A comparative study of phenolic composition and antioxidant activity in commercial and experimental seedless table grapes cultivated in a Mediterranean climate

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Abstract

Grapes are important sources of phenolic compounds, which have numerous beneficial effects on human health. Scientific advances in the field of genetics have allowed the production of seedless table grape varieties, which are highly demanded by the consumers for their nutritional value and their easy consumption. In this study, total phenolic content (TPC), radical scavenging activity (RSA) and total anthocyanin content (TAC), were determined in 65 seedless table grape varieties (41 experimental and 24 commercial varieties). Due to crop conditions are directly linked to grape phenolic composition and, in order to obtain comparative results for establish varietal differences, all varieties were grown in Spain (under similar cultivation conditions). TPC ranged from 17-250 mg gallic acid equivalents/100 g fresh weight (FW), RSA ranged from 15-269 mg Trolox equivalents/100 g FW and TAC ranged from 0-168 mg cyanidin-3-glucoside/100 g FW. The TPC of the 65 seedless table grapes showed a high correlation (R^2= 0.90) with the antioxidant activity by RSA assay. Ultra-high performance liquid chromatography coupled to an ion-trap mass spectrometry detector was used to identify and quantify some phenolic acids, flavan-3-ols, flavonols and stilbenes in 14 experimental varieties. In some new experimental grapes analysed, the TPC and TAC was similar to those of some common table grapes with seeds, that indicated promising perspectives for their commercialization as potential sources of these bioactive compounds. In some grapes, high concentration of catechin, procyanidin B2, epicatechin, quercetin 3-β-D-glucoside, 4-hydroxybenzoic acid, vanillic acid, caftaric acid and rutin were found.

Keywords: Seedless table grapes; Phenolic content; Anthocyanin content; Radical scavenging activity; Varietal differences; UHPLC-IT-MS/MS

Introduction

During the last decades, there has been a considerable increase in the consumption of table grapes and their derivative products.[1] The grape is today one of the most cultivated fruits worldwide, reaching in 2014 a production of 74.2 million tons.[2] About 55% of grape production is used in the winemaking, whereas the remaining 45% is used us such or fresh derivatives.[3] Several studies have shown that grapes are among the richest sources of phenolic compounds having beneficial effects on human health, among them antioxidant activity, anti-inflammatory, antimicrobial and vasodilatory effects, antimutagenic and/or anticarcinogenic activities, the increase of the immunity and protection actions against cardiovascular and neurodegenerative diseases.[4-7] In grape berries, phenolic compounds are mainly found in seeds (60-70%) and in a minor quantity in skins (28-35%) and pulp (less than 10%).[1,8-10]

Phenolic compounds are secondary metabolites synthesized by plants, as a defense mechanism in response to stressful conditions.[11,12] The biosynthesis of phenolic compounds in grapes is regulated to genetic factors, but also the environmental factors (cultivation, ripening and harvest conditions) produce important differences among them.[1,6,13-15] Regarding previous data on phenolic compounds in grapes, among non-flavonoid compounds, hydroxybenzoic acids are mainly found in grape skins, whereas hydroxycinnamic acids (with higher antioxidant activity) are in the pulp.[1,16] Resveratrol, which has many positive biological effects for human health,[17] is the most important stilbene and is found in the grapes skin. The most prominent flavonoid compounds are flavanols that are found predominantly in seeds, flavonols and anthocyanins, mainly located in the berry grape skin.[1,13] Flavanols and flavonols are the most effective flavonoids in the prevention of oxidation and anthocyanins (glycoside forms of anthocyanidins) are natural pigments that give colorations ranging from red to blue to the skin of the grapes, according to the pH of the medium. They prevent the oxidation of low density lipoproteins.[18]

Table grapes are one of the most important sources of phenolic compounds in the Mediterranean

diet.[19,20] In the last years, traditional seeded varieties have been progressively replaced by seedless varieties. More recently, selection programs of new varieties are starting to use molecular markers to help optimize the process of selection (marker-assisted selection; MAS) most of them focused on seedlessness,[21,22] muscat flavor[23] and resistance to biotic[24] and abiotic stresses or environmental factors.[25] Nowadays, some of these works start to focus on the obtaining healthier varieties. In addition, in order to select grapes with high quality with respect to their phenolic composition, it is important to carry out comparative studies between new grape varieties, obtained by breeding programs, and commercial grape varieties subjected to similar cultivation conditions.

The objective of this work was to study the phenolic composition (TPC and TAC) and in vitro antioxidant capacity (RSA) of 41 experimental and 24 commercial table grapes, cultivated in Spain, in order to identify the most promising varieties with considerable levels of antioxidant activity. The most representative phenolic compounds (including hydroxybenzoic and hydroxycinnamic acids, flavonols, flavan-3-ols and stilbenes) were additionally quantified in 14 experimental varieties by UHPLC-IT-MS/MS.

Materials and methods

Chemicals and solvents

Folin Ciocalteu Reagent (FCR), 2,2-diphenyl-1-picrylhydrazyl (DPPH•), and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (Madrid, España). Potassium chloride, hydrochloric acid, ethanol (EtOH) and methanol (MeOH) LC-MS grade were purchased from Scharlau (Barcelona, España). Ammonium acetate and formic acid LC-MS grade were purchased from Fluka (Busch, Switzerland). Sodium acetate, acetic acid, and anhydrous sodium carbonate were obtained from Panreac Quimica

97 (Castellar del Vallès, Barcelona, España). Water (resistivity 18.2 MΩ cm) was obtained from a
98 Millipore Milli-Q-System (Billerica, MA, USA).
99 All analytical standards were of high purity grade (≥ 90%). Gallic acid, caftaric acid,
100 protocatechuic acid, chlorogenic acid, p-coumaric acid, caffeic acid, ferulic acid, syringic acid,
101 vanillic acid, catechin, epicatechin, epigallocatechin, epigallocatechin gallate, procyanidin B2,
102 quercetin, quercetin 3-β-D-glucoside, rutin, piceid and *trans*-resveratrol were from Sigma-
103 Aldrich (St. Louis, MO, USA), whereas 4-hydroxybenzoic acid were obtained from Acros
104 Organics (Geel, Belgium).
105 Stock standard solutions (1000 mg/L) were prepared by diluting in MeOH adequate
106 amounts of each compound and stored at -18°C. Working solutions (10-100 mg/L) were
107 prepared by appropriate dilution of the stock solutions with MeOH and stored at -18°C. Mixed
108 standard solutions were prepared daily by dilution of suitable volumes of working solutions
109 with MeOH for the analytical method development and its validation (1-25 mg/L).
110
111 **Samples**
112 65 frozen seedless table grape varieties were provided by Encin Grapevine Germplasm
113 Bank, located in Alcalá de Henares, Madrid. From the total grape varieties studied, 41 were
114 obtained by a breeding program by SNFL (identified with codes, SNFL_Nº; http://snfl-
115 group.eu/en/) and this grapes varieties were still in the experimental phase, 16 were commercial
116 grape varieties of SNFL (identified with codes, Comm_SNFL_Nº), 8 were obtained of Encin
117 Grapevine Germplasm Bank; http://www.madrid.org/coleccionvidencin/ (Scarlet (Accesion
118 number; ESP080-BGVCAM1399), Beauty seedless (ESP080-BGVCAM0797), Marroo
119 seedless (ESP080-BGVCAM2796), Corinthe noir (ESP080-BGVCAM1212), Flame seedless
120 (ESP080-BGVCAM2680), Blush seedless (ESP080-BGVCAM2708), Crimson seedless
121 (ESP080-BGVCAM2763) and Autumn Royal (ESP080-BGVCAM2793)). SNFL grape

varieties were grown under the same environmental and cultivation conditions in Murcia (Spain), whereas varieties from Encin Grapevine Germplasm Bank were all of them cultivated in Madrid (Spain). Grape varieties were classified by the visual color of the berry skin in: green-yellow (1 variety), pink (23), red (11), dark-violet (17) and blue-black (13) grapes.

All grape varieties were harvested during the 2015 when they were at the optimum maturity level to be consumed (19 °Brix). Sampling was performed in different days at the same time every day (between 7:00-10:00 am), in order to avoid deviations of data due to daily fluctuations. Taking into account that all grapes do not ripen identically within each cluster and in each clone of the same variety, a process of homogenization of the maturity stage of the harvested grapes, within each cluster, was carried out. For this, between 5-7 different clusters (depending on grape berry size) were taken from five different clones of each variety. The homogenization was performed according to the density of the grape berry separated from the cluster, which was determined by suspending the grape berries in different solutions of increasing concentration of sodium chloride (75-225 g/L). After selecting the most represented density for each variety (around 125–175 g/L NaCl), three packages of 100 g of each sample were frozen until use. Crimson seedless grape variety (first category, from South Africa), acquired in a supermarket in Madrid, was used to perform the optimization of the extraction process in whole grape berries.

