500 v/v in 75 mmol L<sup>-1</sup> NaH<sub>2</sub>PO<sub>4</sub>-Na<sub>2</sub>HPO<sub>4</sub> (pH 7.0), due to their differences in PC content. Later, 50 μL aliquots of diluted samples and Trolox standards (0 - 50 μmol L<sup>-1</sup>) 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<sup>-1</sup> peroxy 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 Ferreyra *et al.* (2019) was employed. ABTS radical cation (ABTS•+) was produced by mixing 2.5 mL of 7 mM ABTS stock solution and 44 μL of 140 mM K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, 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<sup>-1</sup>) or the diluted sample to 2.5 mL of diluted ABTS•+ 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 Ferreyra *et al.* 2019. A stock solution of 61 μM DPPH• 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• solution, shaken and incubated in darkness. Decrease in mixture absorbance was recorded after 30 min with an UV - vis spectrophotometer Cary-50.

### Statistical analysis

Values of PC, TPC and AC 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

### Identification and quantification of bioactive compounds

Table 1 (page 421) shows individual PC from bunch stem extracts of the different varieties. In terms of total low molecular weight PC (LMW-PCs) concentration, expressed as the sum of quantified compounds, samples MB and PN exhibited the highest amounts.

For all the analyzed samples, the flavanol family presented the highest levels, with concentrations ranging between 1442 and 10605 μg g<sup>-1</sup> DW (data not shown). The cultivars that presented highest amounts were Malbec (MB), followed by Pinot Noir (PN) and Chardonnay (CH). The major flavanol compound was (+)-catechin with the highest level in samples MB and PN, representing about 25% of total assessed PC. It was found at levels between 1249 (PV) and 6462 μg g<sup>-1</sup> (MB), in accordance with previously published data (21).

**Table 1.** PC of bunch stem extracts from different *V. vinifera* L. varieties. Average contents ( $\mu\text{g g}^{-1}$  DW), n = 3 replicates. Samples identification: MB= Malbec; CH= Chardonnay; PN= Pinot Noir; SB= Sauvignon Blanc; PV= Petit Verdot; CS= Cabernet Sauvignon; CF= Cabernet Franc; SY= Syrah.

**Tabla 1.** PC de extractos de escobajo de diferentes variedades de *V. vinifera* L. Contenidos promedios ( $\mu\text{g g}^{-1}$  peso seco), n = 3 réplicas. Identificación de muestras: MB= Malbec; CH= Chardonnay; PN= Pinot Noir; SB= Sauvignon Blanc; PV= Petit Verdot; CS= Cabernet Sauvignon; CF= Cabernet.

	MB	CH	PN	SB	PV
<b>Hydroxybenzoic acids</b>					
Gallic acid	75 ± 2	50 ± 1	58 ± 1	90 ± 1	145 ± 2
Syringic acid	90 ± 1	90 ± 3	89 ± 1	32 ± 1	44 ± 1
<b>Hydroxycinnamic acids</b>					
Caftaric acid	2520 ± 9	2967 ± 52	2196 ± 18	891 ± 38	814 ± 6
<b>Stilbenes</b>					
$\epsilon$ -viniferin	107 ± 1	84 ± 7	612 ± 52	1373 ± 7	326 ± 20
<b>Flavanols</b>					
Procyanidin B1	1987 ± 58	1317 ± 60	1230 ± 23	n.d.	n.d.
(+)-catechin	6462 ± 229	4471 ± 46	6204 ± 13	2209 ± 170	1249 ± 15
Procyanidin B2	232 ± 2	217 ± 4	186 ± 1	69 ± 1	57 ± 1
(-)-epicatechin	354 ± 27	100 ± 6	116 ± 3	107 ± 5	23 ± 1
(-)-epigallocatechin gallate	749 ± 28	707 ± 2	701 ± 13	45 ± 2	112 ± 1
(-)-epicatechin gallate	804 ± 40	369 ± 7	735 ± 42	58 ± 3	n.d.
Naringin	18 ± 0.6	10 ± 0.6	19 ± 0.5	19 ± 0.2	n.d.
<b>Flavonols</b>					
Quercetin-3-galactoside	n.d.	1347 ± 20	n.d.	n.d.	16 ± 0.8
Quercetin-3-glucoside	n.d.	57 ± 4	n.d.	19 ± 0.5	10 ± 0.3
Myricetin	71 ± 1	72 ± 1	74 ± 1	n.d.	75 ± 1
Astilbin	1347 ± 20	734 ± 4	937 ± 27	n.d.	63 ± 2
<b>Other compounds</b>					
OH-tyrosol	162 ± 5	140 ± 1	102 ± 4	91 ± 2	145 ± 3
<b>Total LMW-PCs</b>	14978	11531	13257	5002	3080

