Vitis **58** (Special Issue), 59–67 (2019) DOI: 10.5073/vitis.2019.58.special-issue.59-67

Influence of two prefermentative treatments to reduce the ethanol content and pH of red wines obtained from overripe grapes

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Summary

This study researches treatments for reducing the ethanol content and pH of wine, by either adding or replacing a portion of overripe red grape juice with acidified water or with a white grape juice of low potential ethanol content previously treated with cationic exchange. All treatments resulted in wines with lower ethanol content; however, the treatments did not always correct wine acidity effectively and sometimes the wine composition was negatively affected because the other wine components were diluted. Specifically, both adding and substituting with acidified water caused an increase in wine pH and a general dilution of the other wine components, particularly when the water was added. In contrast, adding acidified must, unlike acidified water, significantly reduced wine pH and the dilution effect was lower, especially when a portion of the original must was replaced by a low sugar content white must treated by cationic exchange. Moreover, this practice is not unauthorized and seems not to affect, but rather improve, the sensory quality of the wine.

K e y w o r d s : climate change; ethanol reduction; pH reduction; red winemaking.

Introduction

In recent years the alcohol content and pH of most wines have gradually increased (Godden and Muhlack 2010) probably because winemakers are looking for grapes with high phenolic and/or aromatic maturity (Kontoudakis *et al*. 2010 and 2011a), and also because climate change is increasing this tendency (Jones *et al*. 2005, Mira de Orduña 2010). If the temperature during ripening is higher, the grape pulp matures faster, and the pH and sugar concentration become too high. The period between veraison and industrial maturity is therefore shorter, which leads to an earlier harvest date. This makes it more difficult to determine the appropriate aromatic and phenolic maturity with precision, and frequently leads to obtain unbalanced wines (Zamora 2014). The Australian Wine Research Institute (AWRI) reported an increase in the mean alcohol level from 12.4 % to 14.4 % for red wines and from 12.2 % to 13.2 % for white wines between 1984 and 2008 (Godden and Muhlack 2010). In another example, the alcohol level of Alsace wines increased from 9 % to 12 % between 1970 and 2005 (Duchêne and Schneider 2005). This trend has also been observed in many other wine-producing countries (Schultz and Jones 2010, Van Leeuwen and Darriet 2016).

An excess of alcohol may cause several drawbacks that are associated with slowdowns of alcoholic (Bisson 1999) and malolactic (Lonvaud-Funel *et al*. 1988) fermentations, increases in volatile acidity (Zamora 2009), and alterations in the wine's sensory qualities (Fischer and Noble 2004, Le Berre *et al*. 2007). Moreover, excessive alcohol consumption has negative effects on human health (Grønbæk 2009) and therefore a high ethanol content on the label of a wine bottle can discourage potential consumers who prefer to be responsible and drink a light wine (Saliba *et al*. 2013). Evidently, the wine industry is very concerned with these issues and is therefore interested in producing wines with a moderate alcohol level.

High pH values in wines can also cause certain problems (Patterson 2009). A correct pH is needed for a good sensory balance and correct conservation of the wines. When pH is higher than usual, wines lack freshness (Nagel *et al*. 1982) and usually age faster than desired (Sims and Morris 1984, Kontoudakis *et al*. 2011c). Moreover, the higher the pH the lower the antimicrobial effect of sulfur dioxide (Usseglio-Tomasset 1992), which is probably why the problems caused by volatile phenols and biogenic amines have become more common in recent years (Landete *et al*. 2005, Romano *et al*. 2007). Moreover, the color of red wine is drastically affected by pH since the percentage of the red form of anthocyanins, the flavylium cation, decreases greatly when pH increases (Kontoudakis *et al*. 2011c).

In light of this problem, winemakers can either harvest their grapes when the potential alcohol value and pH are appropriate or they can harvest them when complete phenolic and aromatic maturity has been reached. In the first case, the grapes would not have reached complete maturity. In the second case, the ethanol content and pH of the grapes would probably be excessive. Neither of these options is conducive to obtain high quality wines and winemakers

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are therefore concerned about it. Some ways have been proposed for reducing the increased ethanol content and pH and thus counteracting the impact of climate change on wine production. These include introducing new cultivars and modifying culture techniques (Schultz 2000), harvesting the grapes at an early ripening stage (Schmidtke *et al*. 2012), adding water and mineral acids to the grape juice before fermentation begins (Harbertson *et al*. 2009), using yeasts (*Saccharomyces* and non-*Saccharomyces*) with a low yield in the sugar-ethanol transformation ratio (Ciani and Ferraro 1996, Tilloy *et al*. 2014) and even using glucose oxidase (EC 1.1.3.4) (Pickering *et al*. 1998).

