Effect of inert gas and prefermentative treatment with polyvinylpolypyrrolidone on the phenolic composition of Chilean Sauvignon blanc wines

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Abstract

BACKGROUND: Sauvignon blanc wines are produced under a wide variety of winemaking conditions, some of which include different fruit-ripening levels, cold soaks and the use of fining agents and inert gases. Anecdotal evidence suggests that sensory variations among these wines may have to do with their phenolic composition and concentration. Therefore the aim of this work was to study the effects of different winemaking conditions typically used in Chile on the phenolic composition and concentration of Sauvignon blanc wines.

RESULTS: The use of an inert gas (CO₂) in winemaking produced differences in the proportion of proanthocyanidin fractions. A higher concentration of flavan-3-ol monomers resulted from winemaking in the presence of inert gas. This condition also produced a higher content of total phenols and low-molecular-weight phenolic compounds. Low doses of polyvinylpolypyrrolidone (PVPP) in the prefermentative treatments produced wines with a higher content of phenolic compounds. Under these conditions a higher content of polymeric proanthocyanidins was observed.

CONCLUSION: Different winemaking conditions modified the concentration and proportion of proanthocyanidin fractions and the global phenolic composition of the resulting white wines. This should be taken into account by the wineries producing these wines.

Keywords: Sauvignon blanc; phenolic compounds; proanthocyanidins; winemaking; PVPP; inert gases

INTRODUCTION

Sauvignon blanc is one of the most important white wine varieties grown in Chile, with approximately 13,277 ha planted.¹ Some of the characteristics of this variety include its aromatic intensity, freshness, distinctive clarity and brightness.² Phenolic compounds contribute to wine sensory characteristics such as colour, astringency and bitterness and have been associated with beneficial health effects derived from moderate wine consumption.³ Many practices that can affect the chemical composition of wines (e.g. harvest time and prefermentative maceration) and winemaking techniques intended to protect against oxygen exposure are often considered essential for achieving a quality product.⁴⁻⁷ The harvest date of the fruit is important, because the accumulation and extractability of some phenolic compounds change as the grapes ripen.⁸⁻¹² The absence of maceration before fermentation and practices such as pressing of whole clusters are said to produce white wines with greater freshness, mainly because of the higher acidity and the lower concentration of phenols obtained.¹³ Prefermentative maceration of must, especially with skins, is used to increase the aromatic intensity of white wines.⁵,⁶ However, the enrichment of the must in phenolic compounds, particularly flavanols, may lead to an increase in browning and changes in astringency and bitterness.¹⁴⁻¹⁶ Indeed, the sensitivity of white wines to browning is related to their concentration of polyphenols. This phenomenon is perhaps one of the biggest problems in the marketing of these wines, changing them in a few months from a typical pale yellow colour and brightness to more brown tones, with

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altered the organoleptic characteristics that lead to consumer rejection.17-18

Some examples of techniques to prevent and/or decrease the tendency to brown are softer pressing, must clarification, the use of inert gases and fining with agents that allow the removal of phenols (e.g. polyvinylpolypyrrolidone, PVPP).19 Currently, there is a lack of studies on the effect of treatments that modify the phenolic concentration and composition of wines. Also, anecdotal evidence suggests that sensory variations among these wines may have to do with their phenolic composition and concentration. Therefore, the aim of this work was to study the effect of different winemaking treatments on the phenolic composition of Sauvignon blanc wines.

**EXPERIMENTAL**

**Materials**

Standards of gallic acid, protocatechuic acid, caffeic acid, p-coumaric acid, ferulic acid, caftaric acid, quercetin, (+)-catechin and (−)-epicatechin were purchased from Sigma Chemical Co. (St Louis, MO, USA). Polyethylene membranes of 0.45 and 0.22 µm pore size were acquired from EMD Millipore (Billerica, MA, USA). Vanillin (99%), ethyl acetate, diethyl ether, sodium hydroxide (NaOH), acetic acid, sulfuric acid (H2SO4), ethanol, hydrochloric acid (HCl) and high-performance liquid chromatography (HPLC)-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). All reagents were of analytical grade or higher. Sep-Pak Plus tC18 environmental cartridges (900 mg) and Sep-Pak Plus Short tC18 cartridges (400 mg) were obtained from Waters (Milford, MA, USA). Ultrapure water was obtained from a Purelab Ultra MK2 purification system (Elga, St Albans, UK). Phosphate buffer (pH 7) was acquired from Mallinckrodt Baker (Phillipsburg, NJ, USA). N2 and CO2 gas (inert gas, IG) was supplied by Indura SA (Santiago, Chile).