n.d.: not detected.  
n.d.: no detectado.

However, our results for (+)-catechin were higher than in other reports, with values ranging from 120 to 1858  $\mu\text{g g}^{-1}$  (1, 14). It is interesting to point out that the (+)-catechin concentrations in grape stem are higher than those described in skins and seeds (33). This compound is very labile under oxidative conditions, which generate protective effect over macromolecules in the presence of free radicals (15).

Recent studies have shown that catechin inhibit the growth of foodborne pathogens (*E. coli* and *Salmonella*) and its potential use as antibiotic substitute (20). Its isomer, (-)-epicatechin, was also found with the maximum level of 354  $\mu\text{g g}^{-1}$  DW, which is in agreement with other studies (14, 21). Nevertheless, our results for (-)-epicatechin were lower than those published by Püssa *et al.* (2006), with values ranging from 600 to 1300  $\mu\text{g g}^{-1}$  DW. The differences amongst the data obtained with those available in the literature confirms the influence of viticultural management and environmental factors in the final composition of by-products. The highest concentration of procyanidin B1 and B2 corresponded again to sample MB (1987 and 232  $\mu\text{g g}^{-1}$  DW, respectively). Similar values of these dimmers were found by other authors (14, 29), which have shown these compounds can significantly contribute to the anti-inflammatory capacity of extracts (15). In this work, traces of naringin were identified in this plant material for the first time. Naringin have protective effects against metabolic diseases, therefore it may be used for the prevention and management of these types of diseases (7).

For the hydroxycinnamic acids family, caftaric acid was the most abundant, with levels ranging between 355 and 2967  $\mu\text{g g}^{-1}$  DW, and the CH sample showed the maximum content. This compound has shown able to inhibit the oxidative damage of free radicals at a biological level, so the exploration of this byproduct as potential source of the compound is important



for future biotechnological applications in food systems (18). Howbeit, Dominguez-Perles *et al.* (2016) reported superior values ranging from 2180 to 19460  $\mu\text{g g}^{-1}$  DW. In all the analyzed samples, gallic and syringic acids were found at quantifiable levels. The first compound was the most abundant of this family, with maximum concentration in sample SY (146  $\mu\text{g g}^{-1}$  DW), followed by syringic acid, in agreement with what was informed by Teixeira *et al.* (2014).

Concerning flavonols content, MB showed as the best variety, with level of 1418  $\mu\text{g g}^{-1}$  DW (data not shown). Astilbin resulted as the major flavonol, with concentrations between 63 and 1347  $\mu\text{g g}^{-1}$  DW, which is in agreement with reported by Gouvinhas *et al.* (2019). This compound have a many bioactivities, such as antibacterial and AC, being this last similar with commercial antioxidants used in the food industry (33). Myricetin was characterized for the first time in bunch stems, with a maximum level of 82  $\mu\text{g g}^{-1}$  DW, although traces of their glycosylated derivatives have been previously informed (15). Myricetin have beneficial effects against several human diseases such as cardiovascular pathologies and diabetes mellitus, being also a potent anticarcinogen (5).

