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# Effect of Addition of Commercial Grape Seed Tannins on Phenolic Composition, Chromatic Characteristics, and Antioxidant Activity of Red Wine

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The effect of addition of grape seed tannins on the phenolic composition, chromatic characteristics, and antioxidant activity of red wine was studied. Two highly pure commercial grape seed tannins (GSE100 and GSE300) were selected, and their phenolic compositions were determined. Two types of red wines were made with Castelão/Tinta Miúda (3/2, w/w) grapevine varieties by fermentation on skin using two different maceration times, which correspond to the wines rich and poor in polyphenols, respectively. Each of these wines was used for experimentation with the addition of GSE100 and GSE300 before and immediately after alcoholic fermentation. Phenolic composition, chromatic characteristics, and antioxidant activity of the finished red wines were analyzed by HPLC-DAD, CIElab 76 convention, and DPPH radical test, respectively. The results showed that the addition of grape seed tannins had obvious effects of increasing color intensity and antioxidant activity only in the wines poor in polyphenols. Although GSE300 contained much higher amounts of di- and trimer procyanidins and a lower amount of polymeric proanthocyanidins, it provided effects of increasing the color intensity and antioxidant activity of the wines poor in polyphenols similar to those of GSE100. Furthermore, GSE100 released more gallic acid to wines than GSE300, although no gallic acid was detected in GSE100. Tannins added after alcoholic fermentation had a better effect on phenolic composition of red wine than tannins added before alcoholic fermentation.

**KEYWORDS: Grape seed tannins; red wine; phenolic composition; chromatic characteristics; DPPH radical test**

## INTRODUCTION

Commercial enological tannins have been increasingly used during the past decades. In addition to the antioxidant activities (*1*, *2*), the use of enological tannins in winemaking could provide wine with an improved aroma and sensory profile (*3*, *4*), stabilize the color of red wine, or facilitate the fining of white or rosé wines (*3*, *5*, *6*). Lempereur et al. (*7*) and Kovac et al. (*8*) observed an increase in red color soon after tannin addition due to copigmentation effects.

On the other hand, several authors have reported that the addition of commercial enological tannins to red wines did not improve efficiently the wine sensory quality (*5*, *9*, *10*). The reason for this is probably due to the low quality or insufficient amount of the enological tannins applied. Furthermore, loss of these tannins during the winemaking process could be another possible explanation for little effect of the additional tannin on wine quality.

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The effects of enological tannins on wine quality are highly dependent on their nature and chemical structure. Enological tannins include wood tannins, galls tannins, and grape tannins. Grape tannins, that is, grape proanthocyanidins, are superior to other tannins in enological practice because the chemical structures of grape proanthocyanidins are similar to those of red wine proanthocyanidins (*11*, *12*). The structure of grape proanthocyanidins is made by polymerization of flavan-3-ol subunits, such as (+)-catechin, (−)-epicatechin, and/or (−)-epigallocatechin and epicatechin-3-*O*-gallate. The subunits vary among tannins from grape skins, seeds, and stems (*12*−*14*).

The objective of this work was to study the effect of the addition of grape seed tannins on the phenolic composition, chromatic characteristics, and antioxidant activity of red wine. Considering that the tannin producers provide generally little or no information about the polyphenolic composition of their products and that few published works (*3*) on this subject gave detailed phenolic composition of the used tannin products, in this work, we have first characterized the two different grape seed tannin products. Furthermore, two types of red wines were made from the same grapes by fermentation on skin using different maceration times to produce wines either rich or poor in polyphenols.


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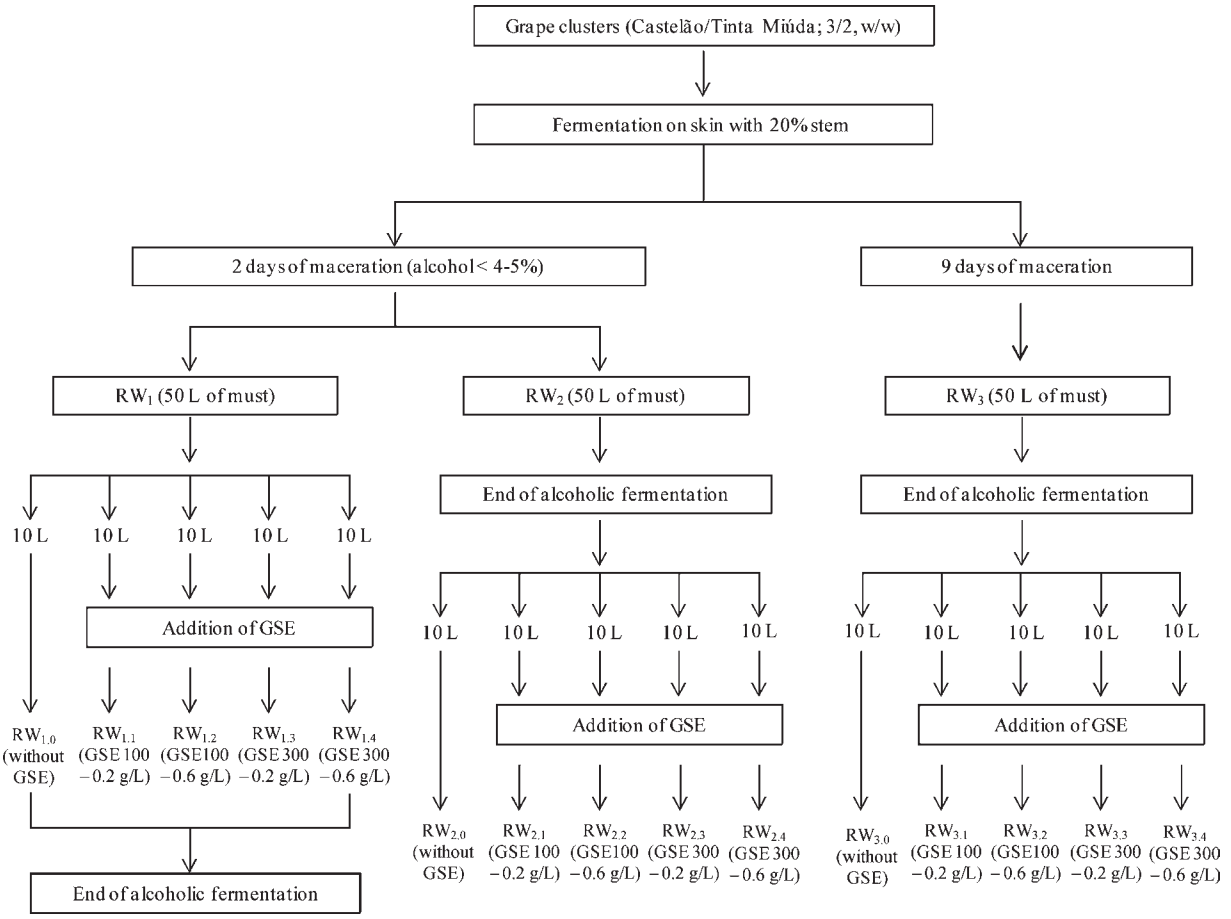


**Figure 1.** Diagram of red wine preparation. Vinifications were performed in duplicate.

## MATERIALS AND METHODS

**Materials.** All organic solvents were of HPLC or analytical grade quality. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich (Steinhem, Germany). Two grape seed tannins with the commercial names GSE100 and GSE300 were provided by Biocrático Lda (Vilar, Portugal). The two commercial tannins were selected and used in this work because they are grape seed tannins and presented the highest purity in polyphenols (>95%) among various commercial enological tannins studied in our previous work (*15*). (+)-Catechin and (−)-epicatechin were purchased from Fluka A.G. (Buchs, Switzerland). Malvidin 3-glucoside was isolated from Pinot Nnoir grape skins as described previously (*16*). Ultrapure water was obtained from a Seralpur PRO 90 CN System (Ransbach-Baumbach, Germany).

