Stilbenes in Tannat, Marselan and Syrah grapes and wines from Uruguay

Guzmán Favre1*, Diego Piccardo1, Sergio Gómez-Alonso2, José Pérez-Navarro2, Esteban García-Romero3, Adela Mena-Morales3 and Gustavo González-Neves1

1Facultad de Agronomía, Universidad de la República, Avenida Garzón 780, C.P., 12900 Montevideo, Uruguay
2Instituto Regional de Investigación Científica Aplicada (IRICA), Universida de Castilla-LaMancha, Avenida Camilo José Cela s/n, 13071 Ciudad Real, Spain
3Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha (IVICAM-IRIAF), Carretera de Albacete s/n, 13700 Tomelloso, Spain

*corresponding author: guzmanfavre@gmail.com

This article is published in cooperation with the 11th OenolIVAS International Symposium, June 25–28 2019, Bordeaux, France
In memory of Prof. Isidro Hermosín-Gutiérrez

ABSTRACT

Aim: The aim of the study was to investigate the stilbene composition of grapes and wines of the Vitis vinifera cultivars Tannat, Marselan and Syrah cultivated in Uruguay. The effects of delaying the harvest on stilbene concentrations were determined, and the stability of stilbenes during wine storage was assessed.

Methods and results: Stilbene concentrations were determined in the grapes and wines of two vintages (2015 and 2016) and two harvest dates for each cultivar. Vinification was carried out by traditional maceration, and samples of the wines of each vintage were analysed in the period from 3 months after devatting to up to 24 months later. After solid-phase extraction, stilbenes were identified and quantified by HPLC-ESI-MS/MS using a multiple reaction-monitoring approach. In the grape berries, stilbene concentrations were between 1.6 and 7.7 mg/kg, depending on grape cultivar, growing season, and in Syrah, harvest date. In the wines, stilbene concentrations were initially between 0.9 and 5.0 mg/L, being highest in Syrah, lowest in Marselan, and intermediate in Tannat. Stilbene concentrations in the Marselan wines were lower than expected based on stilbene concentrations in the grapes from which they were produced, suggesting poor extraction during winemaking. Total stilbene concentrations remained very stable during the analytical period.

Conclusions: Delaying the harvest does not necessarily increase the stilbene content of grapes, but it can do so significantly, as shown for Syrah. For some grape cultivars, such as Marselan, poor extraction of stilbenes during winemaking can limit their concentrations in the resulting wines.

Significance and impact of the study: The results of this study show the relevance of grape cultivar, degree of maturity and storage time may have into stilbenes. They provide reference data on the stilbene composition of grapes and wines produced under Uruguayan winegrowing conditions. The high stability of stilbenes during wine storage is relevant for consumers interested in red wine as a source of bioactive compounds.

KEYWORDS

marselan, piceid, resveratrol, stilbene stability, Syrah, Tannat
INTRODUCTION

Resveratrol is a phytoalexin with a wide range of pharmacological properties (Ingham, 1976; Pannu and Bhatnagar, 2019). It is present in a few plant families, including Vitaceae (Jeandet et al., 2002), in which it is synthesized constitutively (Gatto et al., 2008) but mainly in response to biotic and abiotic agents (Vannozzi et al., 2012; Flamini et al., 2013; Sáez et al., 2018). The trans-resveratrol form (3,5,4′-trihydroxy-trans-stilbene) is the metabolic precursor and structural core of stilbenoids (Sáez et al., 2018), such as cis- and trans-piceid (Waterhouse and Lamuela-Raventós, 1994), viniferins, pterostilbene (Langcake and McCarthy, 1979; Langcake, 1981) and piceatannol (Bavaresco et al., 2002). Resveratrol and its derivatives have attracted attention because of their wide range of chemopreventive effects against different diseases and their potential therapeutic uses (Rauf et al., 2018). Resveratrol interferes with ion transport and associated redox processes (Keylor et al., 2015), characteristics that have been identified as responsible for its activity against pathogens in plants and would underlie its potential in treating human diseases (Lopez-Lluch et al., 2012).

Most studies have shown that stilbenes are synthesized constitutively at only very low levels but accumulate strongly in response to a wide range of biotic and abiotic stresses (Vannozzi et al., 2012). This is reflected by the wide range of stilbene concentrations reported in healthy grapes, depending on grape variety, growing region, exposure to elicitors, and other factors (Gatto et al., 2008; Ruiz-García et al., 2012; Vincenzi et al., 2013; Belmiro et al., 2017). Therefore, it is useful to have reference data on the stilbene-synthesizing capacity of healthy grape berries belonging to different cultivars in different growing regions. To reach the consumer, stilbenes synthesized in the skin of grape berries need to be extracted into must and remain stable in the resulting wine. Therefore, in this article we report, to the best of our knowledge for the first time, data on the stilbene composition of healthy grapes of the varieties Tannat, Marselan and Syrah cultivated in the south of Uruguay, as well as that of the red wines produced from them. The effects on stilbene concentrations of delaying the harvest after technological maturity were evaluated. Furthermore, the stability of the stilbenes during wine ageing, from stabilization to 24 months later, was assessed, thus covering the period of time during which most red wines are consumed.