Despite the different possibilities, currently the most commonly used methods for reducing alcohol content and pH in wines are physical methods (Schmidtke *et al*. 2012). More specifically, to reduce ethanol content, the spinning cone column (Belisario-Sánchez *et al*. 2009) and reverse osmosis (Gil *et al*. 2013) are used, and to reduce pH, the cationic exchange (Lasanta *et al*. 2013) or electrodialysis (Walker *et al*. 2004) are used.

Using unripe grapes harvested during cluster thinning (Kontoudakis *et al*. 2011b) has also been proposed to overcome the problems inherent to overripe red grapes. Briefly, the grape juice of these unripe grapes is fermented and the resulting wine is treated with charcoal and bentonite to eliminate aromas and phenolic compounds. This green wine, which has a very low ethanol content and pH, is used to substitute some of the grape juice of the overripe 'Cabernet Sauvignon', 'Merlot' and 'Bobal' grapes just after destemming and crushing. This procedure has been shown to be very effective for simultaneously reducing ethanol content and pH, it is easy to apply and does not require any additional equipment. Moreover, this procedure improves the color intensity of red wines because it decreases pH very effectively and consequently the proportion of the red form of the anthocyanins, the flavylium cation, increases. Recently Piccardo *et al*. (2019) evaluated the substitution of immature grape must for grape must overripe prior to alcoholic fermentation in Tannat and Pinot noir overripe grapes, reaching similar results

Also recently, Schelezki *et al*. (2018a and b) compared the procedure described by Kontoudakis *et al*. (2011) and a procedure that substitutes a portion of the grape juice with water. Both treatments effectively reduced the ethanol content; however, surprisingly, this article concluded that substitution with water is more suitable than substitution with green wine because the changes in the volatile composition and sensory qualities of the final wine are less pronounced. However, the OIV and most wine producing countries have not authorized adding water, and it can be analytically detected (Thomas *et al*. 2013).

Given the interest of these kinds of treatments in the current context of climate change, this work aimed to study the effect of using acidified water or a low-sugar white grape juice, previously treated with cation exchange resins, as strategies for reducing the ethanol content and pH of overripe red grapes. The acidified water and the treated grape juice were either added directly or they replaced a portion of red grape juice.

Material and Methods

Chemicals: Methanol, acetonitrile, formic acid, and acetic acid were of HPLC grade (> 99 %) and purchased from Panreac (Barcelona, Spain). Acetaldehyde (> 99.5 %), phloroglucinol (> 99 %), ascorbic acid (> 99 %), sodium acetate (> 99 %), and ammonium formate (> 99 %) were purchased from Sigma-Aldrich (Madrid, Spain). Absolute ethanol and hydrochloric acid (37 %) were purchased from Panreac. Malvidin-3-O-glucoside chloride (≥ 95 %), proanthocyanidin dimer B2 (≥ 90 %), (+)-catechin (≥ 99 %), (−)-epicatechin (≥ 99 %), (−)-epigallocatechin (≥ 98 %), and (−)-epicatechin- 3-O-gallate (≥ 97.5 %) were purchased from Extrasynthese (Genay, France). A pullulan calibration kit Shodex P-82 (P-5, MW = 5.9 kDa; P-10, MW = 11.8 kDa; P-20, MW = 22.8 kDa; P-50, MW = 47.5 kDa; P-100, MW = 112 kDa; P-200, MW = 212 kDa; P-400, MW = 404 kDa; P-800, MW = 788 kDa) was obtained from Waters (Barcelona, Spain), while a pullulan 1.3 kDa and four dextrans Bio-Chemika (12, 25, 50, and 80 kDa) were obtained from Fluka (St. Louis, MO, USA). A Winescan TM Autosampler 79000 infrared analyzer (Foss, USA) and the Foss Integrator software version 154 (Foss, Denmark) were used to determine the alcohol content, total acidity and pH of the wines. The polysaccharides used as external standards for quantification were pectins from citrus fruit (≥ 90 %) and dextrans synthesized by Leuconostoc mesenteroides (≥ 99.9 %) purchased from Sigma-Aldrich (St. Louis, MO, USA).

Equipment: The HPLC analyses were performed using an Agilent 1200 series liquid chromatograph equipped with a G1362A refractive index detector (RID), a G1315D diode array detector (DAD), a G1311A quaternary pump, a G1316A column oven, and a G1329A autosampler (Agilent Technologies, Santa Clara, CA, USA). All the spectrophotometric measurements were performed using a Helios Alpha UV−vis spectrophotometer (Thermo Fisher Scientific Inc., Waltman, MA, USA).