**Preparation of Red Wines Rich or Poor in Polyphenols with the Addition of Tannins.** The procedure of preparation of red wines rich or poor polyphenols is diagrammed in **Figure 1**. Briefly, Castelão and Tinta Miúda grape varieties (*Vitis vinifera* L.) from the Estremadura region of Portugal harvested at enological maturity during September−October of 2007 were used in this work. To perform vinifications in duplicate, grape clusters were randomly separated into two equal parts, each of which had a proportion of Castelão and Tinta Miúda grapes 3:2 (w/w). Each lot of grape clusters was crushed using a destemmer−crusher and collected together with 20% of total stems back in a stainless steel tank. The must was treated with sulfur dioxide (80 mg/L). For each lot, after 2 days of maceration when alcoholic fermentation did not begin, 100 L of the must was isolated (drained) from the tank to stop further contact with pomace and divided into two parts: one part ($RW_1$) was subdivided into 5 × 10 L and amended with different amounts of grape seed tannins (0.2 and 0.6 g/L) as indicated (**Figure 1**) before undergoing alcoholic fermentation at 25 °C; the other part ($RW_2$) was also divided into 5 × 10 L but the addition of different amounts of grape seed tannins (0.2 and 0.6 g/L) was made only after alcoholic fermentation was finished. The amount of GSE100 or GSE300 (0.2 g/L) applied to red wine corresponds to the dose recommended by the company, and a higher concentration (0.6 g/L) of GSE100 or GSE300 was chosen for comparison purposes. On the other hand, the mush in the tank continuously underwent maceration/fermentation using classic winemaking technology. After 9 days of maceration, when fermentation was finished, 5 × 10 L of wine ($RW_3$) was isolated, and to each 10 L lot was added grape seed tannins (0.2 and 0.6 g/L) as shown in **Figure 1**. After 3 months of conservation, the wines $RW_1$, $RW_2$, and $RW_3$ were racked, treated with sulfur dioxide (30 mg/L), and stored at room temperature. The second racking was carried out at 6 months of conservation with the addition of sulfur dioxide (30 mg/L). After another 4 months of maturation, the wine was bottled and stored at a wine cellar before analysis. $RW_1$ and $RW_2$ correspond to the wines poor in polyphenols, and $RW_3$ corresponds to the wine rich in polyphenols.

**Characterization of Grape Seed Tannin Products.** Quantification of total oligomer and total polymer proanthocyanidins in the two grape seed tannin products (GSE100 and GSE300) was performed by isolation of oligomeric and polymeric proanthocyanidins fractions using Sep-Pak C18 cartridges as described (*17*), followed by modified vanillin assay (*18*). Analysis of their individual catechins and procyanidins and evaluation of their antioxidant activity were carried out using the methods described below.

**HPLC Analysis of Individual Catechins and Procyanidins.** Quantification of individual catechins and procyanidins (dimers and trimers) was performed by prefractionation of wine samples on Sep-Pak C18 cartridges according to the procedure proposed by Sun et al. (*17*), followed by HPLC analysis. Briefly, red wine was dealcoholized under vacuum at 35 °C. Three milliliters of dealcoholized wines or 1 mL of grape seed tannin products (3 mg/mL) was loaded onto the two water-preconditioned Sep-Pak cartridges connected in series: the superior one is a tC18 Sep-Pak and the inferior is a C18 Sep-Pak. Elution was carried out with 25 mL of ethyl acetate to elute oligomeric polyphenols including essentially catechins, oligomeric proanthocyanidins, phenolic acids, and stilbenes. The ethyl acetate fraction (i.e., oligomeric polyphenols fraction) was evaporated to dryness, recovered first by about 3−4 mL of methanol, re-evaporated to

dryness, and then recovered by exactly 1 mL of 10% methanol in water. This solution was filtered prior to HPLC-DAD analysis. The HPLC-DAD apparatus used in this work was a Waters system, equipped with a quaternary pump (Waters 600), a controller (Waters 600), a thermostat controlling the column temperature, and an autosampler (Waters 717 plus), and a photodiode array detector (Waters 996) coupled to a data processing computer (Millennium 32). Detection ranged from 200 to 600 nm, 280 nm being for detection of individual catechins and procyanidins. The column was a cartridge of LiChrospher 100 RP18 (5 $\mu$m; 250 × 4 mm; Merck, Darmstadt, Germany). The column temperature was 30 °C. The flow rate was fixed at 1 mL/min. Two elution solvents, A (water/formic acid; 98:2, v/v) and B (acetonitrile/water/formic acid; 70:28:2, v/v/v), were used with the following elution program: gradient elution from 0 to 6% of B in 40 min, from 6 to 13% of B in 6 min, from 13 to 20% of B in 24 min, from 20 to 30% of B in 10 min, from 30 to 50% of B in 5 min, isocratic elution with 50% of B in 10 min, from 50 to 100% of B in 5 min, and isocratic elution with 100% of B in 15 min, followed by washing and conditioning of the column to initial conditions. Calibration curves were established with corresponding catechins and procyanidins standards. The latter were isolated from grape seed extract as described previously (*19*).

**Quantification of Phenolic Acids, Stibenes, Total Individual Phenolics, and Total Polyphenols.** Quantification of phenolic acids, total individual phenolics (TIP), and total polyphenols (TP) was carried out by direct injection of wine samples or GSE water solution (3 g/L in water) to HPLC without sample preparation. The HPLC equipment and elution conditions were identical to those described above. Detection ranged from 200 to 600 nm, 270, 309, and 320 nm being for detection of, respectively, gallic acid, coumaric acid, and caffeic acid, 285 nm for detection of *cis*-resveratrol and *cis*-piceid, and 307 nm for detection of *trans*-resveratrol and *trans*-piceid. Calibration curves of phenolic acids, *trans*-resveratrol and *trans*-piceid, were established with respective standards. *cis*-Resveratrol and *cis*-piceid were obtained by isomerization of their respective *trans*-standards as described previously (*20*), and their calibration curves were constructed by assumption that the reduction in *trans*-stilbenes by irradiation was equimolar to the generation of their *cis*-isomers as described by Goldberg et al. (*21*). TIP was determined by measuring all individual peak areas and using (+)-catechin as reference standard. Quantification of TP was performed by integration of total baseline peak areas throughout the elution period, and the amount was expressed using (+)-catechin as reference standard.

**HPLC Analysis of Anthocyanins and Their Derivatives.** Anthocyanins and their derivatives were determined by direct injection of wine samples to HPLC without sample preparation. The HPLC equipment was identical to that described above. The column (250 × 4 mm) was a cartridge of 4 $\mu$m Superspher 100 RP18 (Merck). The mobile phase flow rate was fixed at 0.7 mL/min. Detection ranged from 200 to 650 nm, 525 nm being for detection of anthocyanins and their derivatives. The column temperature was set at 30 °C. Elution conditions were as follows: solvents A (formic acid/water; 5:95, v/v) and B (acetonitrile/water/formic acid; 30:65:5, v/v/v) were used; gradient elution from 25 to 85% B in 70 min, isocratic elution with 85% B in 15 min, followed by washing and re-equilibration of the column to the initial conditions. Each of the anthocyanins and their derivatives was quantified by using malvidin-3-glucoside as reference standard.