MATERIALS AND METHODS

1. Vineyards, cultivars and grapes

The experiments were carried out using the Vitis vinifera L. cv. Tannat, Marselan and Syrah cultivated under similar crop conditions in commercial vineyards in the south of Uruguay. Two vintages were studied: 2015 and 2016. The grapes were harvested at technological maturity (according to winegrowing criteria) and also, once for each cultivar, at a later date (Table 1). In 2015, one vineyard of Marselan, one vineyard of Syrah and two vineyards of Tannat were used. In one of the Tannat vineyards (34°37′S, 56°17′W), two harvests were carried out at different dates, whereas in the other (34°36′S, 56°15′W), as in the Marselan vineyard (34°37′S, 56°13′W) and the Syrah vineyard (34°37′S, 56°17′W), the harvest was carried out at technological maturity only. In 2016, the same vineyards were used for the experiments, except for the Tannat vineyard that had been harvested twice the previous year. Thus, in 2016, one harvest of Tannat was carried out at technological maturity, whereas two harvests of Syrah and Marselan were carried out at different dates. In total, there were five harvests for each vintage.

Climatic data for the period of grape maturity are presented in Table 1; these were collected by the climatic station closest to the vineyards (INIA-Las Brujas; 34°40′S, 56°20′W). All harvests were made by hand, and the clusters carefully transported in plastic boxes (each containing 20 kg) to the experimental winery of the Universidad de la República. There, two 70-kg batches of grapes were randomly separated from each harvest for vinification. Just before crushing, a sample of 100 grapes was randomly collected from the clusters, in clusters of three to five berries taken from different parts of randomly chosen bunches. To avoid bias related to size or aspect, the 100 berries in each sample were scattered over a 50 cm × 50 cm cm of must samples were taken to determine skin dry weight and then stored at -18 °C in
TABLE 1. Basic chemical parameters of the grapes at harvesta.

| Grape sample | Harvest date     | Sugars (g/L)b | Acidity (g/L)c | pHd | GD10 | He (hs) |
|--------------|-----------------|---------------|----------------|-----|------|---------|
| Syrah        | 12 February 2015| 216 ± 1.1 e   | 4.13 ± 0.00 a  | 3.64 ± 0.03 d | 544 | 413     |
| Marselan     | 20 February 2015| 232 ± 1.1 c   | 6.97 ± 0.12 d  | 3.33 ± 0.03 b 635 486 |
| Tannat 1     | 2 March 2015    | 250 ± 1.6 a   | 5.56 ± 0.08 b,c | 3.46 ± 0.03 c 762 579 |
| Tannat 2 H1  | 20 February 2015| 221 ± 1.1 d   | 5.63 ± 0.08 c  | 3.26 ± 0.02 a 635 486 |
| Tannat 2 H2  | 2 March 2015    | 245 ± 2.6 b   | 5.43 ± 0.09 d  | 3.40 ± 0.02 c 762 579 |
| Syrah H1     | 22 February 2016| 196 ± 3.0 d   | 5.79 ± 0.04 c  | 3.39 ± 0.01 b 739 532 |
| Syrah H2     | 1 March 2016    | 216 ± 1.1 b   | 4.47 ± 0.06 e  | 3.47 ± 0.06 a 834 599 |
| Tannat 1     | 9 March 2016    | 208 ± 1.7 c   | 7.82 ± 0.00 a  | 3.16 ± 0.03 c 908 660 |
| Marselan H1  | 3 March 2016    | 249 ± 3.0 a   | 6.05 ± 0.11 b  | 3.36 ± 0.01 b 853 616 |
| Marselan H2  | 9 March 2016    | 253 ± 1.9 a   | 5.29 ± 0.13 d  | 3.39 ± 0.01 b 908 660 |

GD10: growing degree days accumulated from 1 January until harvest; H1 and H2, first and second harvest, respectively; He (hs), heliophancy expressed in accumulated hours of direct solar radiation in the same period (INIA, 2019); Tannat 1 and Tannat 2, grapes from two different closely situated Tannat vineyards.

aData are expressed as the mean (n = 2) ± SD. The different letters in columns 3-5 indicate statistically significant differences between means (p < 0.05), according to Tukey test.

bSugar concentrations were determined by refractometry.

cAcidity was determined by titration and is expressed as g/L of tartaric acid.

dpH was determined by potentiometry.

OENO One 2020, 1, 27-36

© 2020 International Viticulture and Enology Society - IVES

bags containing silica gel. These determinations enabled the concentrations of stilbenes to be calculated in terms of mg/kg of grape or skin.

The basic chemical parameters of the grapes were determined using the must collected immediately after each grape crushing (see Table 1).

2. Winemaking

Each 70-kg batch of grapes was vinified, for a total of 10 vinifications for each vintage. The grapes were destemmed and crushed with an Alfa 60 R crusher (Italcom, Piazzola Sul Brenta, Italy), and stainless-steel tanks (each with a capacity of 100 L) were used for barrelling. Potassium metabisulfite was added (50 mg SO2/L), and kept in 10-L glass containers.

Wines were produced by classic fermentation on the skins (maceration occurring simultaneously with alcoholic fermentation) for 8 days for Tannat and Marselan, and 7 days for Syrah (1 day less because of its lower phenolic potential, according to the proposal of González-Neves et al., 2004). Alongside the macerations, two pumpings-over followed by punching the cap were carried out daily until pressing. At devatting, fermentation was complete in all cases. Pressing was carried out with a stainless-steel manual press. Free-run juices and press juices were mixed, separated from lees, stabilized by adding SO2 (50 mg/L), and kept in 10-L glass containers.