**Instrumentation**

pH was measured using an 8417 N pH meter (Hanna Instruments, Smithfield, RI, USA). Phenolic analyses were performed with an HPLC 1100 Series system (Agilent Technologies, Santa Clara, CA, USA) consisting of a G1315B photodiode array detector (DAD), a QuatPump G1311A quaternary pump, a G1379A degasser and a G1329A autosampler. A reverse phase Nova-Pak C18 column (4 µm, 3.9 mm i.d. × 300 mm; Waters) was used for HPLC-DAD analysis of individual phenolic compounds. Absorbances were measured using a UV/VIS 1700 Pharmaspec UV–visible spectrophotometer (Shimadzu, Kyoto, Japan).

**Grapes and experimental vineyard**

Grapes were harvested in 2009 from a self-rooted *Vitis vinifera* L. cv. Sauvignon blanc vineyard planted in 1999 in the Maipo Valley (central region of Chile). The vines were planted at a spacing of 2 m between vines and 2 m between rows, equivalent to a vine density of 2500 vines ha−1. The row orientation was east–west. The vines were pruned to 10 shoots m−1 on a unilateral cordon and trained to a vertical shoot-positioning (VSP) system. The cordon wire was 90 cm above ground, with two sets of dual shoot-positioning wires fixed at 135 and 180 cm above ground. A drip system provided supplemental irrigation. All cultural practices were consistent with those for white wine varieties in the Maipo Valley of Chile.

This work describes two independent assays.

**Assay 1. Effect of winemaking in presence (IG [+]) versus absence (IG [−]) of inert gas on phenolic composition of Sauvignon blanc wines**

For winemaking in the presence of inert gas (IG [+]), the mechanically harvested grapes were received in the cellar, sulfited with 30 mg L−1 potassium metabisulfite, de-stemmed for 8 h in a PF 350 pneumatic press of 35 000 L capacity (Della Toffola, Treviso, Italy) and then pressed. From the pneumatic press, the must was drained into an intermediate tank for cold gravity settling and further processing. All steps were performed in an environment where oxygen exposure was reduced by using CO2 (dry ice or gas) in the tanks, press and pumps. PVPP was added to the must at 80 g hL−1 in order to reduce the concentration of phenols extracted during maceration. When a turbidity of 60 NTU as measured with a 1100P turbidimeter (Hach, Loveland, CO, USA) was reached, the must was fermented in three stainless steel tanks, each of 1000 L capacity, using a yeast inoculum of 25 g hL−1 (*Saccharomyces cerevisiae* (var. *bayanus*) EC1118 yeast strain). The fermentation process was kept at a temperature of 11–13 °C for approximately 16–18 days. At the end of the alcoholic fermentation, when fermentable sugars reached less than 2 g L−1, the wine was transferred to another stainless steel tank for stabilisation under a protected inert gas environment (CO2). Finally, the wine was finely filtered and machine-bottled after adjusting the free SO2 level to 30 mg L−1. The same winemaking procedure, with the exception of the use of inert gas during the whole process, was used for treatment IG [−].

**Assay 2. Effect of different prefermentative treatments on phenolic composition of Sauvignon blanc wines**

Because the traditional method of Sauvignon blanc wine production involves the use of inert gas as described in assay 1 (IG [+]), also known as reductive vinification, various modifications in the prefermentation stage of the traditional method were introduced in assay 2. The alternative treatments consisted of early harvest of grapes, direct pressing or the use of a lower dose of fining agent, as follows. The control treatment (T0), corresponding to IG [+], was compared with: T1, grapes harvested 10 days before those of T0; T2, wines elaborated without maceration of de-stemmed grapes in the press (direct pressing); T3, musts treated with a low dose of PVPP (10 g hL−1). In all cases, three replicates of 1000 L were used for each treatment.