**Chromatic Characteristics.** Chromatic characteristics of the red wines were determined according to the CIELab universal color appreciation system, using a Cary 100 Bio UV−vis spectrophotometer (Varian, Australia) by determining the transmission data at multiple wavelengths ranging from 380 to 780 nm at 5 nm intervals, using a 2 mm cell. Transmittance values were transformed to a 10 mm cell before the color parameters were calculated. The results were expressed by the cylindrical coordinates $L^*$ (psychometric lightness), $C^*$ (psychometric chroma), and $h$ (hue angle) values and the axes of a three-dimensional color space $a^*$ (measure of redness) and $b^*$ (measure of yellowness). Furthermore, wine color was also expressed by color intensity (CI), hue ($T$), and color composition [$OD_{420}$ (%), $OD_{520}$ (%), $OD_{620}$ (%)], which were calculated, respectively, by $CI = OD_{420} + OD_{520} + OD_{620}$; $T = OD_{420}/OD_{520}$; $OD_{420}$ (%) = $OD_{420}/CI \times 100$; $OD_{520}$ (%) = $OD_{520}/CI \times 100$; and $OD_{620}$ (%) = $OD_{620}/CI \times 100$, where $OD_{420}$, $OD_{520}$, and $OD_{620}$ are the optical density at 420, 520, and 620 nm, respectively.

**Scavenging Activity on 1,1-Diphenyl-2-picrylhydrazyl Radical (DPPH•).** The scavenging effects of wine samples or grape seed tannins products (GSE) on DPPH• were evaluated as previously described (*22*), with slight modification. Briefly, for evaluation of DPPH• scavenging activity of GSE100 or GSE300, a 0.02 mL aliquot of sample solution in methanol (different concentrations) and 3,18 mL of DPPH• solution in methanol (66 $\mu$M) were added directly to a 10 mm cell with stopper. The mixture was immediately shaken vigorously for 10 s by a vortex mixer. Absorbance at 515 nm ($A_{515}$) was recorded continuously against methanol as blank reference, using a Cary 100 Bio UV−vis spectrophotometer (Varian, Australia), during 60 min (until the reaction reached steady state). The initial concentration of DPPH• was calculated for every experiment from a calibration curve made by measuring the absorbance at 515 nm of standard samples of DPPH• at different concentrations. The percentage of the DPPH• remaining at steady state, which was calculated as $\%\ DPPH^{\bullet}_{rem} = 100[DPPH^{\bullet}]_T/[DPPH^{\bullet}]_{T=0}$, was plotted against the amount of sample divided by the initial concentration of DPPH•. Each point was acquired in triplicate. A dose response curve was obtained for each GSE product. The antiradical activity is expressed as $EC_{50}$ [(mg/L) of antioxidant/(mg/L) of DPPH•], which is defined as the amount of antioxidant needed to decrease the initial DPPH• concentration by 50%. The results can also be expressed as antiradical power ($ARP = 1/EC_{50}$). For evaluation of DPPH• scavenging activity of the red wines, the manipulation was identical to that described above, but its scavenging activity was expressed as a percentage of inhibition of DPPH• at steady state (% inhibition), that is, $\%\ inhibition = 100([DPPH^{\bullet}]_{T=0} - [DPPH^{\bullet}]_T)/[DPPH^{\bullet}]_{T=0} = 100 \times (A_0 - A_T)/A_0$, where $A_T$ is the absorbance at 515 nm obtained at steady state and $A_0$ is the absorbance at 515 nm of the control sample (0,.02 mL of methanol plus 3.18 mL of DPPH• solution).

**Statistical Analysis.** Vinifications were performed in duplicate with sampling and analysis in duplicate or triplicate, and the data were reported as mean ± standard deviation ($x \pm S$). One-way analysis of variance and comparison of means (LSD, 99% level) were carried out using Statistica v '98 edition (StatSoft Inc., Tulsa, OK).

## RESULTS AND DISCUSSION

Red wine polyphenols originate from the grapes by which the wine is made, due to the pomace-contact maceration during alcoholic fermentation. It has been reported that the levels of polyphenols in red wine depend on the pomace-contact maceration time (*23*−*25*), and the evolution profiles of major groups of polyphenols (i.e., proanthocyanidins and anthocyanins) were quite different: free anthocyanins were extracted at early stages of maceration and reached their maximum amount before the start of alcoholic maceration, whereas proanthocyanins were extracted essentially at later stages of maceration (i.e., alcohol content reached 4−5%), and their amount increased continuously until the end of maceration (*23*, *26*). Thus, to make the two types of wines rich and poor in polyphenols from the same grapes, we isolated the must from the mash with only 2 days of pomace-contact maceration (i.e., just before the start of alcoholic fermentation) and the must from the same mash with 9 days of pomace-contact maceration (i.e., when alcoholic fermentation was finished).

On the other hand, considering that grape tannins are superior to other tannins in enological practice (*12*) and that the usually used commercial tannins contained high percentages of impurities, we selected, in this work, two highly pure grape seed tannins, GSE100 and GSE300, both having purity in polyphenols of >95%. Furthermore, the phenolic composition and antioxidant activity of grape seed tannins GSE100 and GSE300 were determined in this work, and the results are presented in **Table 1**.

**Table 1** shows that GSE300 contained much higher concentrations of monomer and oligomer procyanidins and lower concentrations of polymeric polyphenols than GSE100. However, the antioxidant activities of the two GSE products were not markedly different, although GSE300 presented a little lower antioxidant activity. These results would suggest that the two products have similar effects on the antioxidant activity of wine. In other words,

**Table 1.** Composition and Concentration (Milligrams per Gram) of Two Commercial Grape Seed Tannins (GSE100 and GSE300) in Phenolic Compounds and Antioxidant Activity[a]

| | | phenolic composition | | | | | | | | | | | | | antioxidant activity | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | catechins and di- and triprocyanidins | | | | | | | | | | | | | |
| commercial tannin | | gallic acid | B3 | B1 | Cat | T2 | B4 | B2 | B2-3-O-G | Epi | B2-3′-O-G | B1-3-O-G | C1 | % oligomer PA | % polymer PA | $EC_{50}$ | ARP ($1/EC_{50}$) |
| GSE100 | *x* | nd | 3.18 | 9.41 | 28.93 | 2.95 | 3.41 | 14.49 | 1.88 | 30.85 | 2.49 | 7.41 | 21.74 | 39.55 | 60.45 | 0.094 | 10.64 |
| | *S* | nd | 0.15 | 0.16 | 0.72 | 0.18 | 5.85 | 3.35 | 0.04 | 0.84 | 0.23 | 0.16 | 0.82 | 0.26 | 0.26 | 0.009 | 0.82 |
| GSE300 | *x* | 1.51 | 14.17 | 23.04 | 99.94 | 6.19 | 10.67 | 43.72 | 1.58 | 79.18 | 3.42 | 6.17 | 32.92 | 62.61 | 37.39 | 0.077 | 12.99 |
| | *S* | 0.02 | 0.78 | 4.37 | 4.85 | 0.10 | 0.23 | 2.51 | 0.52 | 0.85 | 0.46 | 0.15 | 0.26 | 0.25 | 0.25 | 0.008 | 0.38 |

[a] Abbreviations: B3, procyanidin dimers B3; B1, procyanidin dimers B1; Cat, (+)-catechin; T2, procyanidin dimers T2; B4, procyanidin dimers B4; B2, procyanidin dimers B2; B2-3-O-G, procyanidin dimers B2-3-*O*-gallate; Epi, (−)-epicatechin; B2-3′-O-G, procyanidin dimers B2-3′-*O*-gallate; B1-3-O-G, procyanidin dimers B1-3-*O*-gallate; C1, procyanidin trimer C1; PA, proantocyanidins; $EC_{50}$, antiradical activity; ARP, antiradical power; nd, not detected; *x*, mean; *S*, standard deviation.