Grapes and wines: The study was carried out using grapes from 'Tempranillo Tinto' (*Vitis* International Variety Catalogue number *V*IVC 12350) and 'Merlot' Noir (*Vitis* International Variety Catalogue number *V*IVC 7657) cultivars (*Vitis vinifera* L.) from the 2017 vintage. The grapes of both cultivars were manually picked in a commercial vineyard located in Els Guiamets [AOC Montsant; 41° 06' 20.92" (N) and 0° 45' 42.59" (E)] and were harvested at two different ripening stages. The first harvest was carried out when the potential degree of alcohol was between 13.0 and 14.0 %. The second harvest was carried out when the grapes reached optimum phenolic maturity. Specifically, the grapes of the first harvest were picked at 22.8 °Brix ('Merlot') and 23.3 °Brix ('Tempranillo'), whereas the grapes of the second harvest were picked at 25.1 °Brix ('Merlot') and 24.9 °Brix ('Tempranillo').

Cationic exchange treatment of the white grape juice: Just after settling, a white grape juice from 'Macabeo' (*Vitis* International Variety Catalogue number *V*IVC 13127) was treated with an industrial cationic exchange column (FreeK+, Agrovin, Alcazar de San Juan, Spain) to reduce its pH as much as possible. The initial cha-

racteristics of this grape juice were 16.6 °Brix, a titratable acidity of 5.7 $g \cdot L^{-1}$ (expressed as tartaric acid), and a pH of 3.21. After the treatment no changes were observed in the Brix degree but the titratable acidity increased to 8.5 $g \cdot L^{-1}$ and the pH decreased to 2.40.

Winemaking experimental conditions: Fig. 1 illustrates the outline of the experimental conditions. 'Merlot' and 'Tempranillo' grapes were carefully destemmed (Delta, Bucher-Vaslin, Chalonnes-sur-Loire, France) and the intact grapes were randomly distributed in batches of 6 kg to avoid differences due to the heterogeneity of the grapes as much as possible. The grapes of each batch were then crushed with a manual crusher, sulphited (100 mg of $K_2S_2O_5 \cdot kg^{-1}$) and placed in 8-L and 6-L tanks equipped with a submerged cap system (Sampaio *et al*. 2007). The grapes of the first harvest (normal control wine) and a quantity of grapes from the second harvest (overripe control wine) were vinified without any additional treatment. The rest of the grapes from the second harvest were used to study the four different treatments for mitigating the effects of overripening. The treatments applied to these grapes were: addition of acidified water (6 g of tartaric acid $\cdot L^{-1}$), substitution with acidified water (6 g of tartaric acid $\cdot L^{-1}$), addition of acidified must, and substitution with acidified must. The proportion of addition/substitution of acidified water or acidified must was calculated to reduce the ethanol content of the wines by around 1.0 degree. Specifically, 333 mL of acidified water was added to the 'Merlot' grapes, and 335 mL was added to the 'Tempranillo' grapes; 312 mL of acidified water was used to substitute the original 'Merlot' grape must and 314 mL of acidified water was used to substitute the 'Tempranillo' grape must; 980 mL of acidified grape must was added to the 'Merlot' grapes and 1000 mL to the 'Tempranillo' grapes. Finally, 814 mL of 'Merlot' grape must and 828 mL of 'Tempranillo' grape must were substituted with acidified grape must. We made these calculations with the aim of decreasing the alcohol content by 1.0 degree taking into account the potential ethanol content of both the original grapes and the acidified grape must and also that 80 % of the grapes' weight is liquid.

All tanks were immediately inoculated with 200 $mg \cdot kg^{-1}$ yeast (EC1118; Lallemand Inc., Montreal, Canada) and maintained at a room temperature of 25 ± 1 °C until racking. Density and temperature were measured daily to monitor the alcoholic fermentation and two manual punch-downs of the cap were made at around 1060 and 1020 density units to improve color and phenolic extraction. After 14 d of maceration, the wines were racked. Once alcoholic fermentation had completely finished, wines were sulphited (100 mg of $K_2S_2O_5 \cdot L^{-1}$) and kept at 4 °C for three months to allow the tartaric salts to stabilize. Therefore, malolactic fermentation was inhibited to avoid any variations resulting from it. The wines were then bottled and stored in a dark cellar at 15 °C until analysis. All these microvinifications were performed in triplicate.

Standard grape juice and wine analysis: We used the analytical methods recommended by OIV (2012) to determine the sugar concentration, pH and titratable acidity of the grape juices as well as the ethanol content, titratable acidity and pH of the wines. The total anthocyanin content of the wines was estimated with the spectrophotometric method proposed by Niketic-Aleksic and Hrazdina (1972). The total phenolic index (TPI) was estimated by measuring the absorbance at 280 nm (Ribéreau-Gayon *et al.* 2006).