**Chemical and spectrophotometric characterisation of wines**

Titratable acidity was determined by titration with 0.1 mol L−1 NaOH to an end-point pH of 8.2 and expressed as g tartaric acid L−1. pH was measured using a pH meter. Total phenolic content was determined by UV absorbance at 280 nm using gallic acid as a standard and expressed as mg gallic acid equivalent (GAE) L−1.20 Colour intensity was determined by visible absorbance at 420 nm and expressed as absorbance units (a.u.).20

**Fractionation of proanthocyanidins by tC18 Sep-Pak cartridges**

The white wine samples were fractionated using tC18 Sep-Pak cartridges according to the method described by Sun et al.21 Briefly, 50 mL of white wine sample was concentrated to dryness in a rotary evaporator at <30 °C. The residue was dissolved in 20 mL of 67 mmol L−1 phosphate buffer (pH 7). The resulting solution was adjusted to pH 7 with either NaOH or HCl as necessary. Two tC18 Sep-Pak cartridges were assembled (top, Sep-Pak Plus tC18 environmental cartridge (900 mg); bottom,
Determination of total content of flavan-3-ols

The vanillin assay was performed as described by Sun et al. A 2.5 mL aliquot of 1:3 (v/v) H2SO4/methanol solution and a 2.5 mL glycosides, for which standards were unavailable, were assigned. Each major peak in the HPLC chromatograms was characterised et al. and was prepared by replacing the vanillin solution in the reaction mix (FII and FIII fractions). The absorbance was read at 500 nm. A blank was prepared by replacing the vanillin solution in the reaction mix with methanol. The absorbance of the blank was subtracted from the absorbance of the corresponding vanillin-containing sample. Quantification was performed using standard curves prepared from monomers (for FI), oligomers (for FII) and polymers (for FIII) of flavan-3-ols isolated from grape seeds, as previously described.22

HPLC analysis of low-molecular-weight phenolic compounds

White wine samples (50 mL) were extracted with diethyl ether (3 x 20 mL) and ethyl acetate (3 x 20 mL). The resulting extracts were evaporated to dryness at 30 °C, dissolved in 2 mL of 1:1 (v/v) methanol/water and membrane-filtered (0.22 µm pore size).23 Aliquots (30 µL) of the final solution were subjected to reverse phase chromatographic separation at 20 °C using a Nova-Pak C18 column. The photodiode array detector was set from 210 to 360 nm. Two mobile phases were used: A, water/acetic acid (98.2:1.8 v/v); B, water/acetonitrile/acetic acid (78:20:2 v/v/v). A gradient was applied at a flow rate of 1 mL min⁻¹ from 0 to 55 min and 1.2 mL min⁻¹ from 55 to 90 min as follows: 100-20% A from 0 to 55 min, 20-10% A from 55 to 57 min, 10-0% A from 57 to 90 min. Each major peak in the HPLC chromatograms was characterised by both the retention time and the absorption spectrum (from 210 to 360 nm). Procyanidin dimers B1 and B3 and quercetin glycosides, for which standards were unavailable, were assigned by retention time and spectral parameters according to Peña-Neira et al.24,25 Quantitative determinations were made using the external standard method with commercial standards. Calibration curves were produced by injecting standard solutions under the same conditions and range of concentrations as those used in the analysis of samples. Compounds for which no standards were available were quantified by using standard curves for (+)-catechin (procyanidin dimer, procyanidin B1 and procyanidin B3) and quercetin (quercetin-3-galactoside and quercetin-3-glucoside). All qualitative and quantitative analyses of phenolic composition (including extraction) were performed in triplicate.23

Statistical analysis

The two assays were evaluated independently. In the first assay, to study the effect of using inert gas in winemaking on the phenolic composition of wines from cv. Sauvignon blanc, the results were analysed by Student’s t test at a significance level of 95% (P < 0.05). In the second assay, to analyse the effect of modifying the phenolic potential of the must before fermentation, the different treatments were compared with the control using analysis of variance (ANOVA) and Tukey’s honest significant difference (HSD) test at a significance level of 95%. All data were analysed using Statgraphics Centurion Version XV (StatPoint Technologies, Inc., Warrenton, VA, USA).