Sample preparation

The sample preparation was carried out using the optimized extraction protocol developed in whole grape berries, as follows: 20 g of the berries were defrosted and crushed in a grinder. Once obtained a homogeneous crushing of the berries, 0.625 g were weighed by duplicate in falcon tubes where the extraction process took place. For this purpose, 5 mL of the extraction solvent (MeOH) were added (sample/solvent ratio 1:16, w/v) and the mixture was

147 stirred for 1 min in a Vortex (Rx^3, Velp Scientifica, Spain). Then, the samples were centrifuged
148 (Rotofix 32, Hettich zentrifugen, Germany) at 6000 rpm for 10 min. After that, a second
149 extraction of the resulting pellets was completed using the same volume of MeOH, and the
150 combined supernatants for each sample were filtered through 0.45 μm nylon membrane filters
151 and, finally, maintained at -18°C until analysis.
152
153 **Total phenolic content (TPC)**
154 The concentration of total phenolics in extracts was determined according to the Folin-
155 Ciocalteu method[26] with some modifications. A 75 μL aliquot of the sample extract was mixed
156 with 645 μL of Milli-Q water and 30 μL of FCR. Next, 75 μL of 20% (w/v) sodium carbonate
157 and 675 μL of Milli-Q water were added and the total solution was mixed briefly in the vortex.
158 The mixture was incubated for 60 min at room temperature in darkness. At the end of the
159 incubation period, absorbance was measured using a UV-Vis spectrophotometer (Cary 60,
160 Agilent, Spain) at the wavelength of 725 nm. A standard calibration curve was prepared with
161 gallic acid at a concentration range of 10-500 mg/L (w/v). The results were expressed as mg
162 gallic acid equivalents (GAE)/100 g of fresh weight (FW).
163
164 **Radical scavenging activity (RSA)**
165 The free radical $DPPH^{\bullet}$ scavenging activity of grape berry extracts was evaluated by a
166 modified colorimetric method proposed by Brand-Williams, Cuvelier and Berset.[27] In order to
167 estimate the RSA, firstly, a $DPPH^{\bullet}$ solution (40 mg/L, w/v) was freshly prepared in MeOH.
168 Then, 3.9 mL of this $DPPH^{\bullet}$ solution were mixed with 0.1 mL of the extract sample or 0.1 mL
169 of MeOH (blank) and the mixture was shaken in a Vortex. The reaction mixture was left for 60
170 min at room temperature in the dark. After the incubation period, the absorbance was measured

at 517 nm to determine the concentration of remaining DPPH• radical. Therefore, RSA (%) was calculated using the following equation:

$$RSA\ (\%) = \frac{(A_{DPPH} - A_{sample})}{A_{DPPH}} \times 100 \quad (1)$$

where A_{DPPH} is the absorbance of the DPPH• radical in the MeOH solution (blank) and A_{sample} is the absorbance of the DPPH• radical in the grape berry extract (sample). A standard calibration curve was prepared with Trolox at a concentration range of 0.5-400 mg/L (w/v). Finally, RSA of the samples was expressed as mg Trolox equivalents (TE)/100 g of FW.

Total anthocyanin content (TAC)

The total monomeric anthocyanin content of the grape berry extract was determined by using the pH-differential method proposed by Giusti and Wrolstad,[28] with some modifications. A 0.1 mL aliquot of extract was mixed with 0.9 mL of hydrochloric acid-potassium chloride buffer (0.025 M, pH 1.0) or with 0.9 mL of acetic acid-sodium acetate buffer (0.4 M, pH 4.5). Then, absorbance of the extracts were measured at two wavelengths, at 510 nm and 700 nm, against a blank cell filled with Milli-Q water. TAC was calculated and expressed as mg cyanidin-3-glucoside equivalents (cyn-3-glu)/100 g of FW. For this, the total absorbance of the extracts was determined by the equation (2) and TAC was calculated by the equation (3):

$$A_{total} = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5} \quad (2)$$

$$TAC = (A_{total} \times MW \times DF \times 1000)/(\varepsilon \times 1) \quad (3)$$

where MW = 449.2 g/mol, ε = 26900 L/mol·cm for cyn-3-glu and DF = dilution factor.

Chromatographic analysis by UHPLC-IT-MS/MS

An UHPLC system (Dionex UltiMate 3000, Thermo Scientific, MA, USA) coupled to an ion trap mass spectrometer detector (AmaZon series, Bruker, MA, USA) was used for chromatographic separation. An ACE Excel 2 C18-PFP column (100 mm x 2.1 mm, 2µm particle size, ACE, UK) was used as stationary phase, and the column oven temperature was set at 30 °C. The flow rate was 0.25 mL/min and the injection volume was 10 µL. The mobile phase consisted of MeOH (phase A) and Milli-Q water (phase B), both containing 2 mM ammonium acetate and 0.1% formic acid in a gradient elution mode. The initial composition was 20% A and 80% B. Then, a gradient elution was carried out, where phase A increased linearly up to 100% in the first 9 min, and then returned in 2 min to initial conditions. The column was then equilibrated for 1 min prior to next injection, yielding a total analysis time of 12 min. MS acquisition was carried out using an electrospray ionization interface (ESI) operating in negative ion mode. The capillary voltage was held at -4500 V, and the end plate offset at -500 V. The nebulizer was set at 20 psi, the dry temperature at 200 °C, and the dry gas at 10 L/min.[29] The ESI source parameters were initially optimized by direct infusion of standards solutions of each analyte (5 mg/L) at a flow rate of 4 µL/min, and multiple reaction monitoring (MRM) mode was employed for all analytes.

For quantification of phenolic compounds in the selected grape varieties, the analytical parameters of the UHPLC-IT-MS/MS method were studied, including linearity, intraday precision, accuracy, and matrix effects. To evaluate the linearity of the method, matrix-matched calibration curves were obtained for each compound. In this sense, according to the sensitivity of the UHPLC-IT-MS/MS system, the samples were spiked with the target compounds at four concentration levels and then, phenolic compounds were extracted from the grapes following the developed method (see sample preparation section). Calibration curves were performed by plotting the peak area of each analyte versus its concentration and were fitted by linear regression analysis. Solvent-based standard calibration curves were also constructed by using

working standard solutions subjected to the analytical proposed method, in order to evaluate matrix effect by comparing the slopes of the matrix-matched and solvent-based standard calibration curves. The accuracy (recovery %) was obtained by spiking the samples with a low concentration level (1 mg/L) of the matrix-matched calibration curve and subjecting them to the proposed method. Recovery values were calculated by comparing the areas of the spiked samples with the areas of simulated samples (samples spiked at the same concentration level but at the end of the extraction process prior to UHPLC-IT-MS/MS analysis), and were applied to quantify all the analytes. The method precision (inter-day) was determined in terms of within-laboratory reproducibility (% RSD) by the analysis of three replicates of two fortification levels (1-10 mg/L) on different days.

Statistical analysis

Evaluation and analysis of data were performed by SPSS for Windows software version 21.0 (IBM, Chicago, IL, USA). Results were subjected to analysis of variance (ANOVA), and significant differences among samples were located using Tukey's Honest Significant Difference (HSD) test; $p \leq 0.05$ was considered significant in all tests. All data were reported as the mean ± SD (n=4). In order to highlight relationships between TPC or TAC and RSA, a linear regression analyses were performed with the statistical program MS Excel (Microsoft Office 2016 Professional). The relative determination coefficient (R^2 adjusted) was reported.

Results and discussion

Optimization of extraction process

In order to optimize the extraction conditions of the phenolic compounds in the whole grape berry samples, different studies were undertaken, including the choice of extraction solvent, the type and time of agitation and the sample/solvent ratio. All these studies were carried out with

246 commercial Crimson seedless grape variety (from South Africa). A compromise between the
247 values obtained for the TPC, TAC and RSA, the time and reagent consumption in the extraction
248 process was taken into account, in order to select the best extraction conditions.

249 Firstly, a study of the extraction solvent was carried out under the following conditions:
250 simple extraction, stirring in vortex (for 1 min) and 1:2 (w/v) sample to solvent ratio. The
251 solvents evaluated were MeOH, EtOH, MeOH:water 1% HCl (6:1, v/v) and MeOH:water
252 (70:30, v/v). These solvents were chosen according to the solubility of the different compounds
253 of interest in organic and acidified media, in order to increase the extraction of the flavonoids.
254 Results obtained in this study (Fig. 1a) showed that the highest value of TPC was achieved with
255 MeOH:water (70:30, v/v), while with 100% MeOH slightly lower values were obtained. In
256 contrast, the highest RSA and TAC results were provided by MeOH extraction. Therefore,
257 MeOH was selected as extraction solvent. Then, a study was carried out to verify if successive
258 extractions with MeOH could increase the yield in the extraction of the phenolic compounds.
259 Results obtained indicated that a double extraction improved the yield of the extraction (Fig.
260 1b) and more extractions did not significantly improved the results. Moreover, this implied a
261 higher cost, more time consumption and the possibility of the phenolics being degraded. Other
262 types of agitation, including stirring in a magnetic plate (Shaker 18 pieces, Ovan, Spain) and in
263 an ultrasonic bath (Elmasonic S 30, Elma, Germany) for 5 min, were tested (Fig. 1b). Results
264 obtained were very similar, so stirring in a vortex for 1 minute (double extraction) were selected
265 as the optimal. Higher stirring times were not tested to avoid heating, oxidation and consequent
266 degradation of the phenolic compounds. Finally, a study was carried out to check the
267 sample/solvent ratio (1:4, 1:8, 1:16 and 1:20, w/v) more suitable to obtain a higher extraction
268 efficiency employing optimal conditions obtained in previous studies. Best results were found
269 for the 1:16 ratio (Fig. 1c).
270

Study of TPC, RSA and TAC in 65 seedless table grape varieties

After extraction optimization, the study of the 65 seedless table grape varieties was carried out. Quantitative and qualitative variations have been observed between grape berries in the phenolic composition according to the degree of maturity, climatic factors and post-harvest storage. For this reason, and in order to obtain comparative results to establish varietal differences, all grape varieties were grown in Spain, on similar cultivation conditions. Grapes were collected when they were at the optimum maturity level to be consumed, following an appropriate sampling process (see samples section). Results obtained for TPC, RSA and TAC are shown in Table 1 and Fig. 2.