HPLC anthocyanin analysis: Reversed-phase HPLC analyses of the anthocyanins were carried out by injecting 40 µL of wine into an Agilent 1200 series liquid chromatograph (HPLC-DAD) and using an Agilent Zorbax Eclipse XDBC18, 4.6 × 250 mm, 5 µm column

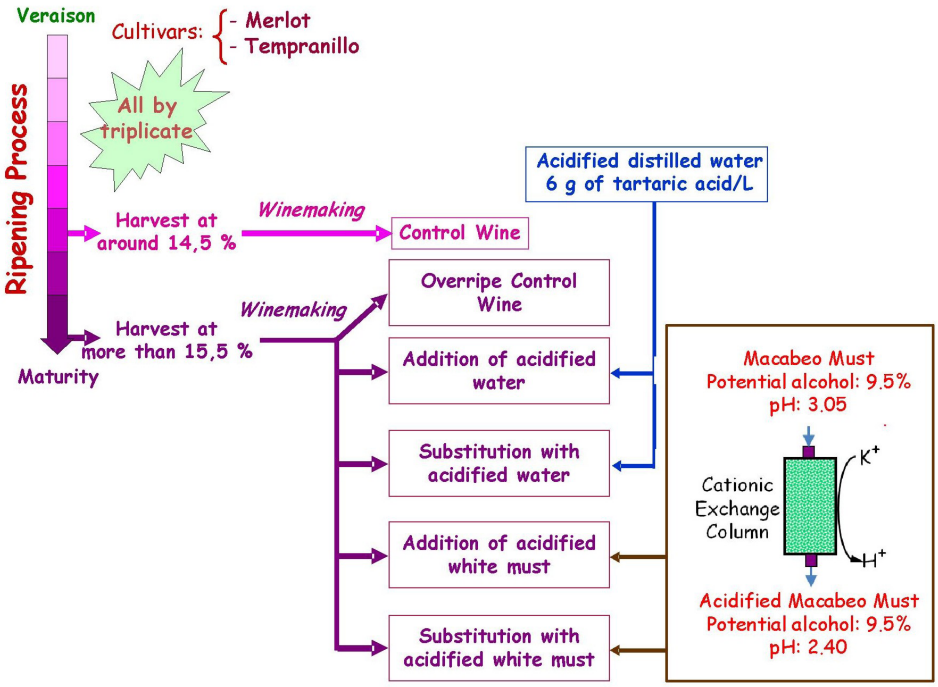

Fig. 1: Experimental design.

(Agilent Technologies). The solvents used were 10 % aqueous formic acid (solvent A) and a mixture of 45 % methanol, 45 % water, and 10 % formic acid (solvent B) in accordance with the method described by Gil *et al*. (2012). Chromatograms were recorded at 530 nm, and anthocyanin standard curves were made using malvidin-3-O-glucoside chloride. Compounds were identified considering the relative retention times between the compounds and by recording their UV spectra with a diode array detector and comparing these with the UV spectra. We quantified the five anthocyanidin-3-monoglucosides of wine (delphinidin, cyanidin, peonidin, petunidin, and malvidin) and their respective acetylated and p-coumaroylated anthocyanins.

HPLC proanthocyanidin analysis: The proanthocyanidins of the wines were extracted and analyzed by acid depolymerization in the presence of an excess of phloroglucinol (Pastor del Rio and Kennedy 2006); the products of the reaction were separated by RP-HPLC-DAD (Kennedy and Jones 2001). Proanthocyanidins were analyzed with an Agilent 1200 Series HPLC equipped with a G1362A refractive index detector (RID), a G1315D DAD, a G1311A quaternary pump, a G1316A column oven and a G1329A autosampler (Agilent Technologies, Santa Clara, CA, USA). The chromatographic system was managed by an Agilent Chem Station (version B.01.03) data processing station.

HPLC polysaccharides analysis: Samples were processed using the methodology described by Ayestarán *et al*. (2004). Briefly, 10 mL of sample was concentrated to a final volume of 2 mL using a vacuum evaporator (Univap 148 100ECH; Progen Scientific, London, UK). The total soluble polysaccharides were precipitated by adding 10 mL cold acidified ethanol (hydrochloric acid 0.3 mol·L^{-1} in absolute ethanol) and kept for 24 h at 4 °C. The samples were then centrifuged (10 000 × g for 15min) and the supernatants discarded. Finally, the precipitates were dissolved in 1mL ultra-pure water, frozen to -20 °C and freeze-dried. The polysaccharides were analyzed as described in Esteruelas *et al*. (2015) by high-resolution size-exclusion chromatography (HRSEC) using a refraction index detector (RID) and an HPLC Agilent 1200 Series system (Agilent Technologies Inc., Santa Clara, CA, USA).

