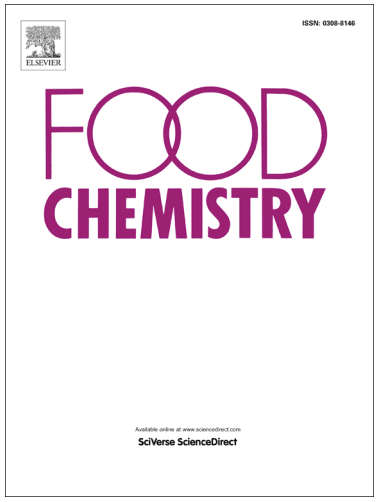
Sulfur free red wines through the use of grapevine shoots: Impact on the wine quality



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PII: S0308-8146(17)31588-1
DOI: https://doi.org/10.1016/j.foodchem.2017.09.111
Reference: FOCH 21780

To appear in: *Food Chemistry*

Received Date: 27 February 2017
Revised Date: 21 September 2017
Accepted Date: 21 September 2017

Please cite this article as: Raposo, R., Chinnici, F., Ruiz-Moreno, M.J., Puertas, B., Cuevas, F.J., Carbú, M., Guerrero, R.F., Ortíz-Somovilla, V., Moreno-Rojas, J.M., Cantos-Villar, E., Sulfur free red wines through the use of grapevine shoots: Impact on the wine quality, *Food Chemistry* (2017), doi: https://doi.org/10.1016/j.foodchem.2017.09.111

Sulfur free red wines through the use of grapevine shoots: Impact on the wine quality

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Abstract

Following a preliminary study to determine the possibility of using a grapevine shoot extract (VIN) as a sustainable alternative to sulfur dioxide (SO_2), in this study, the chromatic features, phenolic composition, and sensory analysis of wines treated with VIN at two concentrations were studied during storage in bottle for the first time. The highest differences were found in phenolic compounds after 12 months of storage in bottle. The VIN wines had a low content of free anthocyanins and were high in vinyl-pyranoanthocyanins, and B-type vitisins. Consequently, they showed better chromatic characteristics. Moreover VIN, especially at high dose, preserved non-anthocyanin phenolic compounds better than SO_2. However, at this high dose some organoleptic properties were affected. VIN, when used at a low dose, is able to preserve wine composition without loss of quality.

Keywords: stilbene, sulfur dioxide, quality wine, polyphenols, color, anthocyanins.

1. Introduction

Wine quality is defined by sensory attributes, which are determined by the physical and chemical characteristics of the wine. Since phenolic compounds are essential constituents of

wine and are responsible for important organoleptic characteristics such as color, astringency and bitterness, they constitute an important quality parameter of red wine. In wine, they are mainly composed of anthocyanins, including monomeric anthocyanins and their derivatives, and non-anthocyanin phenolic compounds which include hydroxybenzoic and hydroxycinnamic acids (and their derivatives), flavanols and flavonols. The color of young red wine is mainly a result of the quantity and quality of monomeric anthocyanins, while astringency and bitterness is related to flavanols and phenolic acids (He et al., 2012a, 2012b). During maturation and wine aging, there is a progressive loss of phenolic compounds due to their participation in a number of chemical reactions, such as degradation, oxidation, precipitation with polysaccharides, condensation with tannins and the formation of other stable anthocyanin-derived pigments. All these reactions can result in significant changes in the color, mouth-feel and flavor properties of red wines (Fulcrand, Dueñas, Salas, & Cheynier, 2006). Anthocyanins are transformed into oligomeric and polymeric pigments through condensation reactions with flavanols, either directly or mediated by aldehydes. Monomeric anthocyanins are also involved in other condensation reactions with pyruvic acid, 4-vinylphenols, hydroxycinnamic acids and acetaldehyde, among others, leading to the so-called pyranoanthocyanins. They can help to preserve the color of red wine due to their stability and resistance to oxidation (Fulcrand et al., 2006; Rentzsch, Schwarz, Winterhalter, & Hermosín-Gutiérrez, 2007; Schwarz, Hofmann, & Winterhalter, 2004). In turn, polymeric pigments, which play an important role in the overall color intensity of aged wines, have been shown to be barely prone to discoloration, in this case produced by SO_2.

SO_2 is the most important and widely used preservative in winemaking due to its well-known antioxidant and antimicrobial properties. However, several human health risks, including dermatitis, urticaria, angioedema, diarrhea, abdominal pain, bronchoconstriction and anaphylaxis, have been associated with its presence in wines (reviewed by Guerrero &

Cantos-Villar, 2015). Consequently, there is a great deal of interest in finding alternatives to SO_2 in winemaking. The use of phenolic compounds, for instance, has been proposed as a promising alternative to SO_2. García-Ruiz and co-workers (García-Ruiz, Moreno-Arribas, Martín-Álvarez, & Bartolomé, 2011) evaluated the antioxidant and antibacterial activity of 18 phenolic compounds mainly present in *Vitis vinifera L.* on different lactic acid bacteria (LAB). They confirmed the potential use of phenolic compounds as preservatives in wine. Furthermore, other authors have reported the use of phenolic extracts of different origins as preservatives in red wines (Salaha, Kallithraka, Marmaras, Koussissi, & Tzourou, 2008).

Today's consumers demand high quality foods that are free from additives, fresh tasting, microbiologically safe and with an extended shelf-life. With this in mind, researchers and the wine industry are looking for natural alternatives to replace SO_2 without significantly changing the quality and safety attributes of wine. In previous research, Vineatrol®, an extract from grapevine-shoot that is particularly rich in stilbenes, was tested as a preservative in red wine (Raposo et al., 2016). Although promising results were achieved, several issues needed further research: first, the optimal dose to avoid a loss of wine quality, while optimizing the preservation of antioxidant properties mainly during storage in bottle; second, important aspects regarding phenolic compounds, mainly in anthocyanins.

In this work, the quality of SO_2 free red wines elaborated with Vineatrol was studied at two doses. Quality parameters and sensory analysis were studied. Special attention was focused on polyphenolic compounds. The wine storage evolution in bottle was also followed.