**Table 2.** Effect of Addition of Grape Seed Tannins on the Concentrations (Milligrams per Liter) of Phenolic Acids, Stilbenes, Total Individual Phenolics (TIP), and Total Polyphenols (TP) in Red Wine[a]

| wine sample | | gallic acid | coumaric acid | caffeic acid | *trans*-resveratrol | *cis*-resveratrol | *trans*-piceid | *cis*-piceid | TIP | TP |
|---|---|---|---|---|---|---|---|---|---|---|
| $RW_{1.0}$ | *x* | 7.20 a | 1.97 b | 4.28 d | 2.66 b | 3.32 a | 6.46 a | 7.08 ab | 420.90 a | 1651.49 a |
| | *S* | 0.01 | 0.01 | 0.01 | 0.01 | 0.55 | 0.08 | 0.01 | 7.92 | 32.31 |
| $RW_{1.1}$ | *x* | 14.48 c | 1.90 b | 3.69 b | 2.64 ab | 2.27 a | 6.93 b | 7.05 ab | 506.98 b | 1789.38 a |
| | *S* | 0.00 | 0.03 | 0.03 | 0.03 | 0.05 | 0.02 | 0.01 | 3.12 | 27.01 |
| $RW_{1.2}$ | *x* | 29.77 e | 1.32 a | 3.23 a | 2.51 a | 4.37 a | 6.87 b | 7.16 b | 557.85 b | 2126.03 b |
| | *S* | 0.26 | 0.04 | 0.07 | 0.04 | 0.01 | 0.00 | 0.01 | 10.61 | 77.13 |
| $RW_{1.3}$ | *x* | 11.52 b | 2.17 b | 4.03 c | 2.75 b | 4.62 a | 6.95 b | 6.89 a | 497.80 b | 1861.21 a |
| | *S* | 0.03 | 0.01 | 0.00 | 0.04 | 0.04 | 0.01 | 0.07 | 32.09 | 77.50 |
| $RW_{1.4}$ | *x* | 19.16 d | 1.44 a | 3.52 b | 2.68 b | 3.70 a | 6.87 b | 7.45 c | 557.13 b | 2194.02 b |
| | *S* | 0.10 | 0.16 | 0.08 | 0.03 | 2.22 | 0.04 | 0.11 | 4.28 | 59.69 |
| $RW_{2.0}$ | *x* | 7.07 a | 2.05 c | 3.67 b | 2.98 a | 4.26 c | 6.89 d | 6.70 a | 492.23 a | 1641.00 a |
| | *S* | 0.86 | 0.00 | 0.01 | 0.02 | 0.01 | 0.03 | 0.00 | 14.47 | 11.37 |
| $RW_{2.1}$ | *x* | 14.40 c | 1.67 bc | 3.64 b | 2.97 a | 4.16 c | 6.82 c | 7.16 b | 576.33 c | 1827.18 b |
| | *S* | 0.15 | 0.00 | 0.01 | 0.27 | 0.05 | 0.00 | 0.04 | 3.54 | 28.21 |
| $RW_{2.2}$ | *x* | 30.01 e | 1.00 a | 3.34 a | 2.73 a | 1.89 a | 6.72 b | 7.52 d | 588.69 c | 2256.21 c |
| | *S* | 0.18 | 0.20 | 0.00 | 0.01 | 0.00 | 0.01 | 0.02 | 4.66 | 12.52 |
| $RW_{2.3}$ | *x* | 11.05 b | 1.82 c | 3.46 ab | 3.29 a | 4.24 c | 6.69 b | 7.09 b | 521.31 ab | 1797.22 b |
| | *S* | 0.10 | 0.00 | 0.02 | 0.56 | 0.05 | 0.01 | 0.01 | 13.98 | 6.91 |
| $RW_{2.4}$ | *x* | 20.88 d | 1.18 ab | 3.37 a | 2.82 a | 2.09 b | 6.56 a | 7.29 c | 550.09 bc | 2229.98 c |
| | *S* | 0.07 | 0.15 | 0.17 | 0.03 | 0.02 | 0.00 | 0.00 | 10.94 | 43.36 |
| $RW_{3.0}$ | *x* | 42.55 a | 5.54 a | 6.84 b | 7.79 a | 14.41 a | 13.70 bc | 10.51 ab | 1137.41 a | 4404.03 a |
| | *S* | 0.18 | 0.20 | 0.04 | 0.15 | 0.02 | 0.05 | 0.09 | 140.03 | 113.32 |
| $RW_{3.1}$ | *x* | 50.89 c | 5.43 a | 6.69 b | 7.84 a | 14.45 a | 13.52 a | 10.88 b | 1054.38 a | 4690.73 b |
| | *S* | 0.11 | 0.39 | 0.01 | 0.08 | 0.21 | 0.03 | 0.25 | 93.66 | 28.56 |
| $RW_{3.2}$ | *x* | 70.22 e | 5.10 a | 6.52 a | 8.43 ab | 18.11 c | 13.68 bc | 34.42 c | 1182.61 a | 5186.77 c |
| | *S* | 0.04 | 0.03 | 0.01 | 0.34 | 0.17 | 0.00 | 0.07 | 35.54 | 6.91 |
| $RW_{3.3}$ | *x* | 50.57 b | 5.58 a | 6.48 a | 8.53 ab | 15.59 b | 13.62 b | 10.64 b | 1044.23 a | 4687.11 b |
| | *S* | 0.09 | 0.06 | 0.07 | 0.30 | 0.07 | 0.00 | 0.20 | 2.27 | 6.12 |
| $RW_{3.4}$ | *x* | 57.79 d | 5.29 a | 6.48 a | 9.22 b | 15.35 b | 13.73 c | 9.95 a | 1097.11 a | 5093.46 c |
| | *S* | 0.10 | 0.06 | 0.04 | 0.06 | 0.10 | 0.01 | 0.05 | 13.68 | 13.75 |

[a] Abbreviations: *x*, mean; *S*, standard deviation. For each winemaking process, mean values followed by the same letter in a column are not significantly different (LSD, 99.9%).

on a weight basis, in vitro antioxidant activity of procyanidins is independent of their degree of polymerization. In fact, the results presented below will give this confirmation.

The effect of addition of GSE on the concentrations of phenolic acids, stilbenes, total individual phenolics, and total polyphenols in red wine is presented in **Table 2**.

For all of these wines, addition of GSE had no significant effect on coumaric acid and caffeic acid, but had significant effect on gallic acid in red wines. In other words, increasing the amount of GSE added increased significantly the gallic acid concentration in red wine.

It should be noted that, although gallic acid was not been detected in GSE100, the addition of GSE100 induced much higher concentration of gallic acid in the wine than the addition of GSE300. The reason for this may be explained by the fact that GSE100 contained a larger amount of polymeric proanthocyanidins (**Table 1**), the molecules of which have a higher percentage of galloylation than the oligomeric ones (*14*, *17*), and during winemaking and storage, galloylated proanthocyanidins would release gallic acids under wine pH medium and thus increase significantly the gallic acid concentration in red wine.

From **Table 2**, it can also be seen that the addition of GSE has no or little effect on the concentrations of stilbenes in red wines, with the exception of *cis*-piceid in $RW_2$. In fact, we did not detect the presence of *cis*- and *trans*-resveratrol or piceid in either GSE product. The difference in stilbene concentration of the wines was probably due to the instability of such compounds during winemaking and storage (*20*).