Sensory analysis: All sensory analyses were performed in the tasting room of the Faculty of Enology in Tarragona (University Rovira i Virgili), which was designed in accordance with UNE87004.197 (Aenor 2010). Official ISO tasting glasses (ISO-3591 1997) were used for the tasting. Each sample consisted of 30 mL of wine at room temperature (20 °C) covered with a clear plastic petri dish to minimize the escape of volatile components. They were randomly coded with three-digit numbers.

A panel of ten trained wine tasters tasted all the samples. The panel was made up of 4 females and 6 males aged between 26 and 58. Two tasting sessions were held, one for 'Merlot' wines and the other for 'Tempranillo' wines. For each sample, tasters were required to evaluate the intensity of seven sensory attributes on a scale of 1 to 10 (1 = "slight intensity", 10 = "maximum intensity"): fruit, vegetal, spicy, acidity, astringency, bitterness and structure. The intensity level of each descriptor was then expressed as the mean value of all the tasters. A sensory training session was held beforehand so that the panelists could agree on the criteria for each of the different sensory attributes. Samples were served randomly to avoid the influence of the tasting order. Tasters were also required to classify the different wines in order of preference (from 1, the one they considered the best, to 6 the one they considered the worst).

Statistics: All analytical data are expressed as the arithmetic mean ± standard deviation of the samples from three replicates. The multifactor analysis of variance (ANOVA) was carried out using XLSTAT software in order to compare the different samples. All sensory data are expressed as the arithmetic mean of the scores of the 10 panelists.

Results and Discussion

Tab. 1 shows the general parameters of the different wines. As expected the sugar content of the must and the ethanol content and pH of the overripe control wines were significantly higher and the titratable acidity significantly lower than in normal control wines. This confirms that the grapes of the two cultivars really ripened between the two harvest dates. The total phenolic indexes of the overripe control 'Merlot' and 'Tempranillo' wines were also significantly higher than those of their corresponding normal controls, which suggest that the total phenolic content and its extractability increased between the two harvest dates. Similar results have been previously reported (Gil *et al.* 2012, Pascual *et al.* 2016). No significant changes were detected in the total anthocyanin concentration measured spectrophotometrically between the two maturation stages; however, a tendency to decrease was observed in both cultivars. This trend is probably because the grapes have already reached the maximal anthocyanin concentration at normal maturity. The HPLC analysis of the anthocyanins (Tab. 2) confirms these data because the total anthocyanin concentration tended to decrease in the overripe control wines, although these differences were only significant in the 'Tempranillo' wine. In general, the proportion of the different anthocyanins was very similar between both control wines (normal grapes and over-ripe grapes) although small but significant differences were observed in two anthocyanins. Specifically paeonidin-3-*O*-glucoside was significantly higher and acetylated malvidin-3-*O*-glucoside significantly lower in the over-ripe control wine. Regarding the different treatments, no large differences were found with respect to the control. It was only observed that acetylated delphinidin-3-*O*-glucoside decreased significantly in the treatments performed with must and that coumarylated malvidin-3-*O*-glucoside increased significantly in all the treatments. In any case, these differences can be considered as not very important in relation to the global composition in anthocyanins of these wines. TPI and anthocyanins have been previously reported to behave similarly throughout the grape ripening process (Pérez-Magarino and Gonzales-San Jose 2004, Pasqual *et al*. 2016).

Tab. 3 shows the total proanthocyanidin concentration of the different wines as well as their mean degree of polymerization (mDP) and the percentages of prodelphi-