2. Material and methods

2.1. Chemicals

Analytical grade methanol and acetic acid were supplied by Panreac (Barcelona, Spain). HPLC-grade acetonitrile, NaOH and acetic acid were supplied by Merck (Darmstadt, Germany). Diethyl ethoxymethylenmalonate (DEEMM), boric acid and acetaldehyde were

supplied by Sigma-Aldrich (Steinheim, Germany). The following chemical standards were purchased from Sigma-Aldrich (Steinheim, Germany): gallic acid, syringic acid, protocatechuic acid, vanillic acid, *trans*-caffeic, ferulic acid, (+)-catechin, (-)-epicatechin, procyanidin B2, myricetin-3-glucoside, myricetin-3-glucuronide, quercetin-3-*O*-glucuronide, quercetin-3-*O*-glucoside, kaempferol-3-*O*-glucosyde, isorhamnetin-3-*O*-glucoside, quercetin, *trans*-resveratrol and piceatannol. ε-Viniferin, ampelopsin A, r-viniferin, r2-viniferin, hopeaphenol, isohopeaphenol, pallidol and ω-viniferin were kindly provided by the GESVAB (Groupe D'Étude des Substances Végétales à Activité Biologique, University of Bordeaux II). Malvidin-3-*O*-glucoside was purchased from Extrasynthese (Genay, France). Ultrapure water from a Milli-Q system (Millipore Corp., Bedford, MA) was used throughout this research.

2.2. Grapevine-shoot extract

Vineatrol® was kindly provided by Actichem S.A. (Montauban, France), the developer and producer this grapevine shoot extract. This extract contains considerable amounts of stilbenoids: 5.66% *trans*-resveratrol, 13.25% ε-viniferin, 3.76% ampelopsin A, 1.44% r-viniferin, 1.22% hopheaphenol, 1.07% ω-viniferin, 1.04% pallidol, 0.97% piceatannol, 0.78% isohopeaphenol and 0.30% r2-viniferin. The total stilbene richness of Vineatrol was 29.5%. Since Vineatrol has low solubility in aqueous media, it was dissolved in wine alcohol (96%) before adding it to the wine.

It can be noted that no other phenolic compounds were detected in Vineatrol.

2.3. Winemaking

Syrah grapes (482 kg) were harvested, destemmed, crushed, and then placed into a Ganimede fermenter (Ganimede®). Previous results described few differences when Vineatrol was used in the Ganimede system compared with the traditional one (Raposo et al., 2016). Thus, for the current study Ganimede system was chosen, simplifying the process and reducing variability.

In the SO_2 free must, alcoholic fermentation (AF) was started after adding yeast (20 g/hL, ES488, Sepsa-Enartis, Spain). After AF, malolactic fermentation was induced with *Oenococcus oeni* (1 g/hL, Challenge Easy ML, Sepsa-Enartis, Spain) and nutrients (20 g/hL Nutriferm ML, Sepsa-Enartis, Spain). Once the malolactic fermentation had finished, the wine was divided into three batches, each one in triplicate, as follows: in the first batch, SO_2 (Sulfosol, Sepsa-Enartis) was added at 50 mg/L (CT), and in the other two, different amounts of Vineatrol were added: VIN-50 when 50 mg/L of the total stilbene content was added, which meant 175 mg/L of Vineatrol (29% richness in stilbenes and 99% solubility), and VIN-100 when 100 mg/L of the total stilbene content was added, which meant 430 mg/L Vineatrol (29% richness in stilbenes and 80% solubility). The wines were stabilized for 8 weeks at 0 ºC, and then racked, filtered (Optical XL, Millipore, France) and bottled. A synthetic closure made of a polymer material was chosen (Excellent cork SL, Valencia, Spain). Finally, the bottled wines were stored under controlled conditions (16 ºC and 80% RH) for 12 months. A randomly-chosen wine bottle sample was analyzed at bottling and another after 12 months of storage in bottle.

2.4. Enological parameters

Ethanol, glycerol, dry extract, total and volatile acidity, pH, organic acids (acetic, citric, tartaric, malic, lactic, and succinic), potassium, total and free SO_2 and acetaldehyde were determined following the official analytical methods established by the International Organization of Vine and Wine (OIV, 2015).The total anthocyans, tannins and total polyphenols index (TPI) were measured following the method described by (Saint-Cricq de Gaulejac, Vivas, & Glories, 1998). All the above parameters were measured at bottling, and only those which had been described to evolve during ageing in bottle were measured again after 12 months of storage in bottle (total and free SO_2, total stilbenes, acetaldehyde, total anthocyans and tannins).

2.5. Chromatic measurements and free, co-pigmented and polymerized anthocyanins

Color intensity (CI = D.O. 420 nm + D.O. 520 nm + D.O. 620 nm) and hue (D.O. 420 nm/D.O 520 nm) were determined by spectrophotometric measurements (Lambda 25, Perkin-Elmer, Massachusetts).

The colorimetric measurements were registered with a Konica-Minolta CM-3600d spectrophotometer (Osaka, Japan), using 2 mm pathlength glasscells and distilled water as reference. The CIELab parameters L* (lightness), a* (redness), b* (yellowness) were determined using the SpectraMagic v.3.61G software (CyberchromeInc, Minolta Co. Ltd), following the recommendations of the Commission Internationale de L'Eclariage (CIE). The standard observer (D10º) and the standard illuminant (D65) were used. Color differences (ΔE^*_{ab}) were calculated as the Euclidean distance between two points in the 3D space defined by L^*, a^*, and b^* (Martínez, Melgosa, Pérez, Hita, & Negueruela, 2001).

Free anthocyanins (FA), co-pigmented anthocyanins (CA), and polymeric pigments (PP) color fractions were determined following the method proposed by Boulton (1996). The wine samples were previously centrifuged and the pH adjusted to 3.60. Data from UV-Vis analysis were expressed as absorbance units (AU) with 10 mm path length and corrected by dilution, according to Bolton's protocol.

2.6. Analysis of phenolic compounds by HPLC

The analysis of the phenolic compounds was carried out by RP–HPLC/DAD.The wine was filtered at 0.22 µm and directly injected (20 µL) into a Jasco apparatus (Tokyo, Japan) equipped with a binary gradient pump (PU 1580), a Rheodyne valve (Cotati, CA), a photodiode detector (PU MD 910), a fluorescence detector (PU FP 2020 Plus) and an oven (model 7981, Jones Chromatography, Hengoed Mid Glamorgan, UK).The column was a Phenomenex (Torrance, USA) Synergy Hydro-RP 80A 25 cm x 3.0 mm ID.

The anthocyanin phenolic compounds were measured in the wines following the method described by Chinnici, Sonni, Natali, Galassi, & Riponi (2009). The elution solvents for anthocyanin analysis were 10% formic acid in HPLC grade water (solvent A) and 10% formic acid, 45% CH_3CN, 45% HPLC grade water (solvent B) with a flow of 0.47 mL/min. Quantification was performed at 525 nm for monomeric anthocyanins, 490 nm for B-type vitisins, 505 nm for A-type vitisins and vinyl-pyranoanthocyanins and 545 nm for direct and ethyl-bridged adducts.The concentration of each compound was expressed as malvidin-3-glucoside equivalents (mg/L).