3. Analytical procedures

Analyses were carried out at the Laboratory of Instrumental Analysis at the Regional Institute of Applied Scientific Research, Castilla-La Mancha University, Spain, and the Institute of Vine and Wine of Castilla-La Mancha, Spain. All solvents used were HPLC quality, and the chemicals were analytical grade (purity ≥ 99%). Water was Milli-Q quality. The trans-piceid isomer was purchased from PhytoLab (Vestenbergsgreuth, Germany), and trans-resveratrol from Sigma Aldrich (Tres Cantos, Madrid, Spain). These compounds were converted to their respective cis isomers by UV irradiation (366 nm light for 5 min in quartz vials).

4. Preparation of samples for stilbene analysis

Each freeze-dried skin sample was subjected to extraction using 100 mL of a mixture of CH3OH/H2O/HCOOH (50:48.5:1.5, v/v/v), with a homogenizer (DIAX 900; Heidolph, Schwabach, Germany) at 10,000 rpm for 3 min followed by centrifugation at 2500 g at 5 °C for 5 min. The supernatant was separated and conserved, and the pellet was subjected to two more extractions. The three supernatants obtained were mixed, their volume was recorded, and they were stored at -18 °C until analysis. The results of previous studies carried out under similar conditions confirm that two extractions using grape skin
pellet yield nearly 99 % of the polyphenol content of the grapes (Castillo-Muñoz et al., 2009).

Stilbenes and flavan-3-ols (the later not the focus of the present study) were isolated from the wines and hydromethanolic extracts using solid-phase extraction on C18 cartridges (Sep-pak Plus C18, Waters Corporation, Milford, MA, USA; cartridges filled with 1000 mg of adsorbent). A mixture of 2 mL of each wine with 6 mL of water was passed through the cartridge, which had previously been conditioned with 5 mL of methanol and 5 mL of water. In the case of the skin extracts, the mixture passing through the cartridge consisted of 12 mL of water with 2 mL of the hydromethanolic extracts. After drying of the cartridge under reduced pressure, 15 mL of methanol and 5 mL of ethyl acetate were added to recover the adsorbed polyphenols. These solvents were evaporated in a rotary evaporator (at 35 °C), and then the residue was redissolved in 2 mL of methanol.

5. Identification and quantification of stilbenes using multiple reaction-monitoring HPLC-ESI-MS/MS

Analyses were carried out using an HPLC Agilent 1200 series system equipped with DAD (Agilent, Waldbronn, Germany) and coupled to an AB Sciex 3200 QTRAP (Applied Biosystems, Waltham, MA, USA) with triple-quadrupole, turbo spray ionization (electrospray assisted by a thermonebulization) mass spectroscopy system (ESI-MS/MS). The chromatographic system was managed with an Agilent Chem Station (version B.01.03) data-processing unit, and the mass spectra data were processed using Analyst MSD software (Applied Biosystems, version 1.5).

Samples of 20 μL were injected into an Ascentis C18 reverse-phase column (150 mm × 4.6 mm; particle size, 2.7 μm), with the temperature maintained at 16 °C. The solvents were methanol, water and formic acid (solvent A, 2:97:1, v/v/v; solvent B, 100:0:0, v/v/v), and the flow rate was 0.30 mL/min. The gradient for solvent B was as follows: 0 min, 5 %; 2 min, 5 %; 25 min, 30 %; 40 min, 55 %; 50 min, 65 %; 55 min, 95 %; 65 min, 95 %; 70 min, 5 %; and 80 min, 5 %. The Ion Trap ESI-MS/MS detector was used in negative-ion mode, and the MS conditions were as follows: ion spray voltage, -4000 V; ion source temperature, 400 °C; collision gas, high; curtain gas, 15 psi; ion source gas 1, 50 (arbitrary units); ion source gas 2, 50 (arbitrary units); declustering potential, -35 V; entrance potential, -10 V; collision energy, -30 V; and collision cell exit potential, -3 V. Standards of trans-resveratrol and trans-piceid, as well as their cis isomers, were used for identification and quantification, which was achieved by reference to calibration curves covering the range of concentrations expected in the samples. The multiple reaction-monitoring ion chromatograms were obtained after selection of the m/z transitions expected for the compounds under study: cis- and

![FIGURE 1. Multiple reaction-monitoring ion chromatogram.](image-url)

The chromatogram was obtained for a wine sample at m/z transitions selected to: A, trans- resveratrol (empty peak) and cis-resveratrol (filled peak) (m/z, 227/143-227/185); and B, trans-piceid (empty peak) and cis-piceid (filled peak) (m/z, 389/227-389/185).
trans-resveratrol, 227/143-227/185; and cis- and trans-piceid, 389/227-389/185 (Figure 1).

6. Statistical data analysis

The results were subjected to ANOVA with separation of media through the Tukey test (significance level, 0.05). The program used was InfoStat (2016, professional version).

RESULTS AND DISCUSSION

1. Concentration of stilbenes in grapes

Table 2 shows stilbene concentrations per unit of skin mass, enabling analysis of the stilbene-synthesizing capacity of the grapes, and per unit of grape berry mass, an expression more suitable for enological and practical considerations.

In the 2015 vintage, Syrah skin had a very high stilbene concentration, much higher than the concentrations in the other grape varieties. The non-Syrah grapes showed similar stilbene-synthesizing capacity despite being from different cultivars, and in the case of Tannat, being harvested from two different vineyards (Tannat 1 and 2) or at different degrees of maturity (Tannat 2 H1 and H2).