Total phenolic content

TPC found in the 65 grape varieties was in the range between 17-250 mg of GAE/100 g FW (Table 1). The smallest value was provided by the SNFL_39 pink variety, while the highest value was provided by the Scarlet blue-black variety. Grapes classified by visual color exhibited TPC between 17–111, 23–94, 46-198 and 70-250 mg of GAE/100 g FW for pink, red, dark-violet and blue-black varieties, respectively. The TPC for SNFL_03 (green-yellow grapes) was 74 mg of GAE/100 g FW. Several studies assert that TPC in grapes with less color is low, because these varieties do not have anthocyanins in their skins, which contribute strongly to the TPC.[17] In our study, results showed that the green-yellow grape variety had a TPC greater than many pink, red and dark-violet grapes. These results are in agreement with Colombo et al. (2019) who reported that some white seedless table grapes had higher TPC than red seedless varieties (e.g. Centennial and Canner *vs* Beauty and King's Ruby). Phenolic compounds (flavanols and flavonols) are predominant in green-yellow berries, which can be responsible of the high TPC of the SNFL_03 experimental hybrid variety analysed in our study. A similar situation was observed in the pink berries, which had a higher TPC than most of the red and

dark-violet grapes. Even, the pink berry SNFL_05 (111 mg of GAE/100 g FW) had higher TPC than five of the blue-black berries analyzed (Marroo seedless, Beauty seedless, SNFL_16, SNFL_09 and SNFL_33). On the other hand, certain dark-violet berries had TPC lower than some red berries, as occurred with SNFL_19, which had a TPC lower than seven of the red berries studied. Despite of that, the general trend was that the increase in the grape berry coloration provides a higher TPC.

The presence of seeds in the berry contribute with a remarkable amount of phenolic compounds in grapes, increasing the TPC.[13,31,32] For this reason, there are necessary studies on new hybrid varieties to identify better seedless table grapes with high phenolic content. In this sense, seedless grapes with better quality in terms of phenolic composition and antioxidant activity may present great potential for the food industry, as well as health-conscious consumers. Results reported in Table 1 showed that, in general, experimental hybrid varieties and commercial hybrid grape varieties (from SNFL) had higher TPC values than some common commercial grape varieties (Crimson seedless and Autumn Royal). Thus, all red grapes studied had higher TPC (2-4 more times) than Crimson seedless grapes (22.7 mg of GAE/100 g FW). In addition, Autumn Royal grapes had lower TPC (118 mg of GAE/100 g FW) than many experimental and commercial hybrid dark-violet (e.g. Corinthe noir: 198 mg of GAE/100 g FW) and blue-black (e.g. Scarlet: 250 mg of GAE/100 g FW) varieties. These results indicate that if these varieties are consumed regularly, they could contribute significantly to the intake of bioactive phenolic compounds.

Radical scavenging activity

The RSA of the 65 grape varieties of the study (Table 1) was in the range of 15-269 mg of TE/100 g FW. The highest value of the antioxidant capacity was provided by Scarlet (blue-black berry), while SNFL_28 (pink berry) had the lowest value. In general, grapes with a greater

antioxidant capacity were those classified as dark-violet and blue-black, which may be due to the higher content of phenolic compounds of these varieties. However, there were some exceptions, such as SNFL_05 (pink berry), which provided 131 mg of TE/100 g FW (similar to many dark-violet and blue-black berries) probably due to its high TPC. Other example is SNFL_22 (red grape berry) with RSA higher than many dark-violet and blue-black berries (Table 1). In contrast, there were dark-violet and blue-black berries that had lower RSA values than some red, pink and green-yellow grapes. The exceptionally high antioxidant capacity of SNFL_11 (dark-violet) and SNFL_06 (blue-black) in comparison to their TPC were noteworthy. This fact may be due to the phenolic composition of these grapes, having phenolic compounds with a very high antioxidant capacity. In addition, it is possible that these varieties are rich in other highly antioxidant compounds, such as ascorbic acid.[33] Probably, some kind of synergy between antioxidant vitamins and phenolic compounds may increase the antioxidant capacity in these varieties. This is possible because the DPPH$^{\bullet}$ radical method measures antioxidant capacity due to all the compounds that have this activity in the sample, so RSA measured in grapes may be attributed to their TPC and to other antioxidant compounds with free radical scavenging ability.[33] Finally, as it can be seen in Table 1, some experimental SNFL hybrid grape varieties had better values of RSA compared to other commercial grape varieties. For example the experimental dark-violet grapes SNFL_10 and SNFL_11 (185 and 259 mg of TE/100 g FW) and blue-black grapes SNFL_08 and SNFL_06 (193 and 244 mg of TE/ 100 g FW) had RSA higher than the Autumn Royal variety (172 mg of TE/100 g FW). Compared to other seedless table grapes analyzed in previous works,[14,30] these new experimental varieties show very high RSA, so they have a significant antioxidant potential. On the basis of these results, it can be suggested that some of the new grape varieties developed by SNFL have a high potential antioxidant capacity that is linked to its equally high content of phenolic compounds.

346

347 **Total anthocyanin content**

348 Fig. 2 shows the TAC of the studied varieties, grouped by its berry color (in ascending
349 order of TAC). As it can be seen, results ranged between 0-168 mg of cyn-3-glu equivalents/100
350 g FW (Table 1). TAC of the grapes was found closely related to their visual coloration of the
351 berry. Thus, Scarlet (blue-black) provided the highest value of TAC. In contrast, SNFL_03
352 (green-yellow), Blush seedless and SNFL_20 (pink berries) had a null or very low TAC. Pink
353 berries provided TAC between 3-19 mg of cyn-3-glu equivalents/100 g FW. The pink grape
354 berry with the highest TAC was SNFL_29, which presented an intermediate TPC and RSA
355 when compared with other pink berries (Table 1). An opposite example may be the SNFL_05
356 (pink berry), which had a small TAC but the highest TPC of the pink berries, in addition to an
357 enormous RSA (the largest of the pink, red and many dark violet berries). Red berries showed
358 TAC between 3-24 mg of cyn-3-glu equivalents/100 g FW, and dark-violets possessed, in
359 general, a greater TAC (between11-59 mg of cyn-3-glu equivalents/100 g FW) due to the more
360 intense coloration of these varieties. However, SNFL_17, SNFL_10 and SNFL_11 varieties
361 provided very low TAC, despite to the fact that they were the varieties with the highest TPC
362 and RSA (Table 1). This result indicates that the TPC of these varieties is mainly provided by
363 another type of phenolic compounds (different from the anthocyanins) which have a high
364 antioxidant capacity. Finally, blue-black berries provided TAC ranged between 5-168 mg of
365 cyn-3-glu equivalents/100 g FW, and 7 of the 13 blue-black varieties had higher TAC compared
366 to the other grape varieties analysed. Scarlet variety possessed the highest TAC, and also the
367 highest TPC and RSA (Table 1). However, Autumn Royal and SNFL_06 varieties, despite
368 having smaller TAC, showed a good TPC and antioxidant capacity. These results indicate that
369 anthocyanins are not the main contributors to the RSA in these grapes.

As regards to the results of the works carried out by other authors[14,17,30,34] who analyzed different wine and table grapes (with and without seeds), it can be say that many of the experimental grape varieties obtained by a hybridization process studied in this work are able to achieve the same (or higher) TPC, RSA and TAC to those of grapes with seeds that are commonly commercialized. However, at this point, it is noteworthy that when comparing the TPC, RSA and TAC of Crimson seedless variety from South Africa (Fig. 1c, sample/solvent ratio 1:16, w/v) with those of the Crimson seedless variety from Spain (Table 1), it was observed that the values were approximately 3 times higher for TPC and RSA, and almost double for TAC in Crimson seedless grapes from South Africa. These results clearly demonstrate the importance of all those factors related to the crop, in order to obtain comparable results allowing the establishment of varietal differences, because phenolic content of grapes is strongly affected by both genotype and environmental factors.