The non-anthocyanin phenolic compounds were measured in the wines following the method described by Chinnici, Natali, Bellachioma, Versari, & Riponi (2015). The elution solvents were 2% acetic acid in HPLC grade water (solvent A) and 2% acetic acid, 98% CH_3CN, (solvent B). The gradient elution was as follows: initial flow 95% A for 15 min, 90% A for 25 min, 82% A for 32 min, 80% A for 37min, 70% A for 42 min; 50% A for 47 min; 0% A for 49 min. The column temperature was 30 ºC with a flow of 0.5 mL/min. Detection and quantification were carried out as follows: at 280 nm for gallic acid and syringic acid, at 256 nm for protocatechuic acid and vanillic acid, at 308 nmfor *trans-p*-coumaric acid and *trans-p*-coutaric acid, at 324 nm for *trans*-caffeic acid, *trans*-caftaric acid, ferulic acid GRP, at 365 nm for flavonols. Caffeic acid and coumaric derivatives were quantified as caffeic and coumaric acid equivalents respectively. The other compounds were quantified with their respective standards. Flavanols were quantified by fluorescence at an excitation wavelength of 280 nm and an emission wavelength of 315nm.

2.7. Analysis of stilbenes by HPLC

The stilbenes were measured in the wines by HPLC following the method described by Guerrero, Puertas, Jiménez, Cacho, & Cantos-Villar (2010). The samples (20 µL) (wine and Vineatrol) were analyzed by using a Waters HPLC system with a model 1525 pump and a

Waters 996 Photodiode Array Detector. Separations were performed on a Mediterranea Sea18 column (Tecknokroma, Barcelona, Spain) (RP-C18, 25×0.46 cm; 5 μm particle size) and a guard column of the same material, at 30 °C. The mobile phases consisted of a water:methanol:acetic acid mixture, solvent A 88:10:2 and solvent B 8:90:2 at a flow rate of 1 mL/min. Peaks were identified by comparing their retention time and UV-Vis spectra with standard compounds. Quantification was carried out at 280 nm for Ampelopsin A, isohopheaphenol, hopeaphenol, pallidol as ampelopsin A; *trans*-resveratrol was determined at 306 nm with its standard; piceatannol, r2-viniferin, ε-viniferin, r-viniferin and ω-viniferin were quantified at 320 nm as ε-viniferin. Concentrations were expressed as mg/L.

2.8. Sensory analysis

The wines (50 mL) were presented in transparent glasses distributed in a randomized order. They were evaluated in terms of 13 descriptors: odor intensity, red fruit, black fruit, jammy fruit, candy/sweet, woody/smoky, flavor intensity, bitterness, alcohol, astringency, balanced, persistence and global quality. The descriptors were scored on a continuous scale from 0 to 10 (0: absence of a descriptor, 10: maximum intensity). The wine sensory analysis was performed by twelve trained panelists.

2.9. Statistics

Ethanol, glycerol, dry extract, total and volatile acidity, pH, organic acids, potassium, total and free SO_2, total stilbenes, total anthocyans, tannins and acetaldehyde were analyzed by means of a one-way analysis of variance ($p < 0.05$). Color related parameters, co-pigmentation and phenolic compounds were analyzed by a two-way ANOVA ($p < 0.05$), but in the case of non-detected compounds, one-way analysis of variance was applied. Fischer's LSD tests ($p < 0.05$) were used as comparison tests when samples were significantly different after the ANOVA.

The data were subjected to analysis of variance using Shapiro Wilk's and Levene's tests for normality and homocedasticity requirements. The univariate analysis (ANOVA) was performed using Statistix software (v 9.0, Analytical Software, FL, USA).

3. Results and discussion

3.1. Enological parameters

The enological parameters of the wines are shown in Table 1. At bottling, no significant differences were found in glycerol, pH, volatile acidity, acetic acid, citric acid, tartaric acid, malic acid, lactic acid, succinic acid, potassium and acetaldehyde. Ethanol was significantly higher in the VIN-treated wines due to the low solubility of VIN, it was necessary to dissolve it in wine alcohol before adding it to the wine. The VIN wines at high doses (VIN-100) had the highest dry extract, which could be due to the addition of VIN (solid powder). The total acidity was slightly lower in the CT wines. As expected, the CT wines contained significant concentrations of total and free sulfur dioxide, while stilbenes were mainly detected in the VIN wines. The VIN wines showed lower total anthocyans and higher tannin contents. This is in agreement with previous results obtained in our laboratory, where a low dose of VIN also decreased anthocyanin content (Raposo et al., 2016). Regarding tannins, no possible addition of these compounds could have occurred due to VIN addition as no tannins were found when analyzing the extract (VIN composition, section 2.2).

After 12 months of storage in bottle, and apart from the preservatives (SO_2 or total stilbenes), only differences in total anthocyans and tannins were observed among wines (Table 1). Total anthocyans, tannins and acetaldehyde considerably decreased compared to levels at bottling, which could be partly due to pyranoanthocyanin formation and direct condensation products (Boido, Alcalde-Eon, Carrau, Dellacassa, & Rivas-Gonzalo, 2006; Fulcrand et al., 2006). The decrease was proportional in all wines, independent of the preservative used, maintaining the differences found at bottling, except for tannins. The decrease in tannins was slightly higher

in the VIN-50 wines. Regarding preservatives, the concentration of SO_2 in the CT wines decreased by about 62% on average, in agreement with Waterhouse et al., (2016) and Ugliano et al., (2012), while VIN treated wines showed a limited loss of stilbenes (19% lost on average).

3.2. Chromatic measurements and free, co-pigmented and polymerized anthocyanins

Sample chromatic parameters were analyzed at bottling and after 12 months of storage in bottle in the wines (Table 2). Two-way ANOVA was applied on these data using *treatment* (CT, VIN-50 and VIN-100) and *storage* (bottling and 12 months) as variables.