In the 2016 vintage, Tannat skin had a similar stilbene concentration to that of Syrah skin from the first harvest, and Marselan skin had the lowest concentrations of all the samples analysed. In Marselan there were no significant changes in stilbene synthesis between the two harvest dates (Marselan H1 and H2), similar to the results for Tannat in the previous year. Relating the synthesis of stilbenes to climatic variables was not an aim of the present study; however, we note that it was not possible to relate the stilbene synthesis results for Tannat and Marselan to the accumulation of growing degree days or sunlight in the periods between harvests (see Table 1). In contrast, in Syrah, stilbenes accumulated at a high rate between harvests; consequently, stilbene concentration in Syrah skin from the second harvest was the highest recorded for grape skins from the 2016 vintage. Grape genotypes conferring higher resveratrol production also have greater synthesis of transcripts related to enzymes involved in stilbene synthesis, thus enabling high rates of stilbene accumulation during maturation (Gatto et al., 2008).

The great variability in stilbene concentration between samples of skin from grapes of the same cultivar meant that it was not possible for statistical differences to be detected in all cases. Such variability is expected because stilbenes are synthetized constitutively at very low concentrations, and many factors elicit their synthesis (Gatto et al., 2008; Flamini et al., 2013; Bavaresco et al., 2016). However, several studies have shown the importance of genetic factors in determining the stilbene-synthesizing capacity of grapes (Bavaresco et al., 2007; Gatto et al., 2008; Gatti et al., 2014), which is consistent with our results. Gatto et al. (2008) proposed classifying cultivars into higher stilbene producers (stilbene concentration, > 2.3 mg/kg of grape berries) and lower stilbene producers (stilbene concentration, 0.2-1.8 mg/kg of grape berries at harvest). In the present study, only Syrah consistently had stilbene concentrations higher than 2.3 mg/kg of grape berries. Furthermore, the concentrations reached levels much higher than those previously published for this cultivar (Sun et al., 2006; Fernández-Marín et al., 2013). In studies carried out by Fernández-Marín et al. (2013), Syrah was notable for both its high basal and its high induced stilbene concentrations. Additionally, resveratrol concentrations have been reported to be higher in Syrah than in Marselan, Cabernet-Sauvignon or Merlot (Shi et al., 2016).

Depending on the sample, stilbenes were present in Tannat and Marselan grapes at concentrations both higher and lower than the 1.8 and 2.3 mg/kg thresholds mentioned above. The Tannat cultivar is characterized by its very high potential for synthesizing polyphenols such as anthocyanins and tannins. For any conclusions to be made regarding stilbene synthesis in Tannat, larger-scale studies of grape and wine from a greater number of Tannat vineyards and under different culture situations are needed. However, in the present study, the results for Tannat were not notable in this regard. Two enzymes, chalcone synthase and stilbene synthase, control the entry points into the flavonoid and stilbene pathways, respectively, and compete for the same substrates (Flamini et al., 2013). The results for Tannat may be due to preferential use of the precursors for flavonoid synthesis; more detailed studies are needed to explore this hypothesis.

The stilbene concentrations found in the present study are much higher than those in other regional reports (Fanzone et al., 2011; de Castilhos et al., 2015). However, it is difficult to compare data obtained using different methodological procedures (Sun et al., 2006).
2. Concentrations of different stilbenes in grapes

Resveratrol exists in two isoforms, cis and trans, and their respective glucosides are cis- and trans-piceid (Flamini et al., 2013; Pannu and Bhatnagar, 2019). In samples from the 2015 vintage, the free resveratrol form tended to be more abundant than the glucosides, whereas the opposite trend was found in samples from the 2016 vintage (see Table 2). Resveratrol glucosides would preferentially be expressed constitutively, being the form used for storage, translocation, modulation of antifungal activity, and protection from oxidative degradation (Flamini et al., 2013), whereas trans-resveratrol would be inducible (Gatto et al., 2008). Therefore, the grapes from 2016 contained a lower proportion of inducible stilbenes, based on the generally lower concentrations of trans-resveratrol in that year compared with 2015.

Grapes with no apparent fungal infection have been reported to contain similar amounts of trans-resveratrol and trans- and cis-piceid, and infected grapes to have a much higher proportion of trans-resveratrol (Roméro-Pérez et al., 2001). In the present study, all the grapes looked healthy, and although it is true that infected berries initially show no signs of fungus, both 2015 and 2016 were particularly dry during the grape maturity period and consequently there was a very low incidence of rot in the vineyards. Therefore, differences in response to fungal infection would not have contributed significantly to the differences reported here.

An additional observation is that trans-piceid was in all cases the dominant stilbene-glucoside isomer, particularly in skins from the 2016 vintage. In grapes, the cis isomer of resveratrol is usually not reported. However, it has been described as only slightly detectable (Jeandet et al., 1995; Moreno et al., 2008). In wines, its presence mainly corresponds to the isomerization that occurs in response to factors such as ultraviolet radiation (Pannu and Bhatnagar, 2019). However, interestingly, in the present study the cis isomer of resveratrol was found in all Syrah skin samples (see Table 2), and its presence could be a characteristic of this cultivar. In a previous study, the cis isomer of piceid was not found in grapes of some cultivars, including Syrah (Sun et al., 2006). However, in the present study it represented a significant proportion of the total stilbene content in all three cultivars studied.

The stilbene profiles of the grapes show important differences between the years (see Table 2). These may be due to multiple factors that trigger modifications in their molecular structure (Moreno et al., 2008; Flamini et al., 2013; Błaszczyk et al., 2019). Considering the results for both vintages together, it was not possible to identify any consistent relations between stilbene profile and grape variety or maturity, which indicates that other factors may have a greater contribution to the determination of stilbene content.