Correlation analysis between TPC, TAC and RSA

Several studies have indicated high correlation between TPC and *in vitro* antioxidant activity of grapes.[17,30,33,35-40] In order to evaluate the results obtained for the varieties analyzed, correlation analysis between TPC and RSA was performed (Fig. 3a). A highly satisfactory correlation (R^2= 0.90) was observed, which indicated an increase in the antioxidant capacity of the grapes due to the increase in the concentration of the phenolic compounds.[41] According to the visual color of the berries, the highest correlation was obtained in red (R^2= 0.91), followed by dark-violet (R^2= 0.85) and pink (R^2= 0.81) grapes. The blue-black berries showed the poorest correlation (R^2= 0.76).

In Fig. 3a, we can distinguish several zones in which certain varieties of grapes predominate, depending on their coloration, although some varieties had different values from those most likely within their coloration (they appeared in other areas of the graph). For

example, the SNFL_03 (green-yellow berry) with high values of TPC and RSA was found in the upper area, near the pink berries. The same situation occurred with the SNFL_05 (pink berry) and SNFL_22 (red berry), which were found in the upper area, near the dark-violet grape berries. Special mention should be made for varieties SNFL_06 (blue-black berries) and SNFL_11 (dark-violet berry), due to their high RSA. Therefore, the commercialization of these varieties can be highly recommended due to their high phenolic content and antioxidant potential.

On the other hand, as it can be seen in Fig. 3b, very low correlation (R^2= 0.42) between the TAC and RSA was observed, just as it was indicated in the study of Meyer et al.[42] Therefore, it was confirmed that anthocyanins were not responsible for the high antioxidant activity estimated, in the varieties studied, with de DPPH assay that measures the free radical scavenging capacity.

Chromatographic analysis by UHPLC-IT-MS/MS

By direct infusion of pure individual standard solutions in the ESI source, fragmentation patterns of analytes were studied. All compounds were ionized in negative mode since better signal intensities of the analytes were achieve than in positive mode. The most abundant ion was selected as precursor ion to obtain the characteristic product ion spectra (MS^2) of each compound and the most intense product ions were monitored, being the more intense used for quantitation. To achieve the chromatographic separation of the target polyphenols, the gradient elution optimized with MeOH as organic solvent was applied, achieving a total run-time analysis of 12 min, and first compounds eluted at 2.1 min (Table 2).

Mass spectrum and analytical parameters of the developed method were studied and results are shown in Table 2. Solvent-based calibration and matrix-matched calibration curves provided excellent linear regression for all analytes, with R^2> 0.993. The slope values of the

matrix-matched calibration curves were higher than the slopes of the solvent-based calibration curves, except for 4-hydroxybenzoic acid, epigallocatechin gallate and piceid, which indicates ion enhancement in the detection of the analytes. On the other hand, ion suppression in the detection for 4-hydroxybenzoic acid, epigallocatechin gallate and piceid because of the influence of the matrix was observed. So, matrix-matched calibration curves were used for quantification of the target compounds in the samples. Precision of the method provided satisfactory results (RSD< 17%) and recovery values between 93-131% were obtained.

Analysis of phenolic compounds in 14 seedless table grape varieties

14 seedless table grape varieties (4 pink, 1 red, 3 dark-violet and 6 blue-black) were analyzed by UHPLC-IT-MS/MS to evaluate its phenolic composition. The identification of the analytes was carried out by means of their retention time and mass spectrum, and for quantitation purposes, their peak areas were subjected to correction with the recovery values and then interpolated into their corresponding matrix-matched calibration curve. Results obtained are listed in Tables 3 and 4.

Considering phenolic acids (Table 3), caftaric (CA), caffeic (CFA) and syringic (SA) acids were identified and quantified in the 14 varieties, whereas gallic (GA), protocatechuic (PA) and *p*-coumaric (*p*-CA) acid were not found in any grape analysed. Important differences were observed among grapes for the CA and SA content. Thus CA ranged between 0.04-0.83 mg/100 g FW (Corinthe noir) and SA ranged between 0.02-0.50 mg/100 g FW (SNFL_07). CFA, chlorogenic (CHA) and ferulic (FA) acids were equally distributed in grape samples (at very low levels), whereas vanilic (VA) acid was only found in dark-violet and blue-black varieties. Exceptionally high amounts of 4-hydroxybenzoic (4-HA) acid were observed in Comm_SNFL_02, SNFL_26 and Scarlet varieties (between 0.9–1.1 mg/100 g FW).

Epicatechin (EC), epigallocatechin (EGC) and epicatechin gallate (EGCG) were the less abundant flavan-3-ol (Table 4). Two exception were the Corinthe noir and Scarlet varieties with 3 and 2.3 mg/100 g FW of EC, respectively. In addition, these two varieties presented significantly higher amounts of catechin (CA) and procyanidin B2 (P-B2), which explain the very good RSA values of these grapes. For flavonols (Table 4), the most common were rutin (R) and quercetin-3-β-glucoside (Q-G), whereas quercetin (Q) was not found in samples analysed (apart from SNFL_08 with 3.1 mg/100 g FW). Finally, for stilbenes whereas piceid (P) was not present in the grapes, *trans*-resveratol (*T*-R) was found in most varieties between 0.03-0.08 mg/100 g FW. An exception was the SNFL_17 variety where the concentration was significantly higher (0.2 mg/100 g FW).

Conclusions

This work reveals that some new experimental hybrid grape varieties can be considered as highly phenolic compound producer. Phenolic compounds showed positive correlation with the antioxidant capacity (radical scavenging activity). However, this correlation was not observed when examining anthocyanin content. High concentration of catechin, procyanidin B2, epicatechin, quercetin 3-β-D-glucoside, 4-hydroxybenzoic acid, vanillic acid, caftaric acid and rutin were found in some grapes. Taking into account that quantitative phenolic composition of grapes is affected by several environmental and agronomical factors, results reported in this study can evidence this fact as the grapes analysed were cultivated under similar conditions. Additionally, these results indicate promising perspectives to obtain healthier seedless grape varieties and proves benefits of breeding programs. These varieties of table grapes, when consumed regularly, could contribute significantly to the intake of bioactive phenolic compounds in the diet.

Declarations

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Conflict of interest

The authors have declared no conflict of interest.

Availability of data and material

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Code availability

Not applicable

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607

Figure Legends

Fig. 1 Study of solvent (a), type and time of agitation (b), and sample/solvent ratio (c) to optimize the whole grape berry extraction conditions. Total phenolic content (TPC) is expressed as mg GAE/100 g FW. Radical scavenging activity (RSA) is expressed as mg TE/100 g FW. Total anthocyanin content (TAC) is expressed as mg cyn-3-glu/100 g FW

Fig. 2 Total anthocyanin content (TAC) obtained for 65 seedless table grape varieties

Fig. 3 Correlation between total phenolic content (TPC) and radical scavenging activity (RSA) (a) and total anthocyanin content (TAC) and RSA (b) of 65 seedless table grape varieties

Table 1. Results obtained for total phenolic content (TPC), radical scavenging activity (RSA) and total anthocyanin content (TAC) of 65 varieties of seedless table grape berries*

Variety	TPC mg GAE/100 g FW	RSA mg TE/100 g FW	TAC mg Cyn-3-glu/100 g FW
Green-yellow grapes			
SNFL_03[1]	74 ± 5^{a}	100 ± 4^{a}	0 ± 4^{a}
Pink grapes			
SNFL_39[1]	17.1 ± 0.4^{a}	33 ± 1^{bcd}	6 ± 2^{abcde}
Comm_SNFL_12[2]	18 ± 2^{a}	22 ± 4^{ab}	8.3 ± 0.1^{efgh}
SNFL_28[1]	20 ± 1^{ab}	15 ± 2^{a}	9 ± 2^{efgh}
SNFL_24[1]	23 ± 2^{ab}	33 ± 3^{bcd}	11 ± 1^{ghi}
SNFL_23[1]	26.8 ± 0.8^{abc}	22.5 ± 0.3^{abc}	4.6 ± 0.7^{abcd}
Comm_SNFL_14[2]	32 ± 4^{bcd}	36 ± 2^{cde}	11 ± 2^{hij}
SNFL_38[1]	33 ± 3^{bcd}	39 ± 5^{def}	6.4 ± 0.2^{bcde}
SNFL_40[1]	36 ± 4^{cd}	36 ± 2^{bcde}	13 ± 2^{ij}
Comm_SNFL_15[2]	37 ± 3^{cd}	53 ± 4^{g}	13.9 ± 0.4^{j}
SNFL_29[1]	39 ± 2^{cd}	46 ± 4^{efg}	18.5 ± 0.9^{k}
SNFL_41[1]	40 ± 2^{de}	31 ± 4^{bcd}	7.3 ± 0.3^{cdef}
SNFL_20[1]	44 ± 4^{de}	29.5 ± 0.5^{bcd}	2.9 ± 0.7^{a}
SNFL_01[1]	52 ± 3^{ef}	42 ± 3^{defg}	6.5 ± 0.2^{bcdef}
Comm_SNFL_13[2]	62 ± 3^{fg}	50 ± 2^{fg}	9.6 ± 0.9^{fgh}
SNFL_27[1]	65 ± 3^{gh}	93 ± 5^{jk}	4.7 ± 0.5^{abcd}
Comm_SNFL_11[2]	70 ± 10^{gh}	67 ± 2^{h}	11.0 ± 0.7^{hij}
Comm_SNFL_03[2]	72 ± 8^{gh}	77 ± 10^{hi}	8 ± 2^{defg}
SNFL_25[1]	75 ± 2^{h}	106 ± 3^{k}	9 ± 1^{efgh}
Comm_SNFL_02[2]	75 ± 7^{gh}	85 ± 4^{ij}	3.6 ± 0.5^{ab}
SNFL_37[1]	75 ± 7^{h}	94 ± 10^{jk}	11.2 ± 0.8^{hij}
Blush seedless[3]	81 ± 14^{ghi}	81 ± 10^{ij}	2.9 ± 0.6^{a}
SNFL_26[1]	98 ± 7^{i}	77 ± 9^{hi}	4.2 ± 0.5^{abc}
SNFL_05[1]	111 ± 5^{j}	131 ± 3^{l}	6.4 ± 0.2^{bcdef}
Red grapes			
CRIMSON seedless[3]	22.7 ± 0.1^{a}	26.0 ± 0.6^{a}	5.6 ± 0.7^{a}
Flame seedless[3]	34 ± 2^{b}	27 ± 9^{a}	3.4 ± 0.6^{a}