Regarding the *treatment* variable, the CT wines showed a lower color intensity (CI) and b*, while showing a higher hue, yellow (%Y) and luminosity (L*) than the VIN wines. Interestingly, no differences were found between the VIN treated wines at different dose (VIN-50, VIN-100). To examine the mechanisms involved in these observations more closely, the contribution of free anthocyanins (FA), co-pigmented anthocyanins (CA) and polymeric pigments (PP) to the total wine color were determined (Table 2). The CT wine showed higher proportion of CA whereas the VIN wines showed higher proportion of PP. In the CT wines, SO_2 may have reacted with several wine constituents such as acetaldehyde, pyruvic acid, anthocyanins or cinnamic compounds. Hence, those reactions may have reduced the rate of polymerization reactions (He et al., 2012b; Jackowetz & Mira de Orduña, 2013; Morata, Gómez-Cordovés, Calderón, & Suárez, 2006).

Regarding the *storage* variable, a stronger influence on color parameters than *treatment* was observed. After 12 months of storage in bottle, CI, %R, L*, a*, FA and CA decreased, while hue, %Y, %B, b* and PP increased, in agreement with results found in wine ageing by several authors (Boido et al., 2006; García-Puente Rivas, Alcalde-Eon, Santos-Buelga, Rivas-Gonzalo, & Escribano-Bailón, 2006; Hermosín-Gutiérrez, Lorenzo, & Espinosa, 2005).

The interaction *treatment x storage* (TxS) was significant in all parameters except %B and CA (Table 2). It meant that the evolution during storage in bottle was different according to the treatment. The most remarkable differences during this evolution between the CT and VIN wines were found in the CI, L*and b* parameters (Supplementary Table). CI increased in the CT wines but decreased in the VIN wines during storage in the bottle; In CT wines, CI increased due to the release of anthocyanins by SO_2 during the storage in bottle. The CT wines showed a decrease in L* whereas no changes were seen in the VIN wines; and, increases in b* were observed in both the CT and VIN wines, this increase being much greater in the former. The VIN wines showed the largest increase in PP (approximately 2.2-folds) and decrease in FA (approximately 1.4-folds) with regard to at bottling, in contrast with the CT wines, in which the PP increased by 1.8 folds and FA remained constant with regard to its initial values at bottling.

The colorimetric differences (ΔE*) calculated for every pair of wines (VIN wines vs CT), ranged from 7 to 10 CIELAB units (Table 2). These colorimetric differences can be considered to be visually detectable because these values are higher than the estimation of 3.0 CIELAB units (Martinez et al., 2001).

Therefore, it seems that VIN was able to stabilize wine color. VIN contributed to maintaining higher color intensity and darker hue throughout bottling storage (Supplementary Table), apparently promoting a faster and more pronounced polymerization between free anthocyanins (which promptly decreased) and other wine constituents. The nature of some of those condensed pigments is discussed below in the current manuscript.

3.3. Phenolic compounds

Due to the importance of anthocyanins and tannins in the wine quality, further studies on individual phenolic compounds and anthocyanin forms were developed. The nature of some of condensed pigments is also discussed.

3.3.1. Anthocyanins and anthocyanins derived pigments

Following the method developed by Chinnici et al., (2009), twenty eight anthocyanin derivatives were identified in wines as follows (Table 3): delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, delphinidin-3-*O*-acetylglucoside, cyanidin-3-*O*-acetylglucoside, petunidin-3-*O*-acetylglucoside, peonidin-3-*O*-acetylglucoside, malvidin-3-*O*-acetylglucoside, delphinidin-3-*O*-coumaroylglucoside, cyanidin-3-*O*-coumaroylglucoside, malvidin-3-*O*-caffeoylglucoside, petunidin-3-*O*-coumaroylglucoside, peonidin-3-*O*-coumaroylglucoside, malvidin-3-*O*-coumaroylglucoside, malvidin-3-*O*-glucoside-pyruvic acid, malvidin-3-*O*-acetylglucoside-pyruvic acid, malvidin-3-*O*-coumaroylglucoside-pyruvic acid, petunidin-3-*O*-glucoside-pyruvic acid, malvidin-3-*O*-glucoside-acetaldehyde, malvidin-3-*O*-acetylglucoside-acetaldehyde, malvidin-3-*O*-glucoside-4-vinylcatechol, malvidin-3-*O*-glucoside-4-vinylphenol, malvidin-3-*O*-glucoside-4-vinyl(epi)catechin, malvidin-3-*O*-glucoside-(epi)catechin, malvidin-3-*O*-glucoside-ethyl-(epi)catechin (2 isomers). These compounds were grouped into three categories (Table 3, bottom side): (i) monomeric anthocyanins, including glucosides, acetylglucosides, coumaroylglucosides and caffeoylglucosides; (ii) pyranoanthocyanins, which included A-type vitisins, B-type vitisins and vinyl-pyranoanthocyanins; (iii) direct and ethyl bridged adducts.

Regarding the *treatment* variable, the CT wines showed the highest total anthocyanin concentration, mainly due to the higher differences in the concentration of monomeric anthocyanins. Anthocyanin glucosides, acetylglucosides and cinnamoylglucosides were 1.5-fold higher in the CT wines. Free anthocyanins have been described to bind with SO_2, which inhibits their further polymerization (Picinelli, Bakker, & Bridle, 1994). In contrast, the pyranoanthocyanins category was found in lower percentage in the CT wines, in particular B-type vitisins and vinyl-pyranoanthocyanins. This may be explained by the lower amount of

acetaldehyde likely to be available in the CT wines, since SO_2 is known to bind with acetaldehyde (Jackowetz & Mira de Orduña, 2013). In contrast, A-type vitisins were lower in the VIN wines. The pyruvic acid reaction with malvidin-3-*O*-glucoside is less favorable than these with acetaldehyde (Morata et al., 2006), affecting SO_2 to a lesser extent.

It has been reported that the chemical pathway for the formation of pyranoanthocyanins is likely to start with cycloaddition of either yeast metabolites such as pyruvic acid or acetaldehyde, or yeast components such as 8-vinylflavanols, 4-vinylphenols and caffeic acid to the C-4 and 5-OH positions of the anthocyanins (De Freitas & Mateus, 2011; Fulcrand et al., 2006; Schwarz et al., 2004). The SO_2 blocks the C4 position in the C ring of the anthocyanins in the flavilium cation, preventing their formation (He et al., 2012a). The reduced amount of malvidin-3-*O*-glucoside-4-vinylcatechol, also called Pinotin A, in the CT wines may be justified by a lower caffeic acid/malvidin-3-*O*-glucoside ratio (lower caffeic acid, Table 4, and higher malvidin-3-*O*-glucoside) (Rentzsch et al., 2007; Schwarz et al., 2004). The higher stability of the pyranoanthocyanins compared to the anthocyanins monoglucosides is well known (García-Puente Rivas et al., 2006; He et al., 2012b). The fact that the VIN wines showed a higher concentration of pyroanthocyanins supported the color stability found in VIN wines, in agreement with the observations described in Section 3.2.