RESULTS AND DISCUSSION

Assay 1. Effect of winemaking in presence (IG [+]) versus absence (IG [−]) of inert gas on phenolic composition of Sauvignon blanc wines

Table 1 shows the parameters of titratable acidity, pH and phenolic composition of the wine samples. These parameters were in accordance with data from white wines in previous studies.5,6,26 There was a slightly higher content of total phenols in the wines produced in the presence of inert gas. For colour, significant differences were observed, with higher values in wines elaborated in the presence of inert gas. With regard to treatment IG [−], the presence of oxygen could cause the loss of phenolic compounds, probably due to enzymes that degrade these compounds before fermentation.15,16,27,28 This result was corroborated in this study. Although this method is convenient to avoid browning and bitterness in the wine attributable to phenolic compounds, it is important to keep in mind that excessive oxidation in winemaking may also cause a loss of aromatic compounds, which could decrease the positive sensory quality of white wines.7,26,29

Figure 1 shows the monomeric, oligomeric and polymeric proanthocyanidin fractions. Winemaking in the presence of inert gas resulted in wines with a twofold higher concentration of the monomeric fraction of flavan-3-ols compared with winemaking in the absence of inert gas (IG [−]) 1.25 ± 0.11 mg L⁻¹, IG [+]) 2.66 ± 0.06 mg L⁻¹). Higher concentrations of monomers may increase bitterness, which is undesirable in white wines.30 On the contrary, wines elaborated in the presence of inert gas had a significantly higher concentration of polymers (IG [−]) 12.51 mg L⁻¹, IG [+]) 19.26 mg L⁻¹). Using winemaking conditions in the presence of oxygen, one would expect a higher concentration of polymerised flavanols owing to a higher presence of acetaldehyde, which could act as a bridge in flavanol polymerisation reactions.31 However, in this case, oxygen supply resulted in excessive degradation and oxidation of those compounds, thus decreasing their concentrations. In the absence of inert gas, decreases in the monomeric and polymeric fractions were 53 and 35% respectively, with no effect on the oligomeric proanthocyanidin fractions. Winemaking in the presence of inert gas resulted in wines with a twofold higher concentration of the monomeric fraction of flavan-3-ols compared with winemaking in the absence of inert gas (IG [−]) 1.25 ± 0.11 mg L⁻¹, IG [+]) 2.66 ± 0.06 mg L⁻¹). Higher concentrations of monomers may increase bitterness, which is undesirable in white wines.30 On the contrary, wines elaborated in the presence of inert gas had a significantly higher concentration of polymers (IG [−]) 12.51 mg L⁻¹, IG [+]) 19.26 mg L⁻¹). Using winemaking conditions in the presence of oxygen, one would expect a higher concentration of polymerised flavanols owing to a higher presence of acetaldehyde, which could act as a bridge in flavanol polymerisation reactions.31 However, in this case, oxygen supply resulted in excessive degradation and oxidation of those compounds, thus decreasing their concentrations. In the absence of inert gas, decreases in the monomeric and polymeric fractions were 53 and 35% respectively, with no effect on the oligomeric proanthocyanidin fractions. Winemaking in the presence of inert gas resulted in wines with a twofold higher concentration of the monomeric fraction of flavan-3-ols compared with winemaking in the absence of inert gas (IG [−]) 1.25 ± 0.11 mg L⁻¹, IG [+]) 2.66 ± 0.06 mg L⁻¹). Higher concentrations of monomers may increase bitterness, which is undesirable in white wines.30
concentrations and proportions of proanthocyanidins. This result is interesting from the organoleptic point of view, because the observed differences in the various fractions of flavan-3-ols of wines elaborated either in the presence or absence of CO2 could result in differences in mouthfeel properties that should be of interest to wineries.