**Table 3.** Effect of Addition of Grape Seed Tannins on the Concentrations (Milligrams per Liter) of Catechins and Oligomeric Procyanidins in Red Wine[a]

| wine sample | | B3 | B1 | Cat | T2 | B4 | B2 | B2-3-O-G | Epi | B2-3′-O-G | B1-3-O-G | C1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $RW_{1.0}$ | *x* | nd | 9.37 a | 3.56 a | 0.43 a | 4.21 a | 10.39 a | 0.61 a | 4.07 a | 2.76 a | 1.62 a | 0.84 a |
| | *S* | nd | 0.34 | 0.19 | 0.07 | 0.39 | 0.71 | 0.04 | 0.09 | 0.29 | 0.23 | 0.14 |
| $RW_{1.1}$ | *x* | nd | 8.16 a | 6.12 ab | 0.61 a | 4.57 ab | 10.23 a | 0.71 a | 5.75 b | 1.99 a | 0.94 a | 0.67 a |
| | *S* | nd | 0.18 | 0.14 | 0.03 | 0.12 | 0.16 | 0.03 | 0.04 | 0.01 | 0.02 | 0.00 |
| $RW_{1.2}$ | *x* | nd | 8.81 a | 8.78 bc | 0.78 a | 4.48 ab | 8.24 a | 0.77 a | 8.83 c | 2.14 a | 1.01 a | 0.79 a |
| | *S* | nd | 0.06 | 0.51 | 0.13 | 0.26 | 2.28 | 0.07 | 0.10 | 0.32 | 0.70 | 0.10 |
| $RW_{1.3}$ | *x* | nd | 8.43 a | 10.85 c | 0.43 a | 4.39 ab | 8.46 a | 0.71 a | 7.58 c | 2.22 a | 1.26 a | 0.64 a |
| | *S* | nd | 1.56 | 0.23 | 0.02 | 0.47 | 1.53 | 0.04 | 0.54 | 0.34 | 0.48 | 0.06 |
| $RW_{1.4}$ | *x* | nd | 10.65 a | 21.75 c | 0.88 a | 5.58 b | 11.25 a | 1.00 b | 15.82 d | 2.32 a | 1.43 a | 0.92 a |
| | *S* | nd | 0.41 | 1.38 | 0.23 | 0.17 | 0.19 | 0.00 | 0.58 | 0.02 | 0.07 | 0.09 |
| $RW_{2.0}$ | *x* | 2.60 c | 10.95 a | 4.43 a | 1.33 a | 5.96 a | 13.20 b | 1.46 a | 5.65 a | 3.26 a | 2.70 a | 2.61 b |
| | *S* | 0.10 | 1.51 | 0.01 | 0.05 | 0.01 | 0.37 | 0.00 | 0.18 | 0.02 | 0.04 | 0.19 |
| $RW_{2.1}$ | *x* | 0.00 a | 9.16 a | 6.61 a | 0.55 a | 3.80 a | 8.90 a | 0.55 a | 5.51 a | 1.95 a | 1.20 a | 0.50 a |
| | *S* | 0.00 | 0.08 | 0.16 | 0.04 | 0.10 | 0.48 | 0.02 | 0.13 | 0.31 | 0.56 | 0.00 |
| $RW_{2.2}$ | *x* | 1.07 b | 11.35 a | 13.99 b | 1.55 a | 6.39 a | 13.49 b | 1.70 a | 13.40 c | 2.56 a | 1.85 a | 0.60 a |
| | *S* | 0.18 | 1.05 | 0.33 | 0.25 | 0.55 | 1.38 | 0.10 | 0.75 | 0.23 | 0.12 | 0.03 |
| $RW_{2.3}$ | *x* | 1.20 b | 12.99 a | 15.21 b | 0.89 a | 5.14 a | 11.00 ab | 0.76 a | 10.06 b | 2.32 a | 1.61 a | 0.99 a |
| | *S* | 0.17 | 1.29 | 0.32 | 0.19 | 0.03 | 0.05 | 0.02 | 0.16 | 0.38 | 0.64 | 0.53 |
| $RW_{2.4}$ | *x* | 2.09 c | 23.33 b | 16.17 b | 9.93 a | 13.29 a | 17.43 c | 1.92 a | 20.14 d | 2.44 a | 1.94 a | 1.21 a |
| | *S* | 0.40 | 0.65 | 1.56 | 12.14 | 8.98 | 0.32 | 1.45 | 1.61 | 0.67 | 0.74 | 0.01 |
| $RW_{3.0}$ | *x* | 2.46 a | 33.38 a | 24.90 a | 6.80 a | 9.91 a | 28.86 a | 3.05 b | 15.57 a | 4.55 ab | 3.52 a | 2.50 a |
| | *S* | 0.00 | 3.34 | 1.54 | 0.24 | 0.79 | 0.75 | 0.09 | 1.66 | 1.24 | 0.48 | 1.09 |
| $RW_{3.1}$ | *x* | 3.56 bc | 29.33 a | 44.15 b | 9.45 a | 12.22 a | 34.84 b | 6.48 d | 22.99 ab | 7.05 b | 3.82 ab | 6.05 b |
| | *S* | 0.54 | 3.66 | 6.69 | 1.67 | 1.40 | 2.67 | 0.74 | 3.83 | 1.41 | 0.60 | 1.14 |
| $RW_{3.2}$ | *x* | 3.33 ab | 39.94 a | 32.15 ab | 8.77 a | 9.66 a | 31.88 ab | 0.98 a | 18.78 a | 2.87 a | 3.68 ab | 6.72 b |
| | *S* | 0.02 | 0.40 | 0.28 | 0.01 | 0.16 | 0.14 | 0.05 | 0.23 | 0.00 | 0.06 | 0.11 |
| $RW_{3.3}$ | *x* | 2.40 a | 34.79 a | 30.82 a | 7.52 a | 10.37 a | 31.66 ab | 4.52 c | 20.89 a | 4.21 ab | 5.84 c | 5.94 b |
| | *S* | 0.08 | 2.46 | 1.75 | 0.04 | 0.10 | 0.29 | 0.01 | 0.46 | 0.07 | 0.11 | 0.07 |
| $RW_{3.4}$ | *x* | 4.39 c | 39.42 a | 44.66 b | 8.79 a | 11.48 a | 35.73 b | 5.12 cd | 29.52 b | 3.54 a | 5.21 bc | 6.39 b |
| | *S* | 0.10 | 4.27 | 0.14 | 0.44 | 0.19 | 0.14 | 0.03 | 0.91 | 0.02 | 0.45 | 0.06 |

[a] Abbreviations: B3, procyanidin dimers B3; B1, procyanidin dimers B1; Cat, (+)-catechin; T2, procyanidin dimers T2; B4, procyanidin dimers B4; B2, procyanidin dimers B2; B2-3-O-G, procyanidin dimers B2-3-*O*-gallate; Epi, (−)-epicatechin; B2-3′-O-G, procyanidin dimers B2-3′-*O*-gallate; B1-3-O-G, procyanidin dimers B1-3-*O*-gallate; C1, procyanidin trimer C1; nd, not detected; *x*, mean; *S*, standard deviation. For each winemaking process, mean values followed by the same letter in a column are not significantly different (LSD, 99.9% level).

For the wines with 2 days of maceration ($RW_1$ and $RW_2$), addition of both GSE100 and GSE300 increased significantly the concentrations of TIP and TP, and this increase was positively related to the amount of GSE added. For the wine with 9 days of maceration ($RW_3$), the addition of GSE did not increase the concentration of TIP but increased significantly the concentration of TP. The reason for this may be explained by the fact that the amount of individual phenolic compounds contributed by added GSE100 or GSE300 is very little compared to the amount of individual phenolic compounds present in wine macerated for 9 days and thus not sufficient to increase significantly the amount of TIP in that wine. In other words, wine macerated for 9 days contained high concentration of both TP (>4 g/L) and TIP (>1 g/L), whereas the total amount of GSE added was only 0.2 or 0.6 g/L, in which individual phenolic compounds represented only a small percentage and higher oligomeric and polymeric proanthocyanidins represented larger proportions.

