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

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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 synthetized 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 synthetized 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 synthetized 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 were collected from each batch, 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 × 50 cm plain surface on which was marked a numbered grid of 5 × 5 cm squares. A sequence of squares was chosen randomly, and then the grapes in each of these squares were collected until 35 grapes had been obtained.

Each 35-berry sample was weighed and then peeled (the pulp remaining against the skins removed carefully with the help of a rounded-edge blade). The resulting skin sample was gently blotted with a paper towel, weighed (to determine skin fresh weight) and freeze-dried. After freeze-drying, the skin sample was weighed again (to determine skin dry weight) and then stored at -18°C in 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).

Table 1. Basic chemical parameters of the grapes at harvest[a]

Grape sample	Harvest date	Sugars (g/L)[b]	Acidity (g/L)[c]	pH[d]	GD_{10}	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 b	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	4.74 ± 0.06 e	3.47 ± 0.06 a	834	599
Tannat 1	9 March 2016	208 ± 1.7 c	7.42 ± 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

GD_{10}, growing degree days accumulated from 1 January until harvest; H1 and H2, first and second harvest, respectively; He (hs), heliophany 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.

[a]Data 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.

[b]Sugar concentrations were determined by refractometry.

[c]Acidity was determined by titration and is expressed as g/L of tartaric acid.

[d]pH was determined by potentiometry.

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 SO/100 kg of grapes), and the grapes were inoculated with dry active yeast (*Saccharomyces cerevisiae* ex *bayanus* Natuferm 804; OenoBioTech, Paris, France; 20 g/kg of grapes).

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 SO_2 (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 $CH_3OH/H_2O/HCOOH$ (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 *trans*-resveratrol, 227/143-227/185; and *cis*- and *trans*-piceid, 389/227-389/185 (Figure 1).

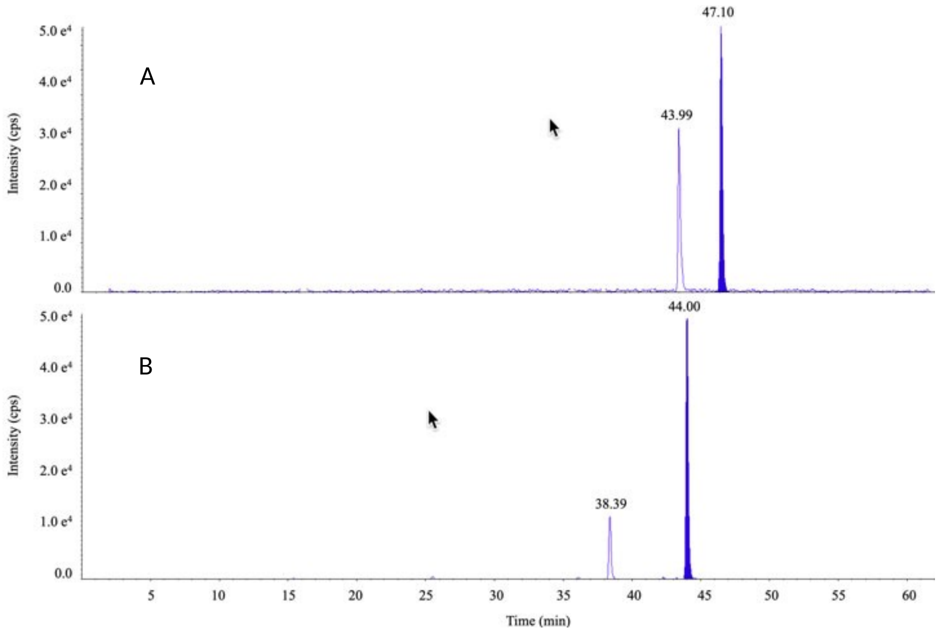

Figure 1. Multiple reaction-monitoring ion chromatogram.

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).

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 in 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.

Table 2. Stilbene composition of grapes at harvest[a]

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 b	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 ± 4.3a	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.

[a]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).

An additional observation is that *trans*-piceid was in all cases the dominant stilbene-glucoside isoform, 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 both vintages, Syrah wines had the highest stilbene concentrations compared with wines 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 2-4). 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.