Table 2 shows the content of the major low-molecular-weight phenols in Sauvignon blanc wines. The hydroxybenzoic acids quantified were gallic and protocatechuic acids. The hydroxycinnamic acids quantified were ferulic, caffeic, cis-caftaric, trans-caftaric, cis-p-Coumaric and trans-p-Coumaric acids. The flavanols quantified were (+)-catechin, (-)-epicatechin, procyanidin B3, procyanidin B1 and procyanidin dimer. The flavonols identified and quantified were quercetin-3-galactoside and quercetin-3-glucoside. All identified compounds were detected in all wines used in this study. Winemaking in the presence of inert gas was associated with slightly higher contents of gallic acid, protocatechuic acid, caffeic acid and cis-p-Coumaric acid. The content of protocatechuic acid was about twofold that observed in wines produced in the absence of inert gas. Regarding flavanols, winemaking in the presence of inert gas was associated with a slightly higher content of (+)-catechin. Quercetin-3-glucoside presented a slightly higher content in wines produced in the presence of inert gas. As previously seen regarding other parameters, pooled data in these wines showed a higher content of the monomeric fraction in wines from treatment IG [+] (Fig. 1) that could be supported by a greater level of monomers determined by HPLC (Table 2). Previous studies have shown a relationship between phenolic compounds such as flavanols and phenolic acids and the varietal aromas of the grape. Several compounds such as (+)-catechin and caffeic acid modify the perception of some varietal aromas in Sauvignon blanc, thus suppressing, accentuating or showing little effect on the perception of the aroma compounds. The higher concentration of polyphenols observed in treatment IG [+] may cause a change in the perception of aromatic compounds, although these results need to be evaluated for the prevailing conditions in Chile. The lower concentration of phenolic compounds observed in treatment IG [–] has also been reported by other authors. Oxidation may be important to reduce the initial content of polyphenols, thus preventing undesirable browning in white wines. Winemaking in the presence of inert gases (reductive condition) is used in a large number of wineries in Chile to adequately preserve the varietal aromas of the cultivar by preventing oxidation of those compounds, although the high concentration of easily oxidisable polyphenols could cause browning and loss of varietal aromas.

The absence of inert gases in winemaking may produce less aromatic and lower-quality wines, although the product may be more resistant to browning.

Assay 2. Effect of different prefermentative treatments on phenolic composition of Sauvignon blanc wines

Table 3 shows the parameters of titratable acidity, pH and phenolic composition of the wines produced after using different prefermentative treatments. Treatment T3 resulted in wines with a lower acidity than the control (T0), while treatments T1 and T3 resulted in wines with a pH value slightly higher than the control (T0). Although in this study the use of a lower dose of PVPP

<table>
<thead>
<tr>
<th>Compound (mg L⁻¹)</th>
<th>IG [–]</th>
<th>IG [+]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>0.65 ± 0.02b</td>
<td>0.77 ± 0.06a</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>0.16 ± 0.04b</td>
<td>0.32 ± 0.03a</td>
</tr>
<tr>
<td>Hydroxycinnamic acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>0.06 ± 0.02a</td>
<td>0.09 ± 0.01a</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>1.00 ± 0.13b</td>
<td>1.28 ± 0.11a</td>
</tr>
<tr>
<td>cis-Caftaric acid</td>
<td>2.21 ± 0.23a</td>
<td>2.59 ± 0.23a</td>
</tr>
<tr>
<td>trans-Caftaric acid</td>
<td>1.63 ± 0.02a</td>
<td>1.84 ± 0.29a</td>
</tr>
<tr>
<td>cis-p-Coumaric acid</td>
<td>0.40 ± 0.05b</td>
<td>0.56 ± 0.06a</td>
</tr>
<tr>
<td>trans-p-Coumaric acid</td>
<td>0.10 ± 0.01a</td>
<td>0.13 ± 0.02a</td>
</tr>
<tr>
<td>Flavonoid phenolics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercetin-3-galactoside</td>
<td>0.04 ± 0.01a</td>
<td>0.03 ± 0.00a</td>
</tr>
<tr>
<td>Quercetin-3-glucoside</td>
<td>0.07 ± 0.01b</td>
<td>0.12 ± 0.01a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation (n = 3). For each compound, different letters denote significant difference between samples (P < 0.05, Student’s t test).
produced a decrease in acidity and an increase in pH, in previous studies by other authors the use of PVPP in the production of white wines did not result in changes in the chemical properties of the wines. The highest contents of total phenols were observed in wines produced under treatments T1 and T3 compared with the control. Thus it is clear that the group treated with the lower dose of PVPP (T3) displayed the highest content of total phenols. These results also agree with results reported by others. Many authors have reported that the fining agent PVPP adsorbs phenolic compounds, thus causing binding and removal of the phenols. This process appears to occur in the control treatment using a higher dose of PVPP (80 g hL\(^{-1}\)) compared with that of treatment T3 (10 g hL\(^{-1}\)), because wines resulting from treatment T3 have a higher content of phenols. Regarding this last result, some Chilean commercial wineries using PVPP to reduce wine browning have also observed a reduction in the aromatic intensity of wines, which is a very important parameter in Sauvignon blanc wines. In that case, lower doses of PVPP used to preserve wine aromas were ineffective in removing phenolic compounds. The curve of accumulation of tannins in red grapes decreases from veraison to maturity, which could explain the lower content of total phenols in the control condition compared with treatment T1, where harvest took place 10 days earlier than the control. Overripening of grapes and a hot climate could cause degradation of a number of compounds such as acids, sugars and phenolics, which may also explain our results. This finding could be especially relevant in the case of grapes from warmer climatic conditions such as the Maipo Valley, a warm valley that specialises in producing high-quality red wines. With regard to colour, treatment T3 resulted in wines with a slightly higher colour than the control condition. The importance of fining treatments and an appropriate date of grape harvest has been demonstrated by previous results. Fining products such as PVPP and adequate grape maturity are important for decreasing the phenolic content that may cause problems in white wines, including excessive bitterness and astringency or browning reactions due to aeration.