Table 1

General parameters of the different wines

Parameter	Cultivar	Normal grapes		Overripe grapes			
		Control wine	Control wine	Addition of acidified water	Substitution of acidified water	Addition of acidified must	Substitution of acidified must
Sugar content of the must ($g \cdot L^{-1}$)	M	241 ± 3.4 A	263 ± 2.0 B	243 ± 2.9 A	248 ± 5.1 A	242 ± 3.4 A	247 ± 2.9 A
	T	245 ± 4.3 A	257 ± 1.7 B	243 ± 5.1 A	251 ± 3.4 A	241 ± 5.1 A	244 ± 3.4 A
Ethanol content (%)	M	14.2 ± 0.2 A	15.4 ± 0.1 B	14.4 ± 0.2 A	14.7 ± 0.3 A	14.3 ± 0.2 A	14.5 ± 0.2 A
	T	14.4 ± 0.5 A	15.3 ± 0.1 B	14.2 ± 0.3 A	14.7 ± 0.2 A	14.3 ± 0.3 A	14.5 ± 0.2 A
Titratable acidity ($g \cdot L^{-1}$)	M	4.77 ± 0.06 E	4.30 ± 0.10 D	2.73 ± 0.12 A	2.77 ± 0.06 A	3.13 ± 0.06 B	3.32 ± 0.03 C
	T	3.90 ± 0.10 E	3.67 ± 0.09 D	2.87 ± 0.15 A	2.77 ± 0.06 A	3.10 ± 0.10 B	3.32 ± 0.03 C
pH	M	3.45 ± 0.02 B	3.56 ± 0.02 C	3.83 ± 0.06 D	3.87 ± 0.12 D	3.30 ± 0.01 A	3.32 ± 0.01 A
	T	3.76 ± 0.03 A	3.94 ± 0.01 C	3.98 ± 0.02 D	3.93 ± 0.05 CD	3.81 ± 0.01 B	3.81 ± 0.03 B
TPI	M	67.6 ± 1.4 B	75.4 ± 1.8 C	63.3 ± 1.1 A	66.1 ± 2.1 AB	67.2 ± 2.8 A	78.6 ± 1.5 B
	T	67.3 ± 0.5 A	75.0 ± 1.9 C	70.1 ± 0.8 B	73.9 ± 1.0 CD	70.3 ± 1.5 B	74.1 ± 2.0 C
Anthocyanins ($mg \cdot L^{-1}$)	M	1096 ± 60 BC	1040 ± 14 B	949 ± 57 A	1029 ± 32 AB	1042 ± 67 B	1152 ± 27 C
	T	897 ± 5 C	845 ± 6 B	825 ± 13 A	840 ± 15 AB	815 ± 13 A	842 ± 10 B
Color intensity	M	15.9 ± 0.4 B	17.0 ± 0.1 C	15.2 ± 0.1 A	17.4 ± 0.1 C	16.9 ± 0.4 C	20.3 ± 0.2 D
	T	8.6 ± 0.1 B	8.1 ± 0.2 A	8.1 ± 0.1 A	8.3 ± 0.1 A	8.2 ± 0.2 A	8.7 ± 0.2 B

All data are expressed as the average values of 3 replicates ± standard deviation. M: 'Merlot'; T: 'Tempranillo'. Different letters indicate the existence of statistical differences ($p < 0.05$). TPI corresponds to the wine total phenolic index.