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

Wine sample	Time since first analysis (months)[a]	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,b	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,b	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.
[a]Time since first analysis (carried out 3 months after pressing).

Table 4. Changes in stilbene composition of wines from the 2016 vintage during storage

Wine sample	Time since first analysis (months)[a]	Total (mg/L)	*trans*-resveratrol	*cis*-resveratrol	*trans*-piceid	*cis*-piceid

			(mg/L)	(mg/L)	(mg/L)	(mg/L)
Syrah H1	0	5.82 ± 0.21 b	1.89 ± 0.15 b	1.04 ± 0.14 a	0.97 ± 0.04 b	1.93 ± 0.16 b
	12	6.57 ± 0.21 b	1.94 ± 0.04 b	1.08 ± 0.08 b	1.35 ± 0.02 b	2.20 ± 0.06 b
Syrah H2	0	12.38 ± 0.40 a	4.10 ± 0.30 a	1.66 ± 0.16 b	2.34 ± 0.08 a	4.28 ± 0.18 a
	12	13.96 ± 0.52 a	4.12 ± 0.21 a	1.92 ± 0.18 a	3.28 ± 0.00 a	4.64 ± 0.13 a
Tannat 1	0	5.69 ± 0.69 b	1.87 ± 0.19 b	0.75 ± 0.01 b	1.14 ± 0.23 b	1.93 ± 0.28 b
	12	6.23 ± 0.64 b	1.77 ± 0.31 b	0.63 ± 0.01 c	1.60 ± 0.16 b	2.23 ± 0.18 b
Marselan H1	0	1.34 ± 0.08 c	0.49 ± 0.01 c	0.20 ± 0.03 c	0.18 ± 0.01c	0.46 ± 0.01 c
	12	1.39 ± 0.16 c	0.42 ± 0.05 c	0.21 ± 0.07 d	0.26 ± 0.00 c	0.51 ± 0.04 c
Marselan H2	0	1.73 ± 0.01 c	0.63 ± 0.01 c	0.26 ± 0.06 c	0.22 ± 0.07 c	0.62 ± 0.03 c
	12	1.76 ± 0.40 c	0.52 ± 0.14 c	0.25 ± 0.01 d	0.33 ± 0.08 c	0.67 ± 0.17 c

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

[a]Time since first analysis (carried out 3 months after pressing).

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 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).

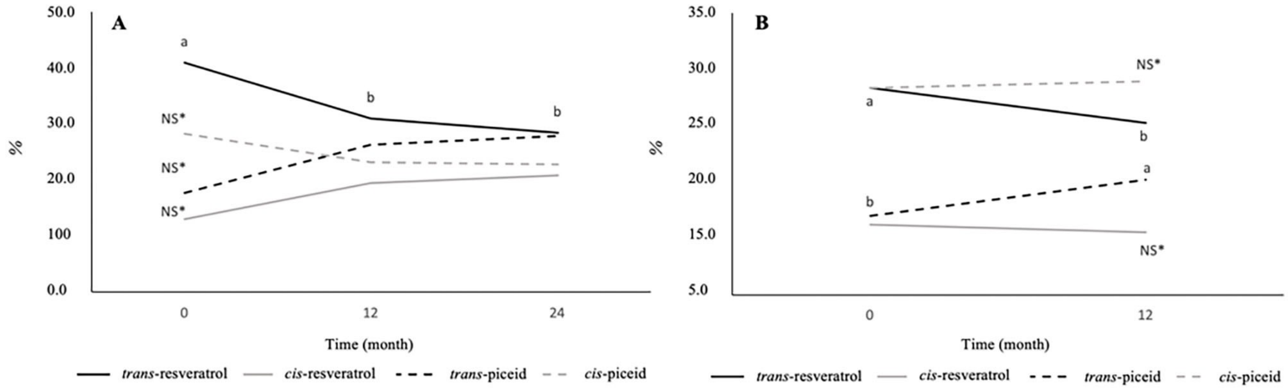

Figure 2. Changes over time in the relative contribution of the different stilbenes in wines from the 2015 vintage (A) and the 2016 vintage (B).
Molar percentages calculated from the data shown in Tables 3 and 4. NS, not significant.

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.

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