Comm_SNFL_05[2]	41 ± 7^{bc}	42 ± 2^{b}	6.0 ± 0.4^{a}
SNFL_21[1]	43 ± 5^{bc}	49 ± 5^{bc}	3.5 ± 0.1^{a}
SNFL_36[1]	47 ± 6^{bc}	51 ± 3^{bc}	3.9 ± 0.7^{a}
SNFL_04[1]	50 ± 5^{bc}	56 ± 4^{cd}	13 ± 1^{b}
Comm_SNFL_06[2]	53 ± 6^{c}	58 ± 5^{cd}	23 ± 1^{d}
SNFL_35[1]	67 ± 4^{d}	62 ± 2^{d}	19 ± 2^{c}
Comm_SNFL_10[2]	67.7 ± 0.7^{d}	73 ± 4^{e}	19 ± 0.9^{c}
Comm_SNFL_16[2]	73 ± 2^{d}	76 ± 5^{e}	24 ± 2^{d}
SNFL_22[1]	94 ± 3^{e}	120 ± 4^{f}	19 ± 2^{c}
Dark violet grapes			
SNFL_19[1]	46 ± 3^{a}	56 ± 6^{a}	19 ± 1^{ab}
SNFL_30[1]	75 ± 3^{b}	70 ± 2^{ab}	19 ± 2^{ab}
SNFL_32[1]	75 ± 2^{b}	74 ± 4^{ab}	14 ± 1^{a}
Comm_SNFL_07[2]	82 ± 4^{b}	96 ± 3^{bcd}	35 ± 2^{d}
SNFL_14[1]	86 ± 8^{bc}	104 ± 7^{cde}	19.9 ± 0.6^{ab}
SNFL_31[1]	95 ± 6^{bc}	112 ± 7^{cde}	31 ± 3^{cd}
SNFL_13[1]	102 ± 5^{bcd}	109 ± 7^{cde}	18.7 ± 0.7^{ab}
Comm_SNFL_08[2]	103 ± 3^{bcd}	127 ± 3^{e}	46.5 ± 0.7^{e}
SNFL_12[1]	111 ± 16^{cde}	88 ± 13^{bc}	59 ± 10^{f}
SNFL_34[1]	114 ± 2^{cde}	123 ± 8de	48 ± 1^{e}
Comm_SNFL_09[2]	125 ± 7^{def}	120 ± 4de	53 ± 6^{ef}
SNFL_18[1]	134 ± 3^{efg}	167 ± 10^{f}	53 ± 5^{ef}
SNFL_15[1]	152 ± 2^{fgh}	163 ± 3^{f}	53 ± 2^{ef}
SNFL_17[1]	158 ± 9^{gh}	159 ± 6^{f}	24 ± 1^{bc}
SNFL_10[1]	161 ± 15^{gh}	185 ± 7^{f}	23.5 ± 0.2^{bc}
SNFL_11[1]	169 ± 24^{h}	259 ± 33^{h}	20 ± 2^{ab}
Corinthe noir[3]	198 ± 15^{i}	228 ± 15^{g}	11 ± 1^{a}
Blue-black grapes			
Marroo seedless[3]	70 ± 9^{a}	88 ± 8^{b}	5.1 ± 0.5^{a}
Beauty seedless[3]	74 ± 11^{a}	67 ± 2^{a}	36 ± 4^{c}
SNFL_16[1]	101 ± 5^{b}	92 ± 4^{b}	36 ± 3^{c}
SNFL_09[1]	108 ± 5^{b}	108 ± 8^{bc}	39 ± 4^{cd}
SNFL_33[1]	110 ± 3^{bc}	124 ± 8^{cd}	19 ± 1^{b}

SNFL_02[1]	115 ± 8^{bc}	118 ± 4^{c}	49 ± 3^{d}
Autumn Royal[3]	118 ± 3^{bc}	172 ± 9^{fg}	68 ± 5^{e}
Comm_SNFL_01[2]	128 ± 9^{cd}	150 ± 8^{e}	95 ± 3^{g}
Comm_SNFL_04[2]	142 ± 8de	153 ± 13^{ef}	80 ± 1^{f}
SNFL_06[1]	147 ± 12^{e}	244 ± 16^{h}	64 ± 7^{e}
SNFL_07[1]	154 ± 3^{e}	142 ± 4de	117 ± 6^{h}
SNFL_08[1]	184 ± 5^{f}	193 ± 8^{g}	97 ± 6^{g}
Scarlet[3]	250 ± 19^{g}	269 ± 34^{hi}	168 ± 23^{i}

[*] Data are expressed as mean ± standard deviation (n = 4)

GAE: Gallic acid equivalents; TE: Trolox equivalents; Cyn-3-glu: Cyanidin-3-glucoside equivalents

[1]= Experimental hybrid grape variety by SNFL

[2]= Commercial SNFL grape variety

[3]= Commercial grape variety of Encin Grapevine Germplasm Bank

[a,b,c,d,e,f,g,h,I,j,k,l] Different letters among colors in the same column indicate statistical significance $p \leq 0.05$.

Table 2. Mass spectrum and analytical parameters studied for the developed UHPLC-IT-MS/MS method for the determination of the target polyphenols in whole grape-berry samples

Analytes	Molecular ion (fragm. ampl); product ions[a] (m/z)	Rt (min)	Linear range (mg/L)	Linearity; R^2 Solvent-based calibration	Linearity; R^2 Matrix-matched calibration	Inter-day precisión (RSD %)		Recovery (%)
						1 (mg/L)	10 (mg/L)	1 (mg/L)
Gallic acid	169 (0.70); 124[b]	2.1	1.0–25.0	y = 765294 x + 1681306; 0.999	y = 1233835 x + 1331075; 0.994	6.46	4.36	93 ± 6
Caftaric acid	311 (0.60); 178, 148[b]	3.3	0.5–10.0	y = 594171 x + 243008; 0.999	y = 81645 x – 11493; 0.999	12.82	12.51	106 ± 5
Protocatechuic acid	153 (0.50); 108[b]	3.5	1.0–25.0	y = 289142 x + 502538; 0.999	y = 47818 x + 22566; 0.998	13.49	3.94	99 ± 7
Chlorogenic acid	353 (0.70); 190[b], 178	4.5	0.1–25.0	y = 1504106 x + 1467118; 0.999	y = 3186983 x + 269095; 0.999	6.16	12.28	115 ± 8
4-Hydroxybenzoic acid	137 (0.50); 106, 93[b]	4.7	5.0–25.0	y = 139657 x + 284269; 0.999	y = 12135 x + 76740; 0.995	5.57	2.59	102 ± 9
p-Coumaric acid	163 (0.70); 118[b]	6.1	1.0–25.0	y = 671543 x + 2468629; 0.980	y = 70397 x + 57442; 0.999	5.75	3.26	102 ± 7
Caffeic acid	179 (0.50); 134[b]	5.3	0.5–25.0	y = 1861112 x + 1843964; 0.999	y = 2631385 x + 505671; 0.999	5.28	6.65	112 ± 14
Ferulic acid	193 (0.60); 177[b], 148, 133	6.4	1.0–25.0	y = 157133 x + 182338; 0.999	y = 264122 x – 1497; 0.999	4.76	4.56	115 ± 4
Syringic acid	197 (0.50); 181[b], 152, 137	5.5	1.0–10.0	y = 109267 x + 30144; 0.999	y = 276892 x + 2007; 0.999	4.31	10.08	102 ± 12
Vanillic acid	167 (0.40); 151[b], 122, 107	5.3	1.0–25.0	y = 38256 x + 58830; 0.999	y = 43017 x + 28270; 0.999	8.98	12.08	100 ± 11
Catechin	289 (0.60); 244[b], 204, 178	4.4	0.5–25.0	y = 195892 x + 436838; 0.999	y = 50603 x + 60362; 0.999	9.27	9.81	106 ± 9
Epicatechin	289 (0.60); 244[b], 204, 178	5.3	1.0–25.0	y = 233298 x + 560388; 0.999	y = 53607 x + 17445; 0.999	8.79	7.83	108 ± 8