Table 2

Anthocyanin concentration of the different wines

Anthocyanins ($mg \cdot L^{-1}$)		Cultivar	Normal grapes		Overripe grapes			
			Control wine	Control wine	Addition of acidified water	Substitution of acidified water	Addition of acidified must	Substitution of acidified must
Total anthocyanins		M	846 ± 55 A	800 ± 23 A	770 ± 42 A	801 ± 36 A	811 ± 14 A	994 ± 14 B
		T	646 ± 65 B	503 ± 27 A	505 ± 36 A	488 ± 8 A	485 ± 14 A	522 ± 59 AB
Non-acylated anthocyanins (%)	Dp-3-*O*-G	M	10.3 ± 0.3 A	10.7 ± 0.2 A	10.4 ± 0.2 A	10.6 ± 0.3 A	10.9 ± 0.3 A	10.9 ± 0.3 A
		T	14.6 ± 0.6 A	13.6 ± 0.7 A	13.4 ± 0.7 A	13.5 ± 0.7 A	14.6 ± 0.3 A	13.6 ± 0.8 A
	Cy-3-*O*-G	M	1.6 ± 0.1 A	1.5 ± 0.1 A	1.5 ± 0.1 A	1.5 ± 0.1 A	1.5 ± 0.1 A	1.6 ± 0.1 A
		T	1.0 ± 0.1 A	1.0 ± 0.0 A	1.0 ± 0.1 A	1.1 ± 0.0 A	1.1 ± 0.1 A	1.0 ± 0.1 A
	Pt-3-*O*-G	M	10.5 ± 0.1 A	10.2 ± 0.1 A	10.0 ± 0.2 A	9.9 ± 0.3 A	10.3 ± 0.2 A	10.4 ± 0.3 A
		T	14.5 ± 0.3 A	13.7 ± 0.7 A	13.5 ± 0.4 A	14.1 ± 0.3 A	13.7 ± 0.3 A	13.7 ± 0.5 A
	Pn-3-*O*-G	M	6.8 ± 0.4 A	8.7 ± 0.2 B	8.9 ± 0.1 B	8.5 ± 0.1 B	8.3 ± 0.1 B	8.2 ± 0.2 B
		T	4.5 ± 0.2 A	4.9 ± 0.4 A	4.7 ± 0.1 A	4.9 ± 0.2 A	4.8 ± 0.3 A	4.7 ± 0.3 A
	Mv-3-*O*-G	M	46.2 ± 2.0 A	45.7 ± 1.8 A	46.0 ± 2.3 A	44.6 ± 0.8 A	42.8 ± 3.6 A	41.9 ± 3.5 A
		T	53.2 ± 2.1 A	56.4 ± 1.2 A	55.3 ± 1.3 A	56.3 ± 0.6 A	54.7 ± 2.1 A	54.6 ± 1.6 A
	Total non-acylated anthocyanins	M	75.5 ± 2.7 A	76.8 ± 1.4 A	76.6 ± 1.5 A	75.1 ± 1.6 A	73.9 ± 2.8 A	73.1 ± 3.2 A
		T	87.8 ± 3.2 A	89.7 ± 3.0 A	87.9 ± 2.2 A	90.0 ± 1.8 A	88.8 ± 1.9 A	87.7 ± 3.2 A
Acetylated anthocyanins (%)	Dp-3-*O*-Ac	M	3.7 ± 0.6 B	3.8 ± 0.7 B	2.8 ± 0.6 AB	2.8 ± 0.5 AB	1.6 ± 0.4 A	1.8 ± 0.4 A
		T	2.2 ± 1.5 A	0.7 ± 0.5 A	1.1 ± 0.5 A	0.7 ± 0.3 A	0.7 ± 0.1 A	1.0 ± 0.7 A
	Cy-3-*O*-Ac	M	2.0 ± 1.3 A	2.7 ± 1.1 A	1.5 ± 0.3 A	2.0 ± 0.3 A	1.3 ± 0.5 A	1.6 ± 0.3 A
		T	1.6 ± 0.8 A	0.6 ± 0.2 A	0.7 ± 0.2 A	0.4 ± 0.4 A	0.6 ± 0.2 A	0.7 ± 0.3 A
	Pt-3-*O*-Ac	M	4.0 ± 2.9 A	2.3 ± 0.8 A	2.1 ± 0.1 A	2.6 ± 0.3 A	2.2 ± 0.4 A	2.4 ± 0.2 A
		T	0.8 ± 0.1 A	0.8 ± 0.3 A	0.9 ± 0.3 A	0.6 ± 0.1 A	1.0 ± 0.1 A	1.5 ± 0.9 A
	Pn-3-*O*-Ac	M	2.2 ± 1.1 A	2.1 ± 0.5 A	1.6 ± 0.3 A	1.9 ± 0.4 A	2.0 ± 0.3 A	2.1 ± 0.1 A
		T	0.5 ± 0.2 A	0.5 ± 0.1 A	0.8 ± 0.4 A	0.5 ± 0.1 A	0.5 ± 0.1 A	0.6 ± 0.1 A
	Mv-3-*O*-Ac	M	10.3 ± 0.2 B	8.7 ± 0.3 A	8.3 ± 0.1 A	8.5 ± 0.1 A	8.4 ± 0.1 A	8.5 ± 0.1 A
		T	4.1 ± 0.3 A	4.5 ± 0.2 A	5.7 ± 1.7 A	4.5 ± 0.2 A	4.8 ± 0.2 A	4.6 ± 0.1 A
	Total acetylated anthocyanins	M	22.1 ± 5.8 A	19.6 ± 3.3 A	16.5 ± 1.6 A	17.8 ± 1.3 A	15.6 ± 1.7 A	16.4 ± 1.4 A
		T	9.1 ± 3.8 A	7.0 ± 1.1 A	9.2 ± 3.1 A	6.7 ± 1.1 A	7.6 ± 1.3 A	8.4 ± 2.0 A
Comarylated anthocyanins (%)	Dp-3-*O*-Cou	M	0.4 ± 0.3 A	0.3 ± 0.1 A	0.3 ± 0.1 A	0.4 ± 0.1 A	0.5 ± 0.1 A	0.4 ± 0.1 A
		T	0.3 ± 0.1 A	0.4 ± 0.1 A	0.6 ± 0.2 A	0.4 ± 0.1 A	0.4 ± 0.1 A	0.5 ± 0.1 A
	Cy-3-*O*-Cou	M	0.1 ± 0.1 A	0.3 ± 0.3 A	0.4 ± 0.1 A	0.5 ± 0.2 A	0.5 ± 0.2 A	0.6 ± 0.3 A
		T	0.1 ± 0.1 A	0.1 ± 0.2 A	0.1 ± 0.1 A	0.1 ± 0.1 A	0.1 ± 0.1 A	0.5 ± 0.3 A
	Pt-3-*O*-Cou	M	0.4 ± 0.1 A	0.5 ± 0.2 A	0.7 ± 0.4 A	0.5 ± 0.2 A	1.0 ± 0.4 A	0.6 ± 0.2 A
		T	0.4 ± 0.1 A	0.3 ± 0.3 A	0.3 ± 0.3 A	0.5 ± 0.1 A	0.5 ± 0.1 A	0.5 ± 0.1 A
	Pn-3-*O*-Cou	M	0.3 ± 0.3 A	0.9 ± 0.4 A	1.3 ± 0.3 A	1.1 ± 0.0 A	0.7 ± 0.6 A	1.4 ± 0.1 A
		T	0.5 ± 0.1 A	0.6 ± 0.1 A	0.2 ± 0.3 A	0.6 ± 0.1 A	0.6 ± 0.1 A	0.6 ± 0.1 A
	Mv-3-*O*-Cou	M	1.3 ± 0.4 A	1.6 ± 1.3 A	4.1 ± 1.7 B	4.6 ± 0.4 B	7.8 ± 0.3 C	7.5 ± 0.1 C
		T	1.7 ± 0.1 A	1.8 ± 0.3 A	1.8 ± 0.1 A	1.8 ± 0.1 A	1.9 ± 0.3 A	1.8 ± 0.2 A
	Total coumarylated anthocyanins	M	2.4 ± 0.6 A	3.6 ± 2.1 A	6.9 ± 1.6 B	7.1 ± 0.6 B	10.5 ± 1.3 A	10.5 ± 0.3 C
		T	3.1 ± 0.4 A	3.3 ± 0.7 A	2.9 ± 0.9 A	3.3 ± 0.1 A	3.6 ± 0.3 A	3.9 ± 0.3 A