The analysis of stilbenes content in bunch stems showed the presence of  $\epsilon$ -viniferin, being SB and PN the best sources of this compound. Previous studies of bunch stems showed values ranging from 47 to 5820  $\mu\text{g g}^{-1}$  DW for different cultivars (6, 26), and the data reported in this paper is within this range. The *trans*-resveratrol dimer  $\epsilon$ -viniferin has been reported as having beneficial health properties as cancer chemo-preventive (25), showing anti-obesity and anti-inflammatory effects *in vitro* and *in vivo* (22). This fact highlights the relevance of the high concentrations found in this work for some samples and the potentiality for future applications.

OH-tyrosol was found in all the analyzed samples at levels higher than 78  $\mu\text{g g}^{-1}$  DW. Its high antioxidant power and preventive capacity in several pathologies (8), demonstrate its importance as phenolics source with health beneficial effects.

Taking into account the grape varieties from where the bunch stems were obtained, the sample from Malbec stand out from the other cultivars in terms of its high content of most PC, especially of the flavanol family. Malbec is the main cultivar of Argentina (37% of the red grape area), making it source of most by-products with biological and technological interesting applications.

### Antioxidant capacity and TPC

Table 2 (page 423), presents the results for the bunch stem extracts. Sample MB showed the maximum levels of TPC both by FC and 280 nm lecture (125 and 85 mg GAE  $\text{g}^{-1}$  DW, respectively), while SB (62 and 47 mg GAE  $\text{g}^{-1}$  DW) had the lowest. Melo *et al.* (2015), obtained similar values of TPC (between 51 and 125 mg GAE  $\text{g}^{-1}$  DW) while Teixeira *et al.* (2014), reported lower values (between 27 and 36 mg GAE  $\text{g}^{-1}$  DW), although from different cultivars. In our work, the highest levels of PC were found in bunch stem extracts of MB and SY.

ORAC, ABTS and DPPH assays were chosen to determine AC in extracts, since they are based in different reaction mechanisms. ORAC employs hydrogen atom transfer reaction, which resembles actual reactions *in situ*, while ABTS and DPPH assays are based on electron transfer (30). As it is shown in table 2 (page 423), the range of AC by ORAC was between 292 (CS) and 1506 (CH)  $\mu\text{mol TE g}^{-1}$  DW. Previous studies reported values of AC ranged from 29 to 1508  $\mu\text{mol TE g}^{-1}$ , but they were obtained from dry stem extracts (4, 28). The samples CS and SB showed the lowest values, while CH and PN had the highest. The AC determined by ABTS and DPPH varied from 504 to 1003  $\mu\text{mol TE g}^{-1}$  DW and from 434 to 1097  $\mu\text{mol TE g}^{-1}$  DW, respectively. Melo *et al.* (2015) found slightly superior values ranging from 805 to 2419  $\mu\text{mol TE g}^{-1}$  DW by ABTS, while DPPH values were in the range of 473 and 1086  $\mu\text{mol TE g}^{-1}$  DW, which are similar to ours.

Because of the potent AC of PC, the correlation coefficient ( $r$ ) between the TPC and AC of bunch stem extracts were studied. A positive correlation between TPC assays measured by FC and 280 ( $p \leq 0.05$ ,  $r \geq 0.89$ ) was observed. The correlation between the TPC measured by both methods (FC and 280 nm) with ORAC values was lower ( $r \leq 0.41$ ). The results are summarized in table 3 (page 423). This poor correlation may be attributed to specific PC present in extracts with a given AC, the action of other phytochemical compounds, and/or the interaction between them (either synergism or antagonism) (3).

**Table 2.** Levels of TPC and AC evaluated by ORAC, ABTS and DPPH assays. Results are expressed as mean  $\pm$  SD, n = 3 replicates. Samples identification as table 1 (page 421).

**Tabla 2.** Niveles de TPC y AC evaluados por los ensayos ORAC, ABTS y DPPH. Los resultados se expresan como media  $\pm$  SD, n = 3 réplicas. Identificación de muestras como en tabla 1 (pág. 421).