**Table 3** presents the effect of the addition of GSE on the concentrations of catechin and dimeric and trimeric procyanidins in red wine.

It can be noted that, for the wines with 2 days of maceration ($RW_1$ and $RW_2$), addition of both GSE100 and GSE300 had no significant effect on di- and trimeric procyanidins in red wines, but increased significantly the concentration of catechin and epicatechin, depending on the amount of GSE added. Furthermore, addition of GSE300 leads to higher amounts of catechins in these two wines than does the addition of GSE100. These circumstances may be explained by the fact that both GSE100 and GSE300 contain higher concentrations of catechin and epicatechin than di- and trimeric procyanidins, with the latter more remarkable (**Table 1**). On the other hand, it should be especially noted that with the addition of higher amounts of GSE100 or GSE300, the concentrations of major di- and trimeric procyanidins in $RW_2$ are higher than those in $RW_1$. These results may indicate that the addition of tannins should be performed at the end of alcoholic fermentation, not before or during fermentation/maceration. The fact that the addition of GSE just before fermentation did not help to keep the added phenolic compounds in wines may be explained by their combination or adsorption with solids and proteins or polymerization or oxidation with other phenolic or nonphenolic compounds during fermentation, which alter or precipitate important parts of catechins and procyanidins (*27*).

For the wine rich in polyphenols ($RW_3$), addition of GSE has, in general, no obvious effect on the contents of catechins and di- and trimeric procyanidins, with the exception of procyanidin C1, for which the addition of both GSE products increased significantly its concentration.

The effect of the addition of GSE on the concentrations of anthocyanins and their derivatives in the red wines is illustrated in **Table 4**.

For the wines poor in polyphenols ($RW_1$ and $RW_2$), the addition of GSE did not affect, in general, the concentrations of anthocyanins and their derivatives in red wines. However, for the wine rich in polyphenols ($RW_3$), the addition of GSE may induce the important reduction of some major anthocyanins

**Table 4.** Effect of Addition of Grape Seed Tannins on the Concentrations (Milligrams per Liter) of Anthocyanins and Their Derivatives in Red Wine[a]

| wine sample | | Dp | Cy | Pt | Pn | Mv | Py | Pncm | Dpcm | Mvac | Ptcm | c-Mvcm | t-Pncm | t-Mvcm |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $RW_{1.0}$ | *x* | 0.56 a | 0.16 a | 0.61 b | 0.98 a | 5.12 b | 1.18 a | 0.29 a | 0.16 ab | 0.16 a | 0.15 a | 0.17 a | 0.15 a | 0.34 b |
| | *S* | 0.11 | 0.00 | 0.06 | 0.01 | 0.03 | 0.02 | 0.02 | 0.00 | 0.00 | 0.00 | 0.03 | 0.00 | 0.03 |
| $RW_{1.1}$ | *x* | 0.72 a | 0.16 a | 0.76 c | 1.14 ab | 5.96 c | 1.21 a | 0.30 a | 0.17 b | 0.16 a | 0.15 a | 0.18 a | 0.17 a | 0.35 b |
| | *S* | 0.06 | 0.01 | 0.01 | 0.03 | 0.01 | 0.02 | 0.03 | 0.00 | 0.01 | 0.00 | 0.01 | 0.02 | 0.01 |
| $RW_{1.2}$ | *x* | 0.53 a | 0.16 a | 0.56 ab | 0.85 a | 3.85 a | 1.21 a | 0.28 a | 0.15 a | 0.16 a | 0.16 a | 0.17 a | 0.16 a | 0.15 a |
| | *S* | 0.04 | 0.00 | 0.01 | 0.15 | 0.12 | 0.02 | 0.01 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 |
| $RW_{1.3}$ | *x* | 0.75 a | 0.16 a | 0.80 c | 1.37 b | 6.51 c | 1.18 a | 0.28 a | 0.16 ab | 0.16 a | 0.20 b | 0.18 a | 0.18 a | 0.39 b |
| | *S* | 0.02 | 0.01 | 0.03 | 0.08 | 0.24 | 0.04 | 0.01 | 0.00 | 0.01 | 0.01 | 0.02 | 0.00 | 0.04 |
| $RW_{1.4}$ | *x* | 0.50 a | 0.15 a | 0.47 a | 0.91 a | 3.60 a | 1.13 a | 0.27 a | 0.16 ab | 0.15 a | 0.15 a | 0.25 a | 0.16 a | 0.23 a |
| | *S* | 0.09 | 0.00 | 0.01 | 0.06 | 0.23 | 0.01 | 0.01 | 0.00 | 0.00 | 0.00 | 0.03 | 0.01 | 0.01 |
| $RW_{2.0}$ | *x* | 0.75 c | 0.15 a | 1.02 b | 1.30 c | 9.11 d | 1.19 a | 0.19 a | 0.17 a | 0.39 a | 0.15 a | 0.15 a | 0.18 a | 0.62 c |
| | *S* | 0.01 | 0.01 | 0.04 | 0.03 | 0.21 | 0.01 | 0.02 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.09 |
| $RW_{2.1}$ | *x* | 0.76 c | 0.16 a | 0.80 ab | 1.09 b | 7.20 c | 1.23 a | 0.32 a | 0.16 a | 0.29 a | 0.16 a | 0.16 ab | 0.29 c | 0.51 abc |
| | *S* | 0.02 | 0.00 | 0.03 | 0.04 | 0.07 | 0.01 | 0.04 | 0.00 | 0.10 | 0.01 | 0.01 | 0.01 | 0.07 |
| $RW_{2.2}$ | *x* | 0.57 b | 0.16 a | 0.67 a | 0.83 a | 5.50 b | 1.21 a | 0.26 a | 0.20 a | 0.20 a | 0.16 a | 0.17 ab | 0.24 b | 0.38 ab |
| | *S* | 0.01 | 0.00 | 0.06 | 0.03 | 0.06 | 0.03 | 0.06 | 0.06 | 0.03 | 0.00 | 0.03 | 0.01 | 0.00 |
| $RW_{2.3}$ | *x* | 0.73 c | 0.16 a | 0.82 ab | 1.13 bc | 7.55 c | 1.14 a | 0.30 a | 0.16 a | 0.26 a | 0.16 a | 0.18 ab | 0.32 c | 0.58 bc |
| | *S* | 0.01 | 0.00 | 0.08 | 0.10 | 0.13 | 0.06 | 0.00 | 0.00 | 0.08 | 0.01 | 0.02 | 0.00 | 0.02 |
| $RW_{2.4}$ | *x* | 0.49 a | 0.15 a | 0.60 a | 0.83 a | 4.95 a | 1.20 a | 0.27 a | 0.15 a | 0.17 a | 0.19 a | 0.23 b | 0.22 b | 0.34 a |
| | *S* | 0.03 | 0.00 | 0.07 | 0.02 | 0.16 | 0.02 | 0.04 | 0.01 | 0.00 | 0.05 | 0.03 | 0.02 | 0.03 |
| $RW_{3.0}$ | *x* | 4.39 b | 0.41 a | 4.83 c | 6.32 d | 32.40 e | 1.77 a | 0.36 b | 0.30 a | 1.04 b | 0.20 a | 0.23 ab | 0.86 b | 2.54 c |
| | *S* | 0.08 | 0.01 | 0.03 | 0.08 | 0.35 | 0.03 | 0.00 | 0.02 | 0.05 | 0.02 | 0.00 | 0.02 | 0.00 |
| $RW_{3.1}$ | *x* | 3.67 a | 0.38 a | 3.94 b | 5.46 c | 27.63 d | 1.74 a | 0.37 b | 0.28 a | 0.84 a | 0.24 a | 0.20 a | 0.74 a | 2.08 b |
| | *S* | 0.05 | 0.00 | 0.10 | 0.00 | 0.30 | 0.17 | 0.02 | 0.02 | 0.02 | 0.01 | 0.00 | 0.01 | 0.04 |
| $RW_{3.2}$ | *x* | 3.60 a | 0.42 a | 3.76 ab | 4.87 b | 24.32 b | 1.84 a | 0.36 b | 0.27 a | 0.80 a | 0.21 a | 0.25 b | 0.70 a | 1.84 a |
| | *S* | 0.00 | 0.11 | 0.15 | 0.03 | 0.33 | 0.13 | 0.00 | 0.01 | 0.02 | 0.03 | 0.00 | 0.01 | 0.04 |
| $RW_{3.3}$ | *x* | 3.67 a | 0.36 a | 3.65 ab | 5.01 b | 25.86 c | 1.82 a | 0.26 a | 0.32 a | 0.78 a | 0.21 a | 0.30 c | 0.66 a | 1.76 a |
| | *S* | 0.14 | 0.01 | 0.09 | 0.07 | 0.53 | 0.03 | 0.01 | 0.10 | 0.09 | 0.01 | 0.02 | 0.04 | 0.07 |
| $RW_{3.4}$ | *x* | 3.38 a | 0.34 a | 3.42 a | 4.65 a | 22.79 a | 1.92 a | 0.38 b | 0.26 a | 0.75 a | 0.22 a | 0.26 b | 0.71 a | 1.71 a |
| | *S* | 0.01 | 0.00 | 0.05 | 0.01 | 0.05 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 |