3. Stilbene concentrations in wines

In other vintages, Syrah wines had the highest stilbene concentrations compared with wines

| Grape sample | Total (mg/kg of skin) | Total (mg/kg of grape) | Stilbene molar profile (%) |
|--------------|-----------------------|------------------------|---------------------------|
|              |                       |                        | trans-resveratrol | cis-resveratrol | trans-piceid | cis-piceid |
| 2015         |                       |                        |                |                |              |            |
| Syrah        | 109.4 ± 37.7 a        | 7.65 ± 2.99 a          | 53.8 ± 2.7 a,b   | 3.0 ± 0.2 a    | 25.4 ± 6.7 a | 17.8 ± 3.8 a |
| Marselan     | 29.4 ± 7.1 b          | 3.24 ± 0.74 a,b       | 56.8 ± 1.1 a,b   | 0.0 ± 0.0 b    | 24.5 ± 1.2 a | 18.7 ± 0.2 a |
| Tannat 1     | 21.1 ± 11.7 b         | 1.73 ± 1.14 a,b       | 62.5 ± 5.4 a     | 0.0 ± 0.0 b    | 28.1 ± 3.87 a | 9.4 ± 1.5 b |
| Tannat 2 H1  | 23.0 ± 1.9 b          | 1.59 ± 0.11 b         | 54.6 ± 0.1 a,b   | 0.0 ± 0.0 b    | 36.3 ± 0.2 a | 9.1 ± 0.1 b |
| Tannat 2 H2  | 24.6 ± 5.9 b          | 2.11 ± 0.64 a,b       | 47.1 ± 0.0 a     | 0.0 ± 0.0 b    | 36.9 ± 1.1 a | 16.0 ± 1.6 a,b |
| 2016         |                       |                        |                |                |              |            |
| Syrah H1     | 34.6 ± 4.3 a          | 2.55 ± 0.32 a         | 36.1 ± 4.5 a     | 4.2 ± 2.0 a    | 46.5 ± 6.9 a | 13.3 ± 0.3 a |
| Syrah H2     | 61.9 ± 42.1 a         | 5.29 ± 3.71 a         | 38.5 ± 3.2 a     | 4.2 ± 2.2 a    | 45.8 ± 4.3 a | 11.5 ± 3.3 a |
| Tannat 1     | 35.1 ± 15.5 a         | 2.57 ± 0.81 a         | 29.2 ± 4.4 a     | 0.0 ± 0.0 b    | 63.4 ± 3.8 a | 7.3 ± 0.6 a |
| Marselan H1  | 16.5 ± 2.3 a          | 1.73 ± 0.31 a         | 41.5 ± 2.2 a     | 0.0 ± 0.0 b    | 41.5 ± 2.3 a | 17.1 ± 0.1 a |
| Marselan H2  | 18.9 ± 2.8 a          | 2.08 ± 0.41 a         | 40.8 ± 7.5 a     | 0.0 ± 0.0 b    | 43.7 ± 12.3 a | 15.5 ± 4.8 a |

H1 and H2, first and second harvest, respectively; Tannat 1 and Tannat 2, grapes from two different closely situated Tannat vineyards.

*Total stilbene content (the sum of both resveratrol isomers plus both resveratrol glucoside isomers) expressed as mg/kg of fresh skin (skin) or mg/kg of grape berries (grape).
produced from grapes of the other cultivars (Tables 3 and 4). Wines produced from Syrah grapes collected in the second harvest in 2016 had a much higher stilbene concentration than those produced from grapes collected in the first harvest (see Table 4), consistent with the results for the corresponding skin samples (see Table 2). However, such correspondence between stilbene concentration in grape skin and that in wine were not found in all cases.

Marselan wines had much lower stilbene concentrations than would be expected from the concentrations in the grapes from which they were produced (see Tables 2 and 4). Stilbenes are extracted at a very low rate during maceration (Sun et al., 2006), and this phenomenon could be more pronounced if the grape variety has characteristics that limit extraction. We have previously confirmed the low extractability of other classes of polyphenols from Marselan (data not shown). In wineries, this cultivar is well known for having a high proportion of skin, whose structure is almost unaffected by winemaking. Further studies are needed to explain these results for Marselan in the present study. However, they highlight the fact that limitations in the extractability of stilbenes from grapes can greatly limit their concentration in wine, independently of their concentrations in the grapes from which the wine was produced. These constraints would be a particular issue for certain grape varieties, such as Marselan.

Overall, we have identified various factors affecting the resveratrol concentration of red wine produced from healthy grapes. These include grape variety, growing season, ease of extraction from the grapes, and at least in Syrah, maturity of the grape berries at harvest.

Most wines in the present study had stilbene concentrations in the ranges previously reported for commercial wines from grape varieties, including Syrah and Cabernet-Sauvignon, cultivated in different regions of South America (e.g. San Francisco valley, Pernambuco, Brazil; Rio Grande do Sul, Brazil; and Mendoza and San Juan, Argentina) (Belmiro et al., 2017). However, they were even higher in the Syrah wines (particularly those of the 2016 vintage) and generally lower in the Marselan wines.