	<b>MB</b> (mg GAE g <sup>-1</sup> DW)	<b>CH</b> (mg GAE g <sup>-1</sup> DW)	<b>PN</b> ( $\mu$ mol TE g <sup>-1</sup> DW)	<b>SB</b> ( $\mu$ mol TE g <sup>-1</sup> DW)	<b>PV</b> ( $\mu$ mol TE g <sup>-1</sup> DW)
MB	125 $\pm$ 2	85 $\pm$ 0.4	784 $\pm$ 24	1003 $\pm$ 43	1047 $\pm$ 18
CH	96 $\pm$ 0.2	62 $\pm$ 0.4	1506 $\pm$ 69	770 $\pm$ 14	822 $\pm$ 13
PN	103 $\pm$ 1	71 $\pm$ 0.5	894 $\pm$ 83	858 $\pm$ 16	867 $\pm$ 30
SB	62 $\pm$ 1	47 $\pm$ 1	340 $\pm$ 15	504 $\pm$ 24	434 $\pm$ 17
PV	73 $\pm$ 0.2	64 $\pm$ 0.3	344 $\pm$ 10	677 $\pm$ 14	617 $\pm$ 25
CS	68 $\pm$ 1	57 $\pm$ 0.3	292 $\pm$ 15	556 $\pm$ 33	562 $\pm$ 6
CF	101 $\pm$ 3	82 $\pm$ 3	625 $\pm$ 17	837 $\pm$ 18	888 $\pm$ 60
SY	111 $\pm$ 6	85 $\pm$ 3	605 $\pm$ 8	918 $\pm$ 19	1097 $\pm$ 77

**Table 3.** Pearson's correlation coefficients between the TPC and the AC in bunch stem extracts (n = 9).

**Tabla 3.** Coeficientes de correlación de Pearson's entre el TPC y la AC en los extractos de escobajo (n=9).

	<b>TPC FC</b>	<b>TPC 280</b>	<b>ORAC</b>	<b>ABTS</b>	<b>DPPH</b>
<b>TPC FC</b>	1	0.891*	0.409	0.985*	0.964*
<b>TPC 280</b>		1	0.066	0.921*	0.924*
<b>ORAC</b>			1	0.380	0.380
<b>ABTS</b>				1	0.970*
<b>DPPH</b>					1

\*, correlation is significant at p  $\leq$  0.05.

\*, la correlación es significativa a p  $\leq$  0,05.

Nevertheless, the correlation between both TPC and the other AC assays (ABTS and DPPH) were high ( $r \geq 0.92$ , table 3, page 423). Our study also showed that values found for ABTS and DPPH ( $r \geq 0.97$ ) methods are well correlated, but there is no good correlation with the results of ORAC ( $r \geq 0.38$ ).

Villaño *et al.* (2005) observed a similar behavior in their report. This study intends to determine the main individual PC responsible of the AC (table 4, page 424). The antioxidant effectiveness of these compounds is proportional to the number of -OH groups present in the aromatic ring(s). At the same time, the mechanism of reaction and availability of antioxidant groups related to the medium-solvent, as well as pH could affect the AC (23). The results showed that caftaric acid had a high correlation with AC measured by ORAC, following procyanidin B2 and (-)-epigallocatechin gallate ( $r=0.86$ ,  $r=0.84$  and  $r=0.83$ ). The high AC of caftaric acid could be explained by the presence of catechol (1,2-dihydroxybenzene) group and side chain conjugated double bonds that allow for delocalisation of the electrons improving stabilize the phenoxy radical formed. Therefore, cinnamic acids are better antioxidants than benzoic acids due to the presence of the -CH=CH-COOH group which enhances the AC more than the -COOH group. In the case of (-)-epigallocatechin gallate respect to procyanidin B2, its lowest correlation could be explained by glycosylation of 3-OH group that reduce the antioxidant properties of compounds (23). In contrast, the results showed that astilbin and syringic acid had strong correlation ( $r = 0.84$  and  $r = 0.78$ , respectively) with AC measured by ABTS and DPPH. In both methods, there are evidence of formation of covalent adducts between the phenolic compounds and cation radical (ABTS or DPPH). Thus, the new compounds exhibit antioxidant properties, which not reflect the antioxidant activity of compound itself, but the sum of antioxidant activities, which have a parent compound and a product of its reaction with the cation radical. This makes prediction of actual antioxidant properties according to chemical structure were complicated (32). However, the antioxidant efficiency of astilbin could be given by catechol group, -OH groups and glycosylation of 3-OH group, making it more effective than its aglicons.