Regarding the *storage* variable, after 12 months of storage in bottle, the wines showed a decrease in their total anthocyanin content, mainly due to anthocyanin-3-*O*-glucosides, anthocyanin-3-*O*-acetylglucosides and anthocyanin-3-*O*-cinnamoylglucosides (Alcalde-Eon, Escribano-Bailón, Santos-Buelga, & Rivas-Gonzalo, 2006; Boido et al., 2006; Monagas, Gómez-Cordovés, & Bartolomé, 2005b). The concentrations of A-type vitisins remained constant while the vinyl-pyranoanthocyanins and direct and ethyl-bridged adducts contents increased, in agreement with other authors (Alcalde-Eon et al., 2006; Asenstorfer, Markides, Iland, & Jones, 2003; Monagas et al., 2005b; Rentzsch et al., 2007).

During wine aging in bottle, monomeric anthocyanins are gradually incorporated into derived pigments such as pyranoanthocyanins (A-type vitisins, B-type vitisins and vinyl-pyranoanthocyanins), which are highly stable, more resilient to pH change, resistant to SO_2 bleaching and oxidative degradation, thus making an important contribution to the color stability of red wines (Håkansson, Pardon, Hayasaka, de Sa, & Herderich, 2003; He et al., 2012b).

Regarding direct and ethyl-bridged adducts, although a slight increase was observed during storage, their contribution to the total anthocyanin concentration remained low. The contribution to the aged red wine color of this class of compounds is still unclear. In fact, while some researchers indicate negligible effects (Alcalde-Eon et al., 2006; Boido et al., 2006; Santos-Buelga & Freitas, 2009), others report a strong correlation between the accumulation of ethyl-bridged adducts and color intensity in aged wines (Chinnici et al., 2009; He et al., 2012b), supposedly related to their high molar extinction coefficients at pH> 3.5.

The interaction *treatment x storage* (TxS) was significant for the following compounds (Table 3): petunidin-3-*O*-acetylglucoside, malvidin-3-*O*-acetylglucoside, peonidin-3-*O*-coumaroylglucoside, malvidin-3-*O*-glucoside-pyruvic acid, petunidin-3-*O*-glucoside-pyruvic acid, malvidin-3-*O*-glucoside-4-vinylcatechol, malvidin-3-*O*-glucoside-4-vinyl(epi)catechin and malvidin-3-*O*-glucoside(epi)catechin. Significant interactions were also found for monomeric anthocyanins (%), pyranoanthocyanins (%) and direct and ethyl bridged adducts (%). These data suggested that the evolution of all the above compounds during *storage* was different depending on the *treatment* (Supplementary Table).

3.3.2. Non-anthocyanin phenolic compounds

Nineteen compounds, belonging to four families, were quantified in wines, according to Chinnici et al., (2009) as follows (Table 4): (i) hydroxybenzoic acids: gallic acid, syringic

acid, protocatechuic acid and vanillic acid; (ii) hydroxycinnamic acids: *trans-p*-coumaric acid, *trans-p*-coutaric acid, *trans*-caffeic acid, *trans*-caftaric acid, ferulic acid and 2-*S*-glutathionyl-caftaric acid (GRP); (iii) flavanols: (+)-catechin, (-)-epicatechin and procyanidin B2; (iv) flavonols: myricetin-3-*O*-glucoside, myricetin-3-*O*-glucuronide, quercetin-3-*O*-glucuronide, quercetin-3-*O*-glucoside, kaempferol-3-*O*-glucoside, isorhamnetin-3-*O*-glucoside.

Regarding the *treatment* variable, hydroxybenzoic acids differentiated each treatment. The content of hydroxybenzoic acids in the wines was as follows in decreasing order: VIN-100 > VIN-50 > CT. It can be noted that hydroxycinnamic acids and flavanols differentiated the treatments into two groups: one group was CT and VIN-50, and the second group, VIN-100. Of noted that caffeic acid, which seems to contribute to color stability and protection against oxidation (Hermosín-Gutiérrez et al., 2005), was higher in VIN treated wines in a dose-dependent mode. Moreover, 2-S-Glutathionyl-*trans*-caftaric acid (also known as grape reaction product, GRP), which is formed when glutathione react with a *o*-quinone to regenerate *o*-diphenol group (Cejudo-Bastante et al., 2010), was similar between treatments. These data allowed us to hypothesize that VIN, especially at high doses, could protect the oxidation of these phenolic compounds. In contrast, no significant differences were found in flavonols among treatments.

Regarding the *storage* variable, after 12 months of storage in bottle, the total hydroxycinnamic acids, total flavanols and flavonols contents in the wines decreased. These decreases may be due to their involvement in oxidation and polymerization reactions (Fulcrand et al., 2006; Hermosín-Gutiérrez et al., 2005; Monagas, Bartolomé, & Gómez-Cordovés, 2005a). Increases in four compounds were observed with ageing. The gallic acid content increased because it can be released from its galloylated precursor (Hermosín-Gutiérrez et al., 2005). Hydroxycinnamic acids increased, while their corresponding esters

decreased in agreement with findings described by other authors (Monagas et al., 2005a; Zafrilla et al., 2003).

The interaction *treatment x storage* (TxS) was significant for all non-anthocyanin phenolic compounds except (+)-catechin, myricetin-3-*O*-glucoside+myricetin-3-*O*-glucuronide and kaempferol-3-*O*-glucoside (Supplementary Table). In fact, decreases in the total non-anthocyanin phenolic compounds during the storage in bottle was observed to differ depending on the wine: 24% in CT wines, 1% in VIN-50 wines, while in VIN-100 wines the initial levels were preserved, which supports the hypothesis of the preservative properties of VIN.

3.4. Sensory analysis

At bottling, wines showed sensory differences as shown in Figure 1a. The VIN wines showed higher scores for 'black fruit', 'jammy fruit', 'candy/sweet' and 'woody/smoky' attributes. However, the only significant differences were found in 'candy/sweet' and 'woody/smoky' attributes for the VIN-100 wines. In tasting, the VIN wines showed the highest scores for astringency and bitterness, which could be related with their higher flavanol content (Table 4). Moreover, the VIN-100 wines were rated the lowest scores for balanced and global quality. No defect was detected by panelist in the sensory analysis.

After 12 months of storage in bottle (Figure 1b), the VIN-100 wines presented the highest scores for 'black fruit', 'jammy fruit','candy/sweet' and'woody/smoky', and the lowest for 'red fruit', but only the 'red fruit' and 'jammy fruit' attributes were significantly different. The differences in taste between the wines were minimized with storage in bottle, only the astringency attribute scoring significantly lower for the CT wines.