[a] Abbreviations: Dp, delphinidin-3-glc; Cy, cyanidin-3-glc; Pt, petunidin-3-glc; Pn, peonidin-3-glc; Mv, malvidin-3-glc; Py, malvidin-3-glc pyruvic derivative; Pncm, peonidin-3-coumaroylglucoside; Dpcm, delphinidin-3-coumaroylglucoside; Mvac, malvidin-3-acetylglucoside; Ptcm, petunidin-3-coumaroylglucoside; c-Mvcm, *cis*-malvidin-3-coumaroylglucoside; t-Pncm, *trans*-peonidin-3-coumaroylglucoside; t-Mvcm, *trans*-malvidin-3-coumaroylglucoside; *x*, mean; *S*, standard deviation. For each winemaking process, mean values followed by the same letter in a column are not significantly different (LSD, 99.9% level).

such as delphinidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside. Such reductions may be explained by the occurrence of interaction between these major anthocyanins with grape seed tannins (proanthocyanidins) during winemaking and storage (*28*). Several previous works have reported the decrease of anthocyanins in contact with individual phenolic compounds (*29*, *16*).

**Table 5** presents the effect of addition of GSE on chromatic characteristics of red wines.

From **Table 5** it can be seen clearly that for the wines rich in polyphenols ($RW_3$), the addition of any GSE products, at different amounts (0.2 or 0.6 g/L), has no effect on the color of the wines. For the wines poor in polyphenols, the addition of any GSE products at lower amounts (0.2 g/L) has no effect on the color of the wine, either. However, it is important to note that for the wine poor in polyphenols, the addition of any GSE products at higher amounts (0.6 g/L) has a significant effect on the color of the wine; the values of the axes of a three-dimensional color space $a^*$ (measure of redness) and $b^*$ (measure of yellowness) of the wines with addition of 0.6 g/L of GSE100 or GSE300 are significantly higher than those of other wines. These results may indicate that (1) the addition of tannins to the wine is necessary only when it is poor in polyphenols or, more precisely, poor in proanthocyanidins, and (2) the amount recommended for the addition of tannins to wine may be not sufficient; higher amounts of tannins may be necessary to effectively improve the wine color properties. Parker et al. (*10*) did not observe the effect of the addition of enological tannins during pre- and postalcoholic fermentation on wine color properties, probably due to the low amount of tannin products applied (0.2 g/L).

The influence of the addition of GSE on antioxidant activity of the wines is shown in **Figure 2**.

It can be seen, from **Figure 2**, that for the wine rich in polyphenols ($RW_3$) the addition of any amount of GSE does not increase its antioxidant activity. However, the addition of grape seed tannins in wines poor in polyphenols ($RW_1$ and $RW_2$) has a significant effect on the antioxidant activity; the antioxidant activity is positively related to the amount of GSE added but independent of the type of GSE added. Statistical analysis showed that the antioxidant activities of the wines poor in polyphenols ($RW_1$ and $RW_2$) are positively correlated with total polyphenols (correlation coefficient $r = 0.960$) but poorly correlated with individual phenolics (correlation coefficient $r = 0.834$).

From these results, we may conclude that the addition of tannins to improve wine color intensity or antioxidant activity is necessary only for those wines poor in polyphenols and that grape seed tannin products added after alcoholic fermentation had a better effect on the phenolic composition of red wine than those added before alcoholic fermentation. Another important conclusion is that, although the two grape seed tannins (GSE100 and GSE300) have the same effect on wine antioxidant activity or similar effects on the major phenolic compounds of wines, GSE100, which contains a high percentage of polymeric polyphenols, releases more gallic acid to wines than GSE300. This fact