4. Changes in total stilbene concentration and relative concentrations of different stilbenes over time

Total stilbene concentration remained very stable over time; there were no significant differences in any of the wines over the period of evaluation (see Tables 3 and 4). Such stability, which is not found for other kinds of polyphenol, is interesting because stilbenes are enologically relevant due mainly to their role as bioactive compounds. Furthermore, the results show that stilbene concentrations just after wine stabilization may reflect the concentrations in wines purchased by potential consumers, because most wines are consumed young, in the time frame of the analyses carried out in the present study (i.e. up to 24 months after stabilization). Although the total stilbene concentrations of wines were stable

### TABLE 3. Changes in stilbene composition of wines from the 2015 vintage during storage.

| Wine sample | Time since first analysis (months) | Total (mg/L) | trans-resveratrol (mg/L) | cis-resveratrol (mg/L) | trans-piceid (mg/L) | cis-piceid (mg/L) |
|-------------|-----------------------------------|--------------|-------------------------|-----------------------|-------------------|------------------|
| Syrah       | 0                                 | 4.97 ± 1.75 a| 2.02 ± 0.60 a           | 0.61 ± 0.23 a         | 0.84 ± 0.29 a     | 1.51 ± 0.64 a    |
|             | 12                                | 4.48 ± 1.13 a| 1.57 ± 0.25 a           | 0.81 ± 0.59 a         | 1.05 ± 0.16 a     | 1.06 ± 0.13 a    |
|             | 24                                | 4.71 ± 0.26 a| 1.58 ± 0.13 a           | 0.68 ± 0.35 a         | 1.32 ± 0.18 a     | 1.14 ± 0.04 a    |
| Marselan    | 0                                 | 0.91 ± 0.18 a| 0.45 ± 0.04 a           | 0.19 ± 0.06 a         | 0.04 ± 0.02 a     | 0.24 ± 0.07 a    |
|             | 12                                | 0.57 ± 0.17 a| 0.20 ± 0.11 a           | 0.34 ± 0.01 a         | 0.01 ± 0.01 a     | 0.03 ± 0.04 b    |
|             | 24                                | 0.57 ± 0.09 a| 0.17 ± 0.01 b           | 0.40 ± 0.09 a         | 0.00 ± 0.00 a     | 0.00 ± 0.00 b    |
| Tannat 1    | 0                                 | 2.04 ± 0.38 a| 0.84 ± 0.06 a           | 0.21 ± 0.06 a         | 0.45 ± 0.14 a     | 0.55 ± 0.12 a    |
|             | 12                                | 2.30 ± 0.46 a| 0.68 ± 0.11 a           | 0.18 ± 0.01 a         | 0.84 ± 0.21 a     | 0.60 ± 0.15 a    |
|             | 24                                | 2.77 ± 0.56 a| 0.72 ± 0.13 a           | 0.21 ± 0.01 a         | 1.05 ± 0.26 a     | 0.79 ± 0.18 a    |
| Tannat P H1 | 0                                 | 2.13 ± 0.03 b| 0.80 ± 0.01 a           | 0.30 ± 0.06 a         | 0.44 ± 0.06 b     | 0.61 ± 0.04 a    |
|             | 12                                | 2.69 ± 0.03 a| 0.78 ± 0.02 a           | 0.23 ± 0.00 a         | 0.85 ± 0.08 a     | 0.84 ± 0.10 a    |
|             | 24                                | 2.61 ± 0.21 a| 0.73 ± 0.03 a           | 0.21 ± 0.03 a         | 0.86 ± 0.13 a     | 0.81 ± 0.08 a    |
| Tannat P H2 | 0                                 | 2.87 ± 0.06 a| 1.07 ± 0.00 a           | 0.23 ± 0.07 a         | 0.73 ± 0.01 b     | 0.85 ± 0.01 a    |
|             | 12                                | 3.38 ± 0.33 a| 0.92 ± 0.10 a           | 0.16 ± 0.01 a         | 1.28 ± 0.12 a     | 1.02 ± 0.11 a    |
|             | 24                                | 3.44 ± 0.28 a| 0.87 ± 0.08 a           | 0.14 ± 0.01 a         | 1.40 ± 0.09 a     | 1.04 ± 0.09 a    |

H1 and H2, first and second harvest, respectively; Tannat 1 and Tannat 2, grapes from two different closely situated Tannat vineyards; Analysis carried out 3 months after pressing.
over this time frame, the relative concentrations of the four forms evaluated were not. This result was as expected based on the literature, as a result of glucoside moiety hydrolysis and the trans/cis isomerization that occurs in wines (Mattivi et al., 1995; Sun et al., 2006; Pannu and Bhatnagar, 2019). However, in the present study, the general trend over the analytical period was a decrease in trans-resveratrol and a corresponding increase in the trans-resveratrol glucoside (Figure 2).

This difference from the expected results may be due to imbalances resulting from the multiple potential reactions in the complex matrix of red wine, particularly when it is still young. In other studies, we found that over 90% of the stilbenes in 3-year-old red wines were in the form of cis-resveratrol (data not shown).

The results of some studies have suggested that trans-resveratrol has higher biological activity than cis-resveratrol, because the lower steric hindrance of its substituents (Anisimova et al., 2011). However, a more extensive review of previous studies has shown that each stilbene form has specific properties depending on the experiment, or even has similar biological activity (Leiro et al., 2004).

**CONCLUSIONS**

Stilbene concentrations in healthy grapes are highly unpredictable; however, some factors affecting concentrations in grape skins and wines have been identified, such as the stilbene-synthesizing capacity of the grape cultivar and differences in ease of extraction of stilbenes during winemaking. The influence of the first factor was particularly apparent for Syrah, which showed much greater stilbene-synthesizing capacity compared with Tannat and Marselan. The influence of ease of extraction was evident in Marselan wines.
which had much lower stilbene concentrations than would be expected based on concentrations in the grapes from which they were produced. Therefore, for this cultivar, it would be interesting to investigate maceration techniques that have been developed to increase phenolic extraction, with stilbene concentration as an indicator of extraction efficiency.