The proportion of monomeric, oligomeric and polymeric fractions of proanthocyanidins with respect to the different prefermentative treatments is shown in Fig. 2. The content of monomeric flavan-3-ols shows that the early harvest treatment (T1) resulted in wines with a higher concentration of monomers, approximately four times higher than the control (T1 4.64 ± 0.33 mg L\(^{-1}\) versus T0 1.27 ± 0.06 mg L\(^{-1}\)). Treatment T2 also resulted in wines with a slightly higher content of monomers compared with the control. Wines elaborated from early harvest grapes contained more monomeric flavan-3-ols, which decreased with ripening. This result was corroborated by measuring the amount of monomeric flavan-3-ols in wines elaborated from grapes harvested approximately 10 days later (control). In this sense, ripening at commercial harvest (T0) was associated with a decrease in the concentration of monomeric flavan-3-ols, which could result in a decrease in bitterness of white wines. In the case of the oligomeric flavan-3-ols, wines produced after treatments T1 and T2 showed slightly higher contents (2.01 ± 0.20 and 2.80 ± 0.08 mg L\(^{-1}\) respectively) compared with the control (1.67 ± 0.00 mg L\(^{-1}\)). After treatment T3 the resulting wine contained a higher concentration (22.79 ± 0.05 mg L\(^{-1}\)) of polymeric flavan-3-ols whereas after treatment T1 the corresponding wine showed a slightly lower content of these flavan-3-ols compared with the control. After treatments T0 and T1 there was an increase in the content of polymeric flavan-3-ols and a decrease in the content of monomeric flavan-3-ols as a result of tannin polymerisation during ripening. By comparing the control treatment with the treatment with a lower dose of PVPP (T3), it was interesting to observe that treatment T3 resulted in wines with a higher content of polymeric flavan-3-ols. This result is consistent with the observation that PVPP can bind small phenols such as monomeric and oligomeric flavan-3-ols.

Table 4 shows the contents of the major low-molecular-weight phenols in Sauvignon blanc white wines made using different prefermentative treatments. From the family of non-flavonoid compounds, two hydroxybenzoic acids and six hydroxycinnamic acids were identified, whereas from the family of flavonoids we identified five flavanols and two flavonols. Only some of these compounds were quantified in all treatments. Thus gallic acid in wines produced using treatments T1, T2 and T3 displayed higher concentrations than in the control wine. Also, wines from the T3 group showed an approximately 17-fold higher content of monomers, approximately four times higher than the control. After treatments T0 and T1 there was an increase in the content of polymeric flavan-3-ols and a decrease in the content of monomeric flavan-3-ols as a result of tannin polymerisation during ripening. By comparing the control treatment with the treatment with a lower dose of PVPP (T3), it was interesting to observe that treatment T3 resulted in wines with a higher content of polymeric flavan-3-ols. This result is consistent with the observation that PVPP can bind small phenols such as monomeric and oligomeric flavan-3-ols.