Epigallocatechin	305 (0.65); 220, 218, 178[b]	4.4	1.0–25.0	y = 125433 x + 76298; 0.999	y = 23653 x + 13087; 0.996	8.82	10.09	110 ± 11
Epigallocatechin gallate	547 (0.65); 330, 168[b]	5.2	10.0–25.0	y = 520436 x – 865429; 0.995	y = 34129 x + 69282; 0.993	16.72	2.67	131 ± 7
Procyanidin B2	577 (1.30); 425[b], 407, 288	4.1	0.1–25.0	y = 420207 x + 617237; 0.999	y = 82071 x + 25987; 0.999	7.80	2.09	96 ± 5
Quercetin	301 (0.55); 178[b], 150	8.6	1.0–25.0	y = 972094 x + 3109582; 0.999	y = 1745703 x + 1250992; 0.995	11.62	9.41	102 ± 19
Quercetin 3- β -D-glucoside	463 (0.65); 300[b]	7.1	1.0–25.0	y = 2135754 x + 2165384; 0.999	y = 2547169 x + 1533471; 0.999	4.65	4.73	115 ± 4
Rutin	609 (1.25); 300[b]	7.0	1.0–25.0	y = 964541 x + 1199333; 0.999	y = 1588903 x + 522363; 0.999	5.74	3.96	125 ± 20
Piceid	389 (0.50); 341, 226[b]	6.2	1.0–25.0	y = 207893 x + 429460; 0.999	y = 12343 x + 30063; 0.999	8.98	8.62	131 ± 24
Trans-resveratrol	227 (0.50); 184[b],158, 142	7.4	1.0–25.0	y = 349274 x + 702310; 0.999	y = 1085409 x + 292697; 0.999	11.50	6.70	104 ± 10

[a] Predominant product ions. Ionization mode is ESI (-).

[b] Ions used for quantitation. Isolation width (*m/z)* is 4.

Chromatographic conditions with the optimized gradient elution: t = 0 min 20% A – 80% B, t = 9 min 100% A, t = 11 min 20% A – 80% B (1 min) (MeOH as mobile phase A and water as mobile phase B, both containing 0.1% formic acid and 2 mM ammonium acetate). The flow rate was 0.25 mL/min.

Table 3. Content of phenolics acids in the selected seedless table grape varieties analyzed by UHPLC-IT-MS/MS

	Phenolic acids (mg/100 g FW)									
Color and variety	GA	CA	PA	CHA	4-HA	*p*-CA	CFA	FA	SA	VA
Pink										
Comm_SNFL_02	< LOQ	0.04 ± 0.01	< LOQ	0.048 ± 0.002	0.9 ± 0.1	< LOQ	0.03 ± 0.01	0.03 ± 0.00	0.04 ± 0.01	< LOQ
SNFL_37	< LOQ	0.04 ± 0.01	< LOQ	0.048 ± 0.003	0.211 ± 0.004	< LOQ	0.031 ± 0.004	0.05 ± 0.02	0.07 ± 0.02	< LOQ
Blush seedless	< LOQ	0.05 ± 0.01	< LOQ	TR	< LOQ	< LOQ	0.02 ± 0.01	TR	0.032 ± 0.001	TR
SNFL_26	< LOQ	0.05 ± 0.02	< LOQ	0.048 ± 0.003	0.9 ± 0.3	< LOQ	0.04 ± 0.01	0.09 ± 0.02	0.07 ± 0.02	< LOQ
Red										
Flame seedless	< LOQ	0.04 ± 0.01	< LOQ	TR	< LOQ	< LOQ	0.02 ± 0.01	TR	0.02 ± 0.01	< LOQ
Dark violet										
SNFL_17	< LOQ	0.06 ± 0.02	< LOQ	0.047 ± 0.002	< LOQ	< LOQ	0.02 ± 0.01	0.05 ± 0.01	0.18 ± 0.06	0.37 ± 0.15
SNFL_10	< LOQ	0.05 ± 0.01	< LOQ	0.047 ± 0.002	0.63 ± 0.50	< LOQ	0.04 ± 0.01	0.033 ± 0.003	0.10 ± 0.04	0.35 ± 0.15
Corinthe noir	< LOQ	0.83 ± 0.07	< LOQ	TR	0.58 ± 0.45	< LOQ	0.04 ± 0.02	0.06 ± 0.00	0.09 ± 0.01	< LOQ
Blue-black										
Marroo seedless	< LOQ	0.07 ± 0.01	< LOQ	TR	< LOQ	< LOQ	0.02 ± 0.01	TR	0.13 ± 0.03	< LOQ
Beauty seedless	< LOQ	0.057 ± 0.004	< LOQ	0.045 ± 0.001	< LOQ	< LOQ	0.02 ± 0.01	TR	0.16 ± 0.05	TR
SNFL_06	< LOQ	0.33 ± 0.06	< LOQ	0.048 ± 0.002	0.45 ± 0.04	< LOQ	0.033 ± 0.003	0.06 ± 0.03	0.20 ± 0.05	< LOQ

SNFL_07	< LOQ	0.04 ± 0.01	< LOQ	0.048 ± 0.003	0.34 ± 0.05	< LOQ	0.03 ± 0.01	TR	0.5 ± 0.1	0.33 ± 0.10
SNFL_08	< LOQ	0.17 ± 0.07	< LOQ	0.051 ± 0.004	0.62 ± 0.22	< LOQ	0.04 ± 0.01	0.04 ± 0.01	0.25 ± 0.05	0.82 ± 0.10
Scarlet	< LOQ	0.34 ± 0.04	< LOQ	TR	1.1 ± 0.3	< LOQ	0.05 ± 0.01	0.06 ± 0.03	0.21 ± 0.05	< LOQ

GA: gallic acid; CA: caftaric acid; PA: protocatechuic acid; CHA: chlorogenic acid; 4-HA: 4-hydroxybenzoic acid; *p*-CA: *p*-coumaric acid; CFA: caffeic acid; FA: ferulic acid; SA: syringic acid; VA: vanillic acid.
< LOQ: detectable but not quantifiable.
TR: traces.

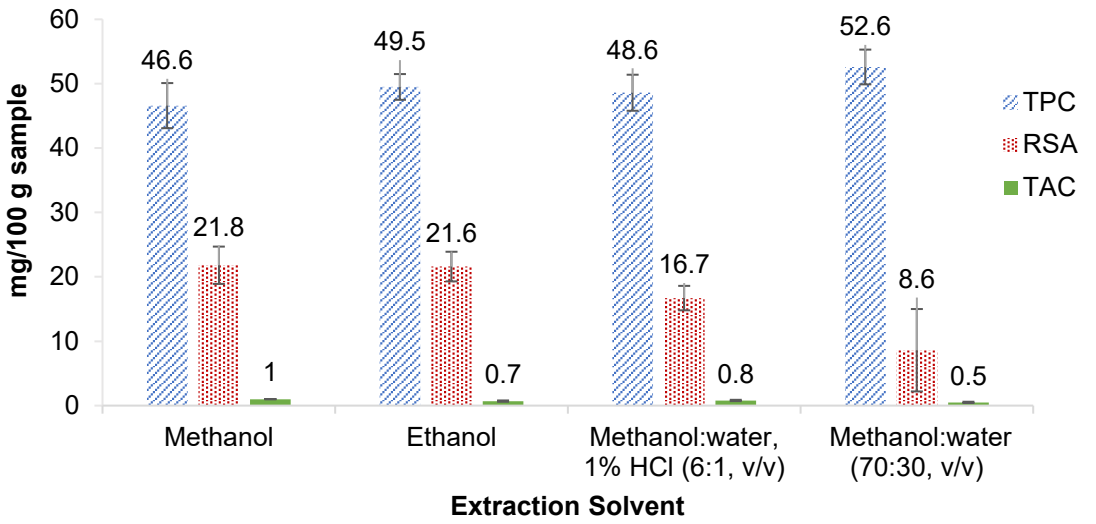
Table 4. Content of flavonoids and stilbenes in the selected seedless table grape varieties analyzed by UHPLC-IT-MS/MS