4. Conclusions

The VIN treated wines showed similar quality parameters to the SO_2 treated wines (CT), but higher color intensity and polymeric pigments were observed in those wines. Differences

were also found in their phenolic composition, especially in the anthocyanin profile. The VIN wines showed lower monomeric anthocyanin concentrations but higher B-type vitisins and vinyl-pyranoanthocyanins. The latest two are highly stable color compounds resistant to oxidation. Moreover, a higher hydroxybenzoic concentration was also observed in the VIN wines. The VIN-100 wines were the richest in hydroxycinnamic acids and flavanols. These phenolics were not due to extract addition as no phenolic compounds apart from stilbene were detected on the extract analysis.

After 12 months of storage in bottle, color features, pigments and phenolic compounds evolved as expected in red wines. However, the extent of this evolution varied between samples depending on both the preservative used (VIN or SO_2) and the dose (VIN-50 or VIN-100). Anthocyanin compounds evolved to a major extent in the VIN wines, which were low in free anthocyanins and high in vinyl-pyranoanthocyanins, and direct and ethyl-bridged adducts. As a consequence, the VIN wines showed a bluer, less red color and higher color intensity. Therefore, it seems that Vineatrol promotes color stabilization reactions and non-anthocyanin phenolic compound preservation. At sensory analysis VIN-100 wines were differentiated from CT and VIN-50 wines by panelists.

The present study demonstrates that the use of a grapevine-shoot extract with 30% stilbenes (Vineatrol®) as a preservative is able to guarantee the quality in red wines with good chromatic parameters and phenolic profile. Although the highest VIN dose (100 mg/L of stilbenes) contributed to preserving better the phenolic acids and flavanols of red wines, they received a lower score at tasting, and thus the lower dose (VIN 50) is recommended. VIN-50 wines can be proposed as red quality wine with added-value, low SO_2 and high bioactive stilbene contents. The results may help in reducing allergenic chemical in red wines, and contributing to sustainability in winemaking by the re-use of a sub-product such as grapevine shoot

Acknowledgements

This work has been funded by INIA and FEDER 2014-2020 "Programa Operativo de Crecimiento Inteligente" (Project RTA2015-00005-C02-01) and "Programa Andalucía se mueve con Europa" (Project AVA.AVA201601.3).. The authors are grateful to Jean-Claude Izard (Actichem SA) for kindly supplying Vineatrol® and to LABCOM for kindly supplying stilbene standards. Ruiz-Moreno and Guerrero thank the European Social Fund (ESF) 2007-2013 *"Andalucía se mueve con Europa*" for the financial support of their contracts.

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FIGURE CAPTIONS

Figure 1. Cobweb diagram of the sensory scores for Syrah wines as affected by treatments (CT, VIN-50 and VIN-100) and storage in bottle (bottling and 12 months).

TABLES

Table 1. Enological parameters in Syrah wines as affected by treatments (CT, VIN-50 and VIN-100) and storage in bottle (bottling and 12 months).

Table 2. Chromatic features in Syrah wines as affected by treatments (CT, VIN-50 and VIN-100) and storage in bottle (bottling and 12 months).

Table 3. Anthocyanin content (mg/L) in Syrah wines as affected by treatments (CT, VIN-50 and VIN-100) and storage in bottle (bottling and 12 months).

Table 4. Non-anthocyanin phenolic compounds (mg/L) in Syrah wines as affected by treatments (CT, VIN-50 and VIN-100) and storage in bottle (bottling and 12 months).

Supplementary Table. Significant differences in chromatic measurements, co-pigmentation, anthocyanins and derivative pigments, non-anthocyanin phenolic compounds in Syrah wines as affected by treatments (CT, VIN-50 and VIN-100) and storage in bottle (bottling and 12 months).

Table 1.

Bottling	CT	VIN-50	VIN-100	LS
Ethanol (% v/v)	15.3 [b]	15.6 [a]	15.5 [a]	***
Glycerol (g/L)	12.13	12.07	12.04	ns
Dry extract (g/L)	27.1 [b]	27.3 [b]	28.0 [a]	**
Total acidity (g/L TH_2)	5.57 [b]	5.69 [a]	5.77 [a]	**
pH	3.63	3.61	3.61	ns
Volatile acidity (g/L AcH)	0.52	0.47	0.45	ns
Acetic acid (g/L)	0.47	0.47	0.47	ns
Citric acid (g/L)	0.15	0.12	0.12	ns
Tartaric acid (g/L)	1.97	2.08	2.07	ns
Malic acid (g/L)	0.13	0.14	0.14	ns
Lactic acid (g/L)	1.20	1.21	1.19	ns
Succinic acid (g/L)	1.18	1.16	1.17	ns
Total SO_2 (mg/L)	43	nd	nd	
Free SO_2 (mg/L)	25	nd	nd	
Total stilbenes[1] (mg/L)	2.21 [c]	45.66 [b]	104.92 [a]	***
Total anthocyans (mg/L)	667 [a]	645 [b]	648 [b]	*
Tannins (g/L catechin)	4.79 [b]	5.52 [a]	5.55 [a]	***
Potassium (mg/L)	1080	1060	1061	ns
Acetaldehyde (mg/L)	9.7	9.7	10.5	ns
12 months	CT	VIN-50	VIN-100	LS
Total SO_2 (mg/L)	17	nd	nd	
Free SO_2 (mg/L)	9	nd	nd	
Total stilbenes[1] (mg/L)	2.77 [c]	35.27 [b]	88.04 [a]	***
Total anthocyans (mg/L)	420 [a]	369 [b]	368 [b]	***
Tannins (g/L catechin)	4.23 [c]	4.55 [b]	4.88 [a]	**
Acetaldehyde (mg/L)	5.4	5.9	5.5	ns

CT, wine with sulfur dioxide added at 50 mg/L; VIN-50 and VIN-100, wines with Vineatrol added at 50 and 100 mg/L of stilbenes respectively. TH_2, tartaric acid; AcH, acetic acid. [1], total stilbenes as the sum of ampelopsin A, pallidol, *trans*-resveratrol, piceatannol, ε-viniferin, r-viniferin and ω-viniferin. The sum and means with different superscript letters in the same column present significant differences according to Fischer's LSD test ($p < 0.05$). Significance level: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns: not significant; nd: not detected.

Table 2.