**Table 4.** Correlation coefficient (r) values of PC concentration *versus* ORAC, ABTS and DPPH values of bunch stem extracts.**Tabla 4.** Valores del coeficiente de correlación (r) de la concentración de PC frente a los valores de ORAC, ABTS y DPPH de los extractos de escobajo.

	ORAC	ABTS	DPPH
<i>Gallic acid</i>	-0.668 *	-0.023	0.035
<i>Syringic acid</i>	0.820 *	0.820 *	0.785 *
<i>Caftaric acid</i>	0.865 *	0.535	0.460
<i>ε-viniferin</i>	-0.402	-0.384	-0.408
<i>Procyanidin B1</i>	0.708 *	0.732 *	0.656
<i>(+)-catechin</i>	0.588	0.793 *	0.775 *
<i>Procyanidin B2</i>	0.839 *	0.664	0.604
<i>(-)-epicatechin</i>	0.124	0.737 *	0.732 *
<i>(-)-epigallocatechin gallate</i>	0.826 *	0.792 *	0.758 *
<i>(-)-epicatechin gallate</i>	0.333	0.542	0.543
<i>Astilbin</i>	0.552	0.840 *	0.742 *
<i>Naringin</i>	0.211	0.231	0.112
<i>Quercetin-3-galactoside</i>	0.493	0.235	0.312
<i>Quercetin-3-glucoside</i>	0.730 *	-0.066	0.031
<i>Myricetin</i>	0.281	0.588	0.623
<i>OH-tyrosol</i>	0.469	0.541	0.416
<i>Total LMW-PCs</i>	0.685 *	0.768 *	0.718 *

\*, correlation is significant at  $p \leq 0.05$

\*, la correlación es significativa a  $p \leq 0,05$

On the other hand, the AC of syringic acid was favored by two methoxy substitutions at the *ortho* position relative to the hydroxyl (23). Apostolou *et al.* (2013) reported that polyphenols (-)-epicatechin and *trans*-caftaric acid were significantly correlated with IC50 values of dried stem extract measured by ABTS.

However, it is not easy to compare the AC data of bunch stem extracts with those reported in other studies, especially because of the differences in the methods and standard units used, type of cultivar and climate conditions, among others (1, 32).

The results reported in the present work indicate that bunch stem extracts constitute a rich source of bioactive PC together with other different biological compounds previously reported, like cellulose, xyloglucans and lignins (15). In addition, the extracts showed great AC, which make them potential alternatives for economic exploitation and utilization in the cosmetic, food and pharmaceutical industries as antioxidant source for several technological purposes (15).

## CONCLUSIONS

Contents of PC in bunch stem extracts obtained from different grape varieties cultivated in Argentina and their correlation with AC are presented. The results showed that bunch stem extracts from *V. vinifera* species constitutes a useful source of bioactive flavanol and hydroxycinnamic acid derivatives, with high AC. One important aspect to consider from this work, is the report, for the first time, of naringin, myricetin and OH-tyrosol. Regarding grape varieties, Malbec was highlighted from the other cultivars for its high PC content. In turn, the information generated here allow for explain the antioxidant properties of extracts and to establish possible synergic effects between compounds. In this sense, bunch stems can be considered an inexpensive and sustainable potential source of bioactive compounds of high value for future applications as functional ingredients.



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#### ACKNOWLEDGEMENTS

This work was supported by ANPCyT (BID PICT 2017-2325 to A.F. and BID PICT 2013-1856 to R.B.). The authors are also grateful to Roy Urvieta for providing the samples. S.F. is recipient of a CONICET doctoral scholarship. A.F. and R.B. are fellows of CONICET.