Table 3. General analytical parameters in wines with different prefermentative treatments

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenols (mg GAE L(^{-1}))</td>
<td>152.52 ± 13.03</td>
<td>191.13 ± 4.44*</td>
<td>141.09 ± 16.90</td>
<td>252.39 ± 7.43*</td>
</tr>
<tr>
<td>Titratable acidity (g tartaric acid L(^{-1}))</td>
<td>7.20 ± 0.00</td>
<td>7.40 ± 0.31</td>
<td>7.40 ± 0.17</td>
<td>6.05 ± 0.09*</td>
</tr>
<tr>
<td>pH</td>
<td>3.17 ± 0.01</td>
<td>3.22 ± 0.01*</td>
<td>3.16 ± 0.01</td>
<td>3.38 ± 0.02*</td>
</tr>
<tr>
<td>Colour (a.u.)</td>
<td>0.045 ± 0.00</td>
<td>0.044 ± 0.00*</td>
<td>0.042 ± 0.00*</td>
<td>0.061 ± 0.00*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation (n = 3). For each parameter, plus signs (+) denote significant difference compared with T0 value (P < 0.05, Tukey’s HSD test). T0, control; T1, early harvest of grapes; T2, direct pressing of grapes; T3, low dose of PVPP; GAE, gallic acid equivalent; a.u., absorbance units.

Figure 2. Monomeric, oligomeric and polymeric proanthocyanidin fractions in wines with different prefermentative treatments: T0, control; T1, early harvest of grapes; T2, direct pressing of grapes; T3, low dose of PVPP. For each fraction, plus signs (+) denote significant difference compared with T0 value (P < 0.05, Tukey’s HSD test).
of protocatechuic acid than the control group. For ferulic acid, treatments T1, T2 and T3 resulted in wines with a slightly higher content than the control, with T3 being the treatment producing wines with the highest concentration (about ninefold higher than the control condition). Also, all experimental treatments resulted in wines with a higher concentration of cis-caftaric acid compared with the control, with treatment T1 being the one resulting in wines with the highest concentration (5.64 ± 0.18 mg L⁻¹). For p-coumaric acid, treatment T3 resulted in wines with the highest content (about 13-fold higher than the control). With regard to the flavanols, only (+)-catechin and (−)-epicatechin were identified in wines of all experimental treatments, with higher concentrations of both compounds compared with those produced after treatment T0. The higher contents of phenolic acids and flavanols found in the early harvest treatment group (T1) may be due to unripe grapes, whose higher content of smaller phenols such as flavanols and phenolic acids would make the wine more bitter.41,42 On the other hand, the higher content of hydroxycinnamic acids with little or no (+)-catechin present in the group subjected to direct pressing (without maceration) is in agreement with reports by other authors showing both a higher concentration of these compounds in free-run Sauvignon blanc juice and a minimal concentration of hydroxycinnamic acids when prolonged skin contact and pressure were used, as in the control treatment.26 In summary, we observed that the treatment with a lower dose of PVPP (T3) resulted in wines with the highest content of low-molecular-weight phenolic compounds. In a study on the browning capacity of white wines, gallic acid, caftaric acid, (+)-catechin and (−)-epicatechin were identified as the main compounds that react with oxygen and cause browning in wine, thus demonstrating that these compounds were readily oxidised.

(−)-Epicatechin was the major browning agent, in addition to caftaric acid. Both polyphenols participated in the production of quinones via the enzymatic activity of polyphenol oxidase in the presence of oxygen.43,44 In that case the lower doses of PVPP caused a lower precipitation of phenolic compounds, especially wine flavanols and hydroxycinnamic acids, which could be easily oxidised and adversely affect the final quality of white wine.

**CONCLUSIONS**

The use of inert gas and prefermentative treatments were able to modify the concentration and composition of phenolic compounds in wines. Data from two assays on the content of low-molecular-weight phenolic compounds showed differences in concentration among various treatments.

The data derived from the fractionation of proanthocyanidins by C18 Sep-Pak cartridges represent a significant chemical analytical finding in white wines made from cv. Sauvignon blanc. Our results show, for the first time to our knowledge, that various specific interventions in the white winemaking process affect the chemical profile of the resulting wines, especially the proanthocyanidin fraction, which should be taken into account by the wineries producing these wines. Further studies on white wines from different geographical origins and produced by other winemaking practices would be necessary to confirm these observations, which will help winemakers to maximise juice quality and recovery for premium wine production.

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