	Treatment				Storage			Interaction TxS
	CT	VIN-50	VIN-100	*p*-value	Bottling	12 Months	*p*-value	*p*-value
CI	15.21 [b]	19.87 [a]	20.12 [a]	***	18.61 [a]	18.19 [b]	*	***
hue	0.602 [a]	0.577 [b]	0.581 [b]	***	0.533 [b]	0.640 [a]	***	***
%Y	33.4 [a]	31.9 [b]	32.0 [b]	***	31.1 [b]	33.8 [a]	***	***
%R	55.5	55.7	55.5	ns	58.3 [a]	52.8 [b]	***	*
%B	11.2	12.4	12.4	ns	10.64 [b]	13.32 [a]	***	ns
L*	38.18 [a]	31.60 [b]	31.34 [b]	***	33.99 [a]	33.43 [b]	***	**
a*	56.90	56.70	56.78	ns	59.52 [a]	54.07 [b]	***	***
b*	9.05 [b]	14.65 [a]	14.78 [a]	***	11.28 [b]	14.37 [a]	***	***
ΔE_{ab}*		8.735	9.005		10.08	7.32		
FA (%)	46.54	47.48	45.84	ns	52.42 [a]	40.83 [b]	***	***
CA (%)	24.56 [a]	15.12 [b]	16.54 [b]	***	24.97 [a]	12.52 [b]	***	ns
PP (%)	29.26 [b]	37.39 [a]	37.62 [a]	***	22.62 [b]	46.90 [a]	***	***

CT, wine with sulfur dioxide added at 50 mg/L; VIN-50 and VIN-100, wines with Vineatrol added 50 and 100 mg/L of stilbenes respectively. CI, color intensity; %Y, percentage of yellow; %R, percentage of red; %B percentage of blue in the total wine color. ΔE_{ab}*, color differences with regard to CT sample. FA, fraction color due free anthocyanins; CA, fraction color due to copigmented anthocyanins; PP, fraction color due to polymeric pigments. The means with different superscript letters in the same column present significant differences according to Fischer's LSD test ($p < 0.05$). Significance level: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns: not significant; nd: not detected.

Table 3.

	Treatment				Storage			Interaction TxS
	CT	VIN-50	VIN-100	*p*-value	Bottling	12 Months	*p*-value	*p*-value
Delphinidin-3-*O*-glucoside	9.01[a]	5.59[b]	5.68[b]	***	9.65[a]	3.87[b]	***	ns
Cyanidin-3-*O*-glucoside	0.41[a]	0.29[b]	0.20[c]	***	0.38[a]	0.21[b]	***	ns
Petunidin-3-*O*-glucoside	21.37[a]	13.82[b]	13.82[b]	***	23.99[a]	8.68[b]	***	ns
Peonidin-3-*O*-glucoside	9.93[a]	6.61[b]	6.67[b]	***	11.33[a]	4.14[b]	***	ns
Malvidin-3-*O*-glucoside	212.8[a]	147.1[b]	148.2[b]	***	249.2[a]	89.5[b]	***	ns
∑ Anthocyanin-3-*O*-glucosides	**253.5[a]**	**173.4[b]**	**174.6[b]**	*******	**294.6[a]**	**106.4[b]**	*******	**ns**
Delphinidin-3-*O*-acetylglucoside	3.42[a]	2.22[b]	2.25[b]	***	3.92[a]	1.33[b]	***	ns
Cyanidin-3-*O*-acetylglucoside	0.49	0.41	0.41	ns	0.85[a]	0.02[b]	***	ns
Petunidin-3-*O*-acetylglucoside	7.41[a]	5.30[b]	5.27[b]	***	8.80[a]	3.18[b]	***	**
Peonidin-3-*O*-acetylglucoside	10.66[a]	7.01[b]	7.02[b]	***	13.69[a]	2.77[b]	***	ns
Malvidin-3-*O*-acetylglucoside	94.34[a]	64.93[b]	65.63[b]	***	116.55[a]	33.38[b]	***	*
∑ Anthocyanin-3-*O*-acetylglucosides	**116.3[a]**	**79.9[b]**	**80.6[b]**	*******	**143.8[a]**	**40.7[b]**	*******	**ns**
Delphinidin-3-*O*-coumaroylglucoside†	1.52[a]	0.99[c]	1.08[b]	***	1.20	nd		
Cyanidin-3-*O*-coumaroylglucoside	1.17[a]	0.83[b]	0.80[b]	***	0.94	0.93	ns	ns
Malvidin-3-*O*-caffeoylglucoside	1.00[a]	0.76[b]	0.76[b]	***	1.29[a]	0.40[b]	***	ns
Petunidin-3-*O*-coumaroylglucoside	3.64[a]	2.31[b]	2.32[b]	***	4.17[a]	1.35[b]	***	ns
Peonidin-3-*O*-coumaroylglucoside	6.20[a]	3.57[b]	3.62[b]	***	6.86[a]	2.07[b]	***	*
Malvidin-3-*O*-coumaroylglucoside	27.12[a]	16.77[b]	17.08[b]	***	31.12[a]	9.52[b]	***	ns
∑ Anthocyanin-3-*O*-cinnamoylglucosides	**39.90[a]**	**24.73[b]**	**25.12[b]**	*******	**45.57[a]**	**14.26[b]**	*******	**ns**
Malvidin-3-*O*-glucoside-pyruvic acid	3.24[a]	2.27[b]	2.29[b]	***	2.09[b]	3.11[a]	***	***
Malvidin-3-*O*-acetylglucoside-pyruvic acid	2.10[a]	1.88[b]	1.87[b]	***	1.60[b]	2.29[a]	***	ns
Malvidin-3-*O*-coumaroylglucoside-pyruvic acid	1.04[a]	0.91[b]	0.89[b]	***	1.38[a]	0.52[b]	***	ns
Petunidin-3-*O*-glucoside-pyruvic acid	1.88[a]	1.35[b]	1.36[b]	***	1.84[a]	1.22[b]	***	**
∑ A-type vitisins	**8.25[a]**	**6.41[b]**	**6.41[b]**	*******	**6.91**	**7.14**	**ns**	*******
Malvidin-3-*O*-glucoside-acetaldehyde	1.70[b]	2.28[a]	2.28[a]	***	2.91[a]	1.26[b]	***	ns
Malvidin-3-*O*-acetylglucoside-acetaldehyde	0.60[b]	1.17[a]	1.21[a]	***	1.06[a]	0.93[b]	**	ns
∑ B-type vitisins	**2.30[b]**	**3.45[a]**	**3.49[a]**	*******	**3.97[a]**	**2.19[b]**	*******	**ns**
Malvidin-3-*O*-glucoside-4-vinylcatechol	0.11[b]	0.25[a]	0.26[a]	***	0.08[b]	0.33[a]	***	***
Malvidin-3-*O*-glucoside-4-vinylphenol	0.32[a]	0.26[b]	0.27[b]	***	0.23[b]	0.34[a]	***	ns
Malvidin-3-*O*-glucoside-4-vinyl(epi)catechin	0.09[b]	0.16[a]	0.17[a]	***	0.03[b]	0.25[a]	***	***
∑ Vinyl-pyranoanthocyanins	**0.52[b]**	**0.67[a]**	**0.70[a]**	*******	**0.34[b]**	**0.92[a]**	*******	*******
Malvidin-3-*O*-glucoside(epi)catechin	1.39[a]	1.02[b]	0.99[b]	***	0.69[b]	1.58[a]	***	***
Malvidin-3-*O*-glucoside-ethyl-(epi)catechin isomer1†	0.72[a]	0.65[b]	0.63[b]	**	nd	0.67		