All data are expressed as the average values of 3 replicates ± standard deviation. M: 'Merlot'; T: 'Tempranillo'. Dp: delphinidin; Cy: cyanidin; Pt: petunidin; Pn: paeonidin; Mv: malvidin; 3-*O*-G: 3-ortho-monoglucoside; Ac: acetylated; Cou: Coumarylated. Different letters indicate the existence of statistical differences ($p < 0.05$).

Table 3

Proanthocyanin concentration and related parameters of the different wines

Parameter	Cultivar	Normal grapes		Overripe grapes			
		Control wine	Control wine	Addition of acidified water	Substitution of acidified water	Addition of acidified must	Substitution of acidified must
Proanthocyanidins	M	1027 ± 74 D	773 ± 70 B	757 ± 29 B	619 ± 22 A	865 ± 18 C	1142 ± 41 D
	T	1248 ± 43 C	1086 ± 43 B	1055 ± 70 AB	945 ± 38 A	1324 ± 84 C	1253 ± 40 C
Mean degree of polymerization (mDP)	M	4.5 ± 0.3 A	4.7 ± 0.3 A	4.7 ± 0.3 A	4.3 ± 0.3 A	4.7 ± 0.2 A	4.9 ± 0.3 A
	T	7.5 ± 0.5 A	6.9 ± 0.4 A	6.5 ± 0.5 A	6.8 ± 0.7 A	6.7 ± 0.8 A	6.6 ± 0.3 A
% Prodelphinidin	M	19.2 ± 0.4 A	21.3 ± 0.4 CB	21.9 ± 0.9 CB	19.5 ± 0.1 A	20.3 ± 0.6 B	22.0 ± 0.7 C
	T	19.2 ± 0.4 A	18.5 ± 0.4 A	19.4 ± 1.3 AB	18.4 ± 0.6 A	20.8 ± 0.9 BC	21.7 ± 0.6 C
% Galloylation	M	10.3 ± 0.6 A	9.6 ± 0.5 A	9.8 ± 0.5 A	10.3 ± 0.6 A	9.2 ± 0.4 A	9.3 ± 0.6 A
	T	5.7 ± 0.4 A	6.1 ± 0.2 A	6.5 ± 0.4 A	6.3 ± 0.6 A	5.8 ± 0.4 A	6.1 ± 0.3 A

All data are expressed as the average values of 3 replicates ± standard deviation. M: 'Merlot'; T: 'Tempranillo'. Different letters indicate the existence of statistical differences ($p < 0.05$).

nidins and galloylation. The results indicate that the total proanthocyanidin concentration was significantly lower in the overripe control wines of the two cultivars than in the corresponding normal control wines. This decrease in the total proanthocyanidin concentration contrasts with TPI, which followed the opposite trend. A possible explanation for these results could be that acid catalysis with phloroglucinol is not completely efficient (Kontoudakis *et al*. 2011c) and thus the proanthocyanidin concentration of overripe grape wines may have been underestimated. No significant differences were found in the mDP or the percentage of galloylation in the proanthocyanidins of the overripe control wine and the normal control wine. In contrast a significant increase in the percentage of prodelphinidins was detected in the overripe control 'Merlot' wine. This indicates that skin proanthocyanidins make a higher contribution when the grapes are riper because prodelphinidins are only present in skin tannins (Souquet *et al*. 1996, Gil *et al*. 2012). This increase was not detected in the 'Tempranillo' wine.

The color intensity of the overripe control wine was significantly higher than in the corresponding normal control wine for 'Merlot' grapes but it was significantly less intense for 'Tempranillo' grapes. This different behavior can be because color not only depends on the wine anthocyanin composition. Other factors such pH and the presence of copigments can exert a very important effect on wine color intensity and hue (Kontoudakis *