Delaying harvest time may or may not have a great impact on grape stilbene concentration, probably depending on the stilbene-synthesizing capacity of the grape variety. The present study also showed stilbene concentrations in wine to be very stable during wine storage, at least during the time frame of the analyses (i.e. from wine stabilization to 24 months later), which is also the period during which most red wines are consumed. To the best of our knowledge, this article is the first report of data on resveratrol and its glucosides in grapes and wines from Uruguay.

Acknowledgements: this work was funded by the Comisión Sectorial de Investigación Científica (CSIC), programme MIA 2015 and 2017; the Agencia Nacional de Investigación e Innovación (ANII), programme Becas de Movilidad tipo Capacitación 2015; and the Comisión Académica de Posgrados (UDELAR) through their grant programme Becas de Apoyo a Docentes para realizar estudios de Posgrado, 2015. We also thank the Spanish Ministerio de Economía y Competitividad for financial support (project AGL2014-56594-C2-2-R), and the Bodegas Pisano Hermanos and Establecimiento Juanicó for the grapes used in the investigation.

REFERENCES

Anisimova N.Y., Kiselevsky M.V., Sosno, A.V., Sadovnikov S.N., Stankov I and Gakh A., 2011. Trans-, cis-, and dihydro-resveratrol: a comparative study. Chemistry Central Journal, 5(1):88. doi:10.1186/1752-153X-5-88

Bavaresco L., Fregoni M., Trevisan M., Mattivi F., Vrhovsek U. and Falchetti R., 2007. Role of the variety and some environmental factors on grape stilbenes. 

Bavaresco L., Fregoni M., Trevisan M., Mattivi F., Gatto P., Vrhovsek U., Muth J., Segala C., Romualdi P., Stefanini M., Moser C., Mattivi F. and Velasco R., 2014. Viticultural performances of different ‘Cabernet-Sauvignon’ clones. ActaHorticulturae, (1046):659-664. doi:10.17660/ActaHortic.2014.1046.90

Bavaresco L., Fregoni M., Trevisan M., Mattivi F., Vrhovsek U. and Falchetti R., 2002. The occurrence of the stilbene piceatannol in grapes. Vitis, 41(3):133-36.

Bavaresco L., Fregoni M., Trevisan M., Mattivi F., Vrhovsek U. and Falchetti R., 2002. The occurrence of the stilbene piceatannol in grapes. Vitis, 41(3):133-36.

Fanzone M., Zamora F., Jofr, V., Assof M. and Peña-Neira A., 2011. Phenolic composition of Malbec grape skins and seeds from Valle de Uco (Mendoza, Argentina) during ripening. Effect of cluster thinning. Journal of Agricultural and Food Chemistry, 59(11):6120-36. doi:10.1021/jf200073k

Fernández-Marín M.I., Guerrero R.F., García-Parrilla M.C., Puertas B., Ramírez P. and Cantos-Villar E., 2013. Terroir and variety: two key factors for obtaining stilbene-enriched grapes. Journal of Food Composition and Analysis, 31(2):191-98. doi:10.1012/jf200073k

Flamini R., Mattivi F., De Rosso M., Arapitsa P., Bavaresco L., 2013. Advanced knowledge of three important classes of grape phenolics: anthocyanins, stilbenes and flavonols Consiglio per La Ricerca e La Sperimentazione in Agricoltura-Centro Di Ricerca Per. International Journal of Molecular Sciences, 14:19651-19669. doi:10.3390/ijms141019651

Gatti M.S., Civardi F., Ferrari N., Fernandes N., van Zeller de Macedo Basto Goncaves M. I. and Bavaresco L., 2014. Viticultural performances of different ‘Cabernet-Sauvignon’ clones. ActaHorticulturae, (1046):659-664. doi:10.17660/ActaHortic.2014.1046.90

Gatto P., Vrhovsek U., Muth J., Segala C., Romualdi C., Fontana P., Pruefer D., Stefanini M., Moser C., Mattivi F. and Velasco R., 2008. Ripening and genotype control stilbene accumulation in healthy grapes. Journal of Agricultural and Food Chemistry, 56(24):11773-11785. doi:10.1021/jf200073k

González-Neves G., Barreiro L., Gil G., Franco J., Ferrer M., Moutounet M. and Carbonneau A., 2004. Anthocyanic composition of Tannat grapes from the south region of Uruguay. Analytica Chimica Acta, 513(1):197-202. doi:10.1016/j.aca.2003.11.078
Ingham J.L., 1976. 3,5,4′-Trihydroxystilbene as a phytoalexin from groundnuts (Arachis hypogaea). Phytochemistry, 15(11):1791-93. doi:10.1016/S0031-9422(00)7494-6

INIA, 2019. Instituto Nacional de Investigación Agropecuaria. In: http://www.inia.uy/investigaci%C3%B3n-e-innovaci%C3%B3n/GRAS/Clima/Banco-datos-agroclimatico. Accessed 25 October 2019

Jeandet P., Bessis R., Maume B.F., Meunier P., Peyron D., and Trollat P., 1995. Effect of enological practices on the resveratrol isomer content of wine. Journal of Agricultural and Food Chemistry, 43(2):316-319. doi:10.1021/jf00050a010