Malvidin-3-*O*-glucoside-ethyl-(epi)catechin isomer2	1.31 [a]	1.10 [b]	1.07 [b]	***	1.55 [a]	0.77 [b]	***	ns
∑ Direct and ethyl-bridged adducts	**3.07** [a]	**2.43** [b]	**2.39** [b]	***	**2.24** [b]	**3.02** [a]	***	***
Σ Total anthocyanins	**423.8** [a]	**290.9** [b]	**293.2** [b]	***	**497.4** [a]	**174.6** [b]	***	**ns**
% monomeric anthocyanins	96.0 [a]	93.8 [b]	93.8 [b]	***	97.3 [a]	91.8 [b]	***	***
% pyranoanthocyanins	3.1 [b]	4.9 [a]	4.9 [a]	***	2.3 [b]	6.4 [a]	***	***
% direct and ethyl bridge adducts	0.9 [b]	1.3 [a]	1.3 [a]	***	0.6 [b]	1.8 [a]	***	***

CT, wine with sulfur dioxide added at 50 mg/L; VIN-50 and VIN-100, wines with Vineatrol added at 50 and 100 mg/L of stilbenes respectively. The sum and means with different superscript letters in the same column present significant differences according to Fischer's LSD test ($p < 0.05$). Significance level: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns: not significant; nd: not detected. †Compounds subjected to a one-way ANOVA.

Table 4.

	Treatment				Storage			Interaction (TxS)
	CT	VIN-50	VIN-100	*p*-value	Bottling	12 months	*p*-value	*p*-value
Gallic acid	9.01^{b}	10.00^{a}	10.64^{a}	**	9.51^{b}	10.25^{a}	*	*
Syringic acid	4.71^{c}	7.20^{b}	7.89^{a}	***	6.60	6.60	ns	***
Protocatechuic acid	0.90^{c}	1.03^{b}	1.14^{a}	***	1.03	1.01	ns	***
Vanillic acid	0.18^{c}	0.24^{b}	0.26^{a}	***	0.30^{a}	0.15^{b}	***	***
∑ *Hydroxybenzoic acids*	**14.79^{c}**	**18.47^{b}**	**19.93^{a}**	*******	**17.44**	**18.02**	**ns**	*******
trans-p-Coumaric acid†	0.47^{b}	0.58^{a}	0.60^{a}	*	nd	0.55		
trans-p-Coutaric acid	7.75	7.85	8.23	ns	8.35^{a}	7.54^{b}	**	*
trans-Caffeic acid	0.32^{c}	0.38^{b}	0.41^{a}	***	0.28^{b}	0.46^{a}	***	***
trans-Caftaric acid	25.37	25.13	26.49	ns	27.24^{a}	24.08^{b}	***	**
Ferulic acid	7.56^{b}	8.11^{b}	9.41^{a}	***	9.03^{a}	7.68^{b}	***	*
GRP	5.89	5.34	5.56	ns	5.31^{b}	5.88^{a}	*	**
∑ *Hydroxycinnamic acids*	**47.12^{b}**	**47.09^{b}**	**50.40^{a}**	**ns**	**50.22^{a}**	**46.19^{b}**	******	******
(+)-Catechin	9.07^{b}	9.34^{b}	10.76^{a}	***	11.32^{a}	8.12^{b}	***	ns
(-)-Epicatechin	10.16^{a}	9.62^{b}	10.38^{a}	**	13.47^{a}	6.63^{b}	***	*
Procyanidin B2	0.85^{c}	1.07^{b}	1.17^{a}	***	1.04	1.02	ns	***
∑ *Flavanols*	**20.08^{b}**	**20.02^{b}**	**22.31^{a}**	*******	**25.84^{a}**	**15.77^{b}**	*******	*****
Myricetin-3-*O*-glucoside + Myricetin-3-*O*-glucuronide	1.60	1.57	1.66	ns	1.73^{a}	1.49^{b}	*	ns
Quercetin-3-*O*-glucuronide	5.33	5.06	5.48	ns	5.47	5.11	ns	*
Quercetin-3-*O*-glucoside	15.65	15.63	16.80	ns	17.14^{a}	14.90^{b}	***	*
Kaempferol-3-*O*-glucoside	0.18	0.18	0.19	ns	0.21^{a}	0.16^{b}	***	ns
Isorhamnetin-3-*O*-glucoside	7.31	7.26	7.74	ns	7.90^{a}	6.97^{b}	***	*
∑ *Flavonols*	**30.06**	**29.69**	**31.86**	**ns**	**32.44^{a}**	**28.63^{b}**	*******	*****
∑ *Total non-anthocyanins*	**112.0^{b}**	**115.3^{b}**	**124.5^{a}**	******	**125.9^{a}**	**108.6^{b}**	*******	******

CT, wine with sulfur dioxide added at 50 mg/L; VIN-50 and VIN-100, wines with Vineatrol added at 50 and 100 mg/L of stilbenes respectively. GRP, grape reaction product (2-S-glutathionyl-caftaric acid). The sum and means with different superscript letters in the same column present significant differences according to Fischer's LSD test ($p < 0.05$). Significance level: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns: not significant; nd: not detected. †Compounds subjected to a one-way ANOVA.

Highlights

Grapevine shoot extract (VIN) stabilized the color of red wines

Monomeric anthocyanins decreased while pyroanthocyanins increased in VIN wines

Hydroxybenzoic and hydroxycinnamic acids and flavanols were preserved in VIN wines

VIN at high dose affected sensory properties