	Flavonoids and stilbenes (mg/100 g FW)									
Color and variety	C	EC	EGC	EGCG	P-B2	Q	Q-G	R	P	T-R
Pink										
Comm_SNFL_02	0.08 ± 0.03	< LOQ	TR	TR	0.2 ± 0.1	< LOQ	0.8 ± 0.1	0.28 ± 0.04	< LOQ	0.03 ± 0.01
SNFL_37	< LOQ	< LOQ	TR	TR	0.12 ± 0.06	< LOQ	0.13 ± 0.03	0.16 ± 0.02	TR	0.04 ± 0.01
Blush seedless	0.08 ± 0.02	< LOQ	TR	TR	2.3 ± 0.5	< LOQ	< LOQ	0.03 ± 0.01	< LOQ	0.05 ± 0.02
SNFL_26	< LOQ	< LOQ	TR	TR	0.006 ± 0.000	< LOQ	< LOQ	0.10 ± 0.02	< LOQ	TR
Red										
Flame seedless	< LOQ	< LOQ	< LOQ	< LOQ	0.15 ± 0.03	< LOQ	0.09 ± 0.03	0.33 ± 0.05	TR	0.03 ± 0.01
Dark violet										
SNFL_17	0.4 ± 0.2	< LOQ	TR	TR	0.4 ± 0.2	< LOQ	0.19 ± 0.06	0.09 ± 0.04	TR	0.20 ± 0.03
SNFL_10	< LOQ	< LOQ	TR	TR	0.17 ± 0.07	< LOQ	0.8 ± 0.4	0.19 ± 0.05	TR	0.03 ± 0.01
Corinthe noir	6 ± 2	3.0 ± 0.5	< LOQ	< LOQ	3.1 ± 0.8	< LOQ	0.8 ± 0.4	0.6 ± 0.2	< LOQ	0.08 ± 0.03
Blue-black										
Marroo seedless	0.43 ± 0.05	< LOQ	TR	TR	0.7 ± 0.2	< LOQ	< LOQ	0.05 ± 0.01	< LOQ	0.05 ± 0.01
Beauty seedless	< LOQ	< LOQ	TR	TR	0.11 ± 0.00	< LOQ	< LOQ	0.11 ± 0.03	TR	0.03 ± 0.01
SNFL_06	< LOQ	< LOQ	TR	< LOQ	0.49 ± 0.08	< LOQ	0.43 ± 0.09	0.06 ± 0.02	< LOQ	TR

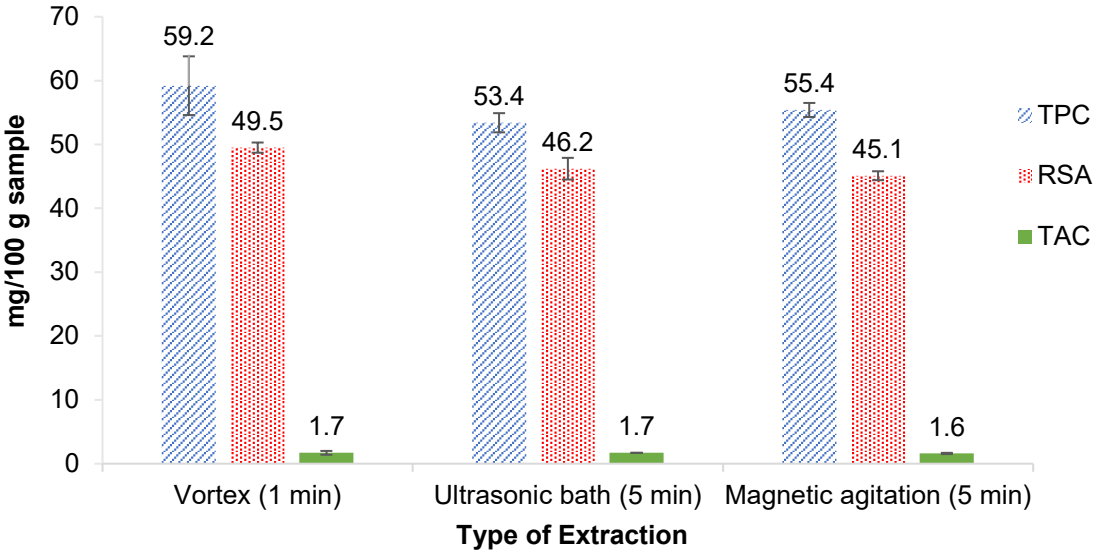
SNFL_07	< LOQ	< LOQ	TR	TR	0.057 ± 0.004	< LOQ	0.08 ± 0.02	< LOQ	< LOQ	0.03 ± 0.01
SNFL_08	< LOQ	< LOQ	TR	< LOQ	0.05 ± 0.02	0.12 ± 0.04	3.1 ± 0.6	0.23 ± 0.05	< LOQ	TR
Scarlet	8 ± 3	2.3 ± 0.2	< LOQ	TR	5 ± 2	< LOQ	0.9 ± 0.1	0.8 ± 0.3	< LOQ	0.07 ± 0.03

C: catechin; EC: epicatechin; EGC: epigallocatechin; EGCG: epigallocatechin gallate; P-B2: procyanidin B2; Q: quercetin; Q-G: quercetin-3-β-D-glucoside; R: rutin; P: piceid; T-R: trans-resveratrol.
< LOQ: detectable but not quantifiable.
TR: traces.

(a)


mg/100 g sample
60
50
40
30
20
10
0
46.6
21.8
1
49.5
21.6
0.7
48.6
16.7
0.8
52.6
8.6
0.5
TPC
RSA
TAC
Methanol
Ethanol
Methanol:water, 1% HCl (6:1, v/v)
Methanol:water (70:30, v/v)
Extraction Solvent

(b)


mg/100 g sample
70
60
50
40
30
20
10
0
59.2
49.5
1.7
53.4
46.2
1.7
55.4
45.1
1.6
TPC
RSA
TAC
Vortex (1 min)
Ultrasonic bath (5 min)
Magnetic agitation (5 min)
Type of Extraction

(c)

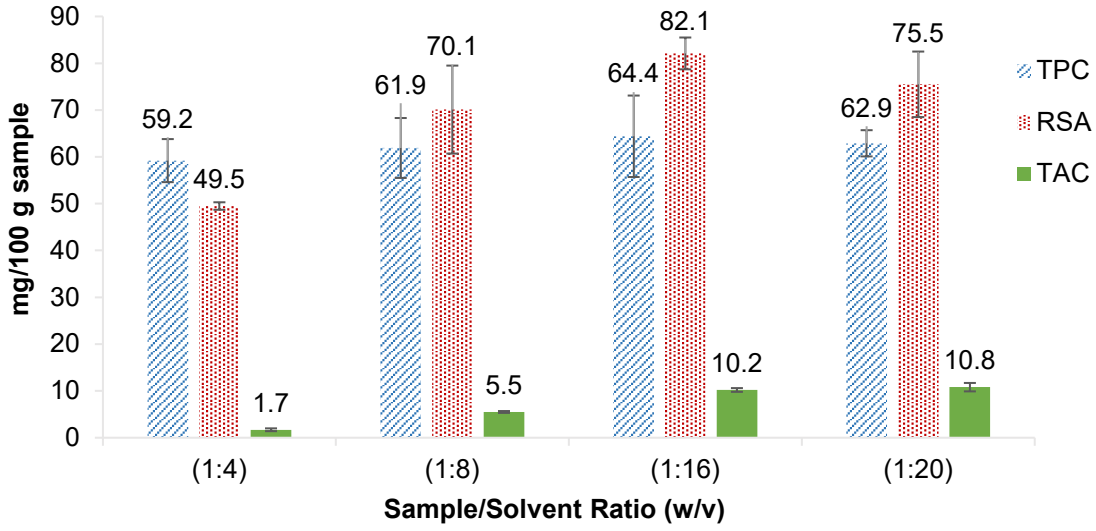

mg/100 g sample
90
80
70
60
50
40
30
20
10
0
59.2
49.5
1.7
61.9
70.1
5.5
64.4
82.1
10.2
62.9
75.5
10.8
TPC
RSA
TAC
(1:4)
(1:8)
(1:16)
(1:20)
Sample/Solvent Ratio (w/v)