**Table 5.** Effect of Addition of Grape Seed Tannins on Chromatic Characteristics of Red Wines[a]

| wine sample | | $L^*$ (%) | $a^*$ | $b^*$ | $h$ | $C^*$ | CI | $T$ | $OD_{420}$ (%) | $OD_{520}$ (%) | $OD_{620}$ (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $RW_{1.0}$ | *x* | 50.4b | 50.08 b | 34.76 ab | 34.76 c | 60.96 ab | 0.99 a | 0.89 c | 42.19 c | 47.59 c | 10.22 a |
| | *S* | 0.29 | 0.04 | 0.15 | 0.14 | 0.05 | 0.01 | 0.00 | 0.00 | 0.09 | 0.09 |
| $RW_{1.1}$ | *x* | 49.4 b | 50.43 b | 34.48 a | 34.36 b | 61.09 b | 1.01 a | 0.89 d | 42.19 c | 47.36 bc | 10.44 a |
| | *S* | 0.09 | 0.03 | 0.02 | 0.01 | 0.04 | 0.00 | 0.00 | 0.01 | 0.01 | 0.00 |
| $RW_{1.2}$ | *x* | 43.7 a | 52.88 c | 35.96 c | 34.22 b | 63.95 d | 1.14 b | 0.88 b | 41.31 b | 47.16 ab | 11.53 b |
| | *S* | 0.45 | 0.01 | 0.09 | 0.07 | 0.05 | 0.01 | 0.00 | 0.05 | 0.07 | 0.12 |
| $RW_{1.3}$ | *x* | 49.7 b | 49.65 a | 34.72 ab | 34.96 c | 60.58 a | 1.00 a | 0.9 e | 42.46 d | 47.01 a | 10.52 a |
| | *S* | 0.06 | 0.01 | 0.02 | 0.01 | 0.02 | 0.00 | 0.00 | 0.01 | 0.02 | 0.01 |
| $RW_{1.4}$ | *x* | 43.8 a | 53.00 c | 34.99 b | 33.43 a | 63.50 c | 1.13 b | 0.86 a | 40.82 a | 47.62 c | 11.56 b |
| | *S* | 0.22 | 0.21 | 0.08 | 0.05 | 0.22 | 0.00 | 0.00 | 0.06 | 0.10 | 0.16 |
| $RW_{2.0}$ | *x* | 54.8 c | 43.46 a | 32.66 a | 36.93 a | 54.36 a | 0.90 a | 1.00 c | 44.81 b | 44.75 a | 10.43 a |
| | *S* | 0.02 | 0.16 | 0.07 | 0.04 | 0.17 | 0.00 | 0.00 | 0.03 | 0.04 | 0.07 |
| $RW_{2.1}$ | *x* | 51.5 b | 46.65 c | 34.60 b | 36.56 a | 58.08 c | 0.97 b | 0.98 ab | 44.38 a | 45.14 a | 10.48 a |
| | *S* | 0.97 | 0.26 | 0.06 | 0.11 | 0.24 | 0.02 | 0.00 | 0.19 | 0.25 | 0.44 |
| $RW_{2.2}$ | *x* | 48.3 a | 48.39 d | 37.40 d | 37.70 b | 61.16 d | 1.05 c | 0.99 bc | 44.34 a | 44.77 a | 10.89 a |
| | *S* | 1.21 | 0.24 | 0.32 | 0.38 | 0.01 | 0.03 | 0.01 | 0.06 | 0.35 | 0.41 |
| $RW_{2.3}$ | *x* | 53.2 bc | 45.70 b | 34.63 b | 37.15 ab | 57.34 b | 0.93 ab | 0.99 c | 44.81 b | 45.04 a | 10.14 a |
| | *S* | 0.20 | 0.02 | 0.08 | 0.05 | 0.07 | 0.00 | 0.00 | 0.02 | 0.05 | 0.07 |
| $RW_{2.4}$ | *x* | 48.5 a | 48.79 d | 36.63 c | 36.90 a | 61.01 d | 1.04 c | 0.98 a | 44.06 a | 45.18 a | 10.76 a |
| | *S* | 0.01 | 0.06 | 0.09 | 0.04 | 0.10 | 0.00 | 0.00 | 0.02 | 0.00 | 0.02 |
| $RW_{3.0}$ | *x* | 14.4 b | 45.75 b | 24.52 b | 28.18 c | 51.91 b | 2.10 a | 0.91 a | 38.65 b | 42.24 b | 19.11 a |
| | *S* | 0.46 | 0.68 | 0.77 | 0.39 | 0.96 | 0.01 | 0.00 | 0.05 | 0.13 | 0.17 |
| $RW_{3.1}$ | *x* | 13.6 ab | 44.83 ab | 23.20 ab | 27.36 b | 50.48 ab | 2.14 b | 0.92 b | 38.62 b | 41.96 a | 19.42 ab |
| | *S* | 0.00 | 0.02 | 0.00 | 0.01 | 0.02 | 0.00 | 0.00 | 0.01 | 0.00 | 0.01 |
| $RW_{3.2}$ | *x* | 13.0 a | 44.19 a | 22.31 a | 26.79 ab | 49.50 a | 2.17 cd | 0.92 c | 38.57 b | 41.77 a | 19.66 b |
| | *S* | 0.04 | 0.06 | 0.06 | 0.03 | 0.08 | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 |
| $RW_{3.3}$ | *x* | 12.8 a | 44.07 a | 21.82 a | 26.34 a | 49.18 a | 2.18 d | 0.91 a | 38.15 a | 41.75 a | 20.10 c |
| | *S* | 0.03 | 0.05 | 0.05 | 0.03 | 0.07 | 0.00 | 0.00 | 0.00 | 0.01 | 0.02 |
| $RW_{3.4}$ | *x* | 13.4 a | 44.49 ab | 22.86a | 27.19 b | 50.02 ab | 2.15 bc | 0.93 d | 38.77 c | 41.78 a | 19.45 ab |
| | *S* | 0.22 | 0.39 | 0.38 | 0.18 | 0.52 | 0.00 | 0.00 | 0.03 | 0.04 | 0.08 |

[a] Abbreviations: $L^*$, clarity ($L^* = 0$ black, $L^* = 1$ in color); $a^*$, red/green color component ($a^* > 0$ red, $a^* < 0$ green); $b^*$, blue/yellow color component ($b^* > 0$ yellow, $b^* < 0$ blue); $h$, hue angle; $C^*$, chroma; CI, color intensity ($CI = OD_{420} + OD_{520} + OD_{620}$); $T$, hue ($T = OD_{420}/OD_{520}$); $OD_{420}$ (%) = ($OD_{420}$/CI) × 100; $OD_{520}$ (%) = ($OD_{520}$/CI) × 100; $OD_{620}$ (%) = ($OD_{620}$/CI) × 100; *x*, mean; *S*, standard deviation. For each winemaking process, mean values followed by the same letter in a column are not significantly different (LSD, 99.9% level).

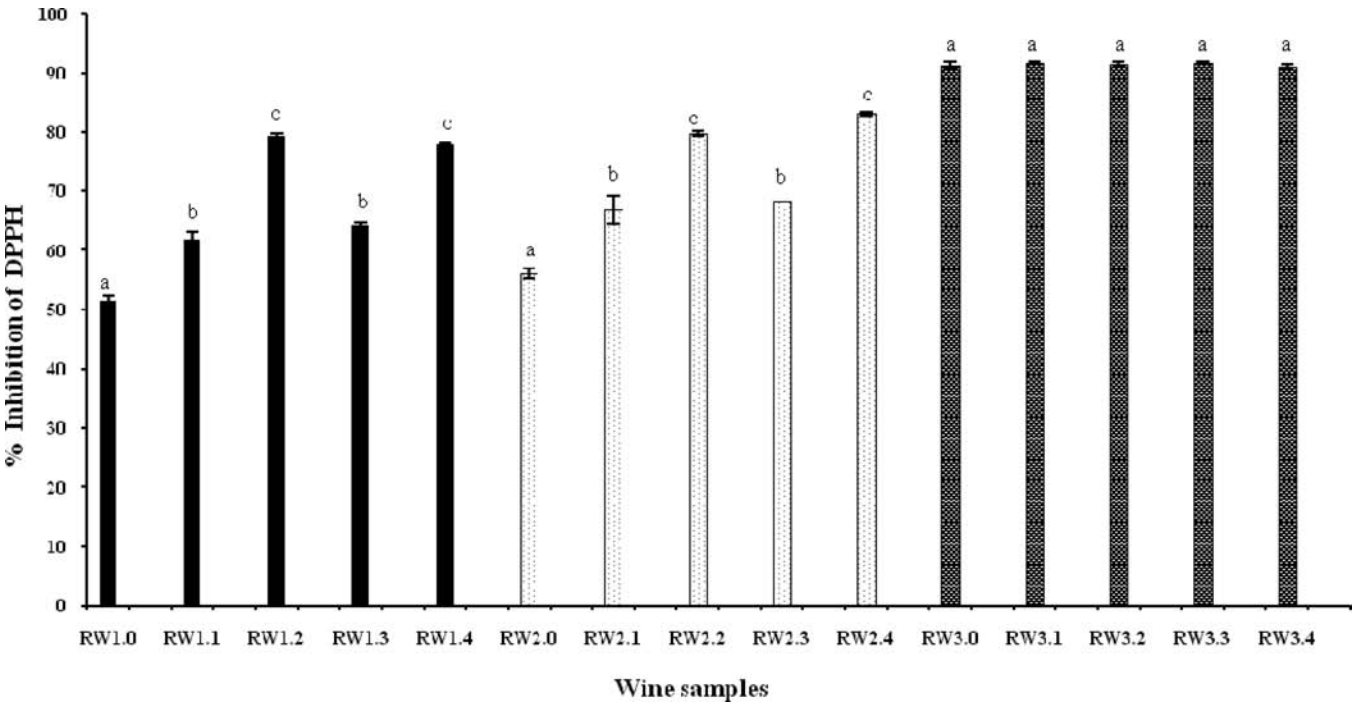


**Figure 2.** Effect of added enological tannins on the antioxidant activity of red wine.

may affect wine sensory properties. Our future work will be done on the effect of the addition of these tannins on the sensory properties, that is, astringency, bitterness, and aromatic profile, of red wines.

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