Jeandet P., Douillet-Breuil A.C., Bessis R., Debord S., Sbaghi M. and Adrian M., 2002. Phytoalexins from the Vitaceae: biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. Journal of Agricultural and Food Chemistry, 50(10):2731-41. doi:10.1021/jf011429s

Keylor M.H., Matsuura B.S. and Stephenson C.R.J., 2015. Chemistry and biology of resveratrol-derived natural products. Chemical Reviews, 115(17):8976-9027. doi:10.1021/cr500689b

Langcake P., 1981. Disease resistance of Vitis spp. and the production of stress metabolites resveratrol, e-viniferin, α-viniferin and pterostilbene. Physiologically Plant Pathology, 1:213-226. doi:10.1016/S0048-4059(81)80043-4

Langcake P. and McCarthy W.V., 1979. The relationship of resveratrol production to infection of grapevine leaves by Botrytis cinerea. Vitis, 18:244-253. doi:10.1016/S0031-9422(00)91470-5

Leiro J., Álvarez E., Arranz J.A., Laguna R., Uriarte E. and Orallo F., 2004. Effects of cis-resveratrol on inflammatory murine macrophages: antioxidant activity and down-regulation of inflammatory genes. Journal of Leukocyte Biology, 75(6):1156-1165. doi:10.1189/jlb.1103561

Lopez-Lluch G., Santa Cruz-Calvo S., and Navas P., 2012. Resveratrol in cancer: cellular and mitochondrial consequences of proton transport inhibition. Current Pharmaceutical Design, 18(10):1338-144. doi:10.2174/138161212799504849

Mattivi F., Reniero F. and Korthammer S., 1995. Isolation, characterization, and evolution in red wine vinification of resveratrol monomers. Journal of Agricultural and Food Chemistry, 43(7):1820-1823. doi:10.1021/jf00055a013

Moreno A., Castro M. and Falqué E., 2008. Evolution of trans- and cis-resveratrol content in red grapes (Vitis vinifera L. cv Mencia, Albarello and Merenzao) during ripening. European Food Research and Technology, 227(3):667-74. doi:10.1007/s00217-007-0770-1

Pannu N. and Bhatnagar A., 2019. Resveratrol: from enhanced biosynthesis and bioavailability to multitargeting chronic diseases. Biomedicine & Pharmacotherapy, 109:2237-2251. doi:10.1016/j.biopsych.2018.11.075

Rauf A., Imran M., Sadiq Butt M., Nadeem M., Peters D.G. and Mubarak M.S., 2018. Resveratrol as an anti-cancer agent: a review. Critical Reviews in Food Science and Nutrition, 58(9):1428-1447. doi:10.1080/10408398.2016.1263597

Roméro-Pérez A.I., Lamuela-Raventós R.M., Adrés-Lacueva C. and de la Torre-Boronat M.C., 2001. Method for the quantitative extraction of resveratrol and piceid isomers in grape berry skins. Effect of powdery mildew on the stilbene content. Journal of Agricultural and Food Chemistry, 49(1):210-215. doi:10.1021/jf0007450

Ruiz-Garcia Y., Hernández-Jiménez A., Gómez-Plaza E., López-Roca J.M., Romero-Cascales I., Martinez-Cutillas A. and Gil-Muñoz R., 2012. Application of BTH and methyl jasmonate during the ripening of grapes (Vitis vinifera L.) and its effects on the stilbene content: preliminary results. Acta Horticulturae, (939):397-401. doi:10.17660/ActaHortic.2012.939.53

Sáez V., Gayoso C., Riquelme R., Pérez J., Vergara C., Mardones C. and von Baer D., 2018. C18 Core-shell column with in-series absorbance and fluorescence detection for simultaneous monitoring of changes in stilbenoid and proanthocyanidin concentrations during grape cane storage. Journal of Chromatography B, 1074:70-78. doi:10.1016/j.jchromb.2017.12.028

Shi P.B., Yue T.X., Ai L.L., Cheng Y.F., Meng J.F., Li M.H. and Zhang Z.W., 2016. Phenolic compound profiles in grape skins of Cabernet-Sauvignon, Merlot, Syrah and Marselan cultivated in the Shacheng area (China). South African Journal of Enology and Viticulture, 37(2):132-38. doi:10.21548/37-2-898

Sun B., Ribes A.M., Leandro M.C., Belchior A.P. and Spranger M.I., 2006. Stilbenes: quantitative extraction from grape skins, contribution of grape solids to wine and variation during wine maturation. Analytica Chimica Acta, 563(1-2):382-90. doi:10.1016/j.aca.2005.12.002

Vannozzi A., Dry I.B., Facsoli M., Zenoni S. and Lucchin M., 2012. Genome-wide analysis of the grapevine stilbene synthase multigenic family: genomic organization and expression profiles upon biotic and abiotic stresses. BMC Plant Biology, 12(1):130. doi:10.1186/1471-2229-12-130

Vincenzi S., Tomasi D., Gaiotti F., Lovat L., Giacosa E., López-Roca J.M., Romero-Cascales I., Martínez-Cutillas A. and von Baer D., 2014. Comparative study of the resveratrol content of twenty-one Italian red grape varieties. South African Journal of Enology and Viticulture, 34(1):30-35. doi:10.21548/34-1-1078

Waterhouse A.L and Lamuela-Raventós R.M., 1994. The occurrence of piceid, a stilbene glucoside, in grape berries. Phytochemistry, 37(2):571-73. doi:10.1016/0031-9422(94)85102-6.