Fig. 1

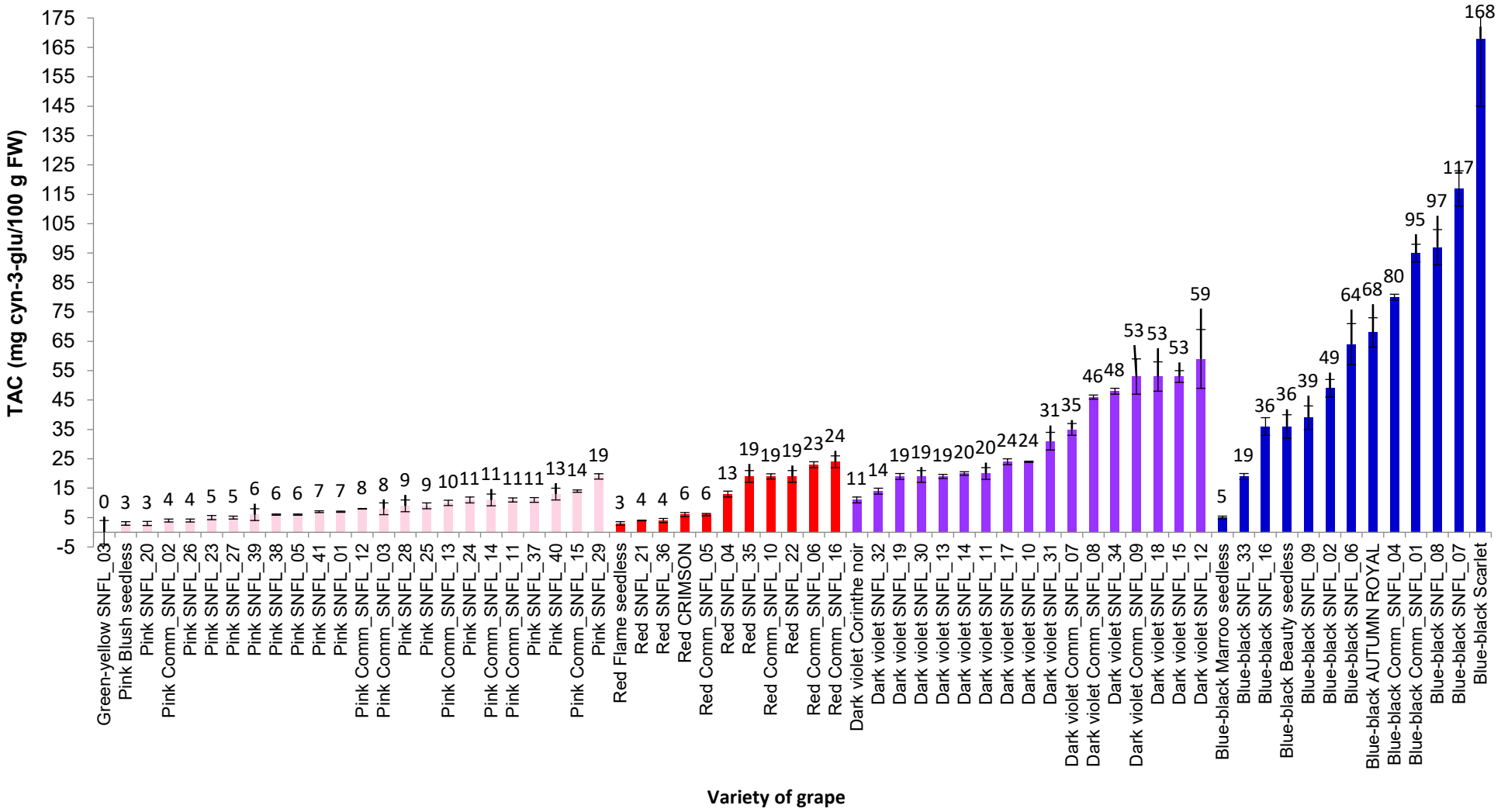

TAC (mg cyn-3-glu/100 g FW)
175
165
155
145
135
125
115
105
95
85
75
65
55
45
35
25
15
5
-5
0 3 3 4 4 5 5 6 6 6 7 7 8 8 9 9 10 11 11 11 11 13 14 19 3 4 4 6 6 13 19 19 19 23 24 11 14 19 19 19 20 20 24 24 31 35 46 48 53 53 53 59 5 19 36 36 39 49 64 68 80 95 97 117 168
Green-yellow SNFL_03
Pink Blush seedless
Pink SNFL_20
Pink Comm_SNFL_02
Pink SNFL_26
Pink SNFL_23
Pink SNFL_27
Pink SNFL_39
Pink SNFL_38
Pink SNFL_05
Pink SNFL_41
Pink SNFL_01
Pink Comm_SNFL_12
Pink Comm_SNFL_03
Pink SNFL_28
Pink SNFL_25
Pink Comm_SNFL_13
Pink SNFL_24
Pink Comm_SNFL_14
Pink Comm_SNFL_11
Pink SNFL_37
Pink SNFL_40
Pink Comm_SNFL_15
Pink SNFL_29
Red Flame seedless
Red SNFL_21
Red SNFL_36
Red CRIMSON
Red Comm_SNFL_05
Red SNFL_04
Red SNFL_35
Red Comm_SNFL_10
Red SNFL_22
Red Comm_SNFL_06
Red Comm_SNFL_16
Dark violet Corinthe noir
Dark violet SNFL_32
Dark violet SNFL_19
Dark violet SNFL_30
Dark violet SNFL_13
Dark violet SNFL_14
Dark violet SNFL_11
Dark violet SNFL_17
Dark violet SNFL_10
Dark violet SNFL_31
Dark violet Comm_SNFL_07
Dark violet Comm_SNFL_08
Dark violet SNFL_34
Dark violet Comm_SNFL_09
Dark violet SNFL_18
Dark violet SNFL_15
Dark violet SNFL_12
Blue-black Marroo seedless
Blue-black SNFL_33
Blue-black SNFL_16
Blue-black Beauty seedless
Blue-black SNFL_09
Blue-black SNFL_02
Blue-black SNFL_06
Blue-black AUTUMN ROYAL
Blue-black Comm_SNFL_04
Blue-black Comm_SNFL_01
Blue-black SNFL_08
Blue-black SNFL_07
Blue-black Scarlet
Variety of grape

Fig. 2

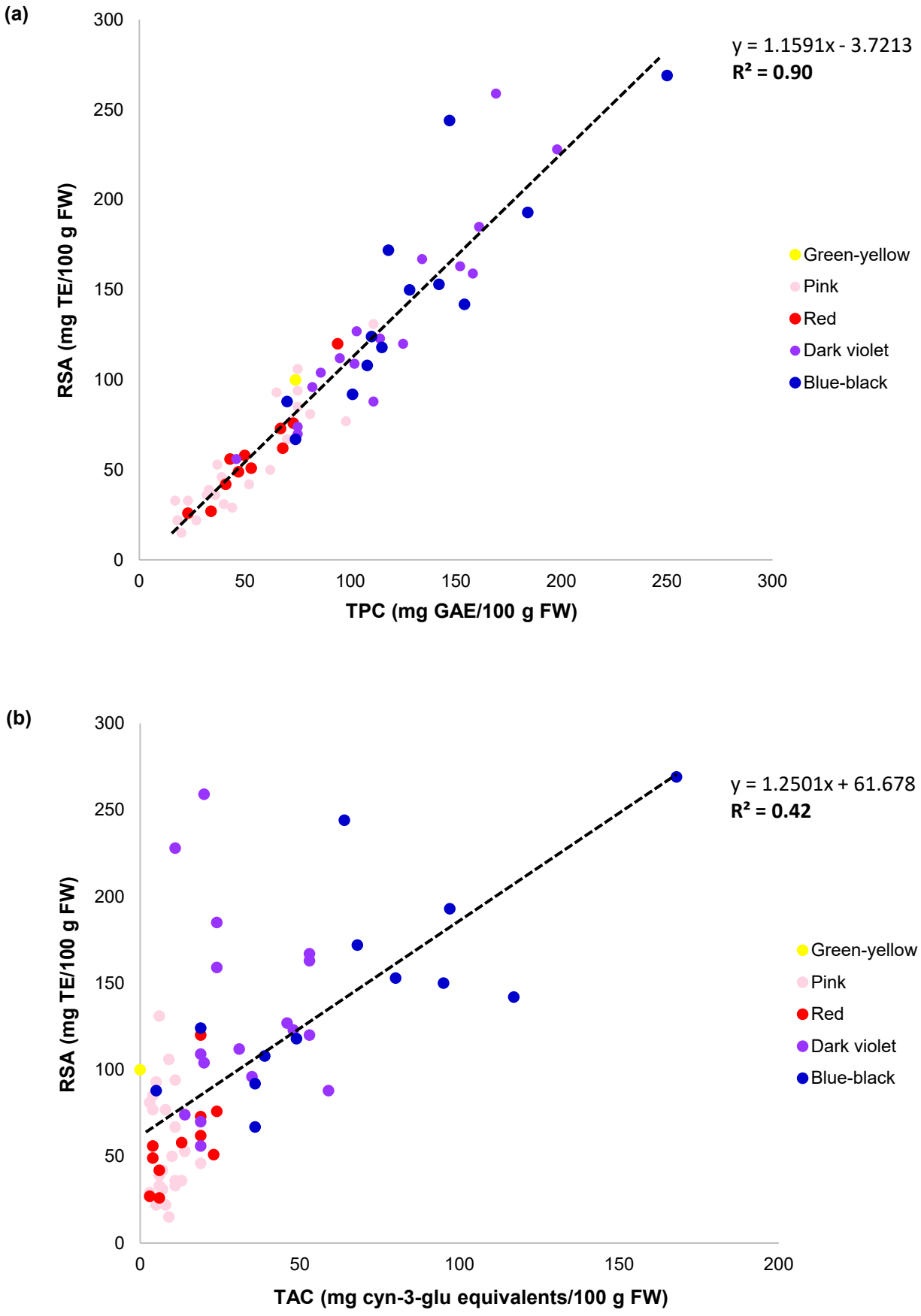

(a)
y = 1.1591x - 3.7213
R² = 0.90
RSA (mg TE/100 g FW)
TPC (mg GAE/100 g FW)
Green-yellow
Pink
Red
Dark violet
Blue-black
(b)
y = 1.2501x + 61.678
R² = 0.42
RSA (mg TE/100 g FW)
TAC (mg cyn-3-glu equivalents/100 g FW)
Green-yellow
Pink
Red
Dark violet
Blue-black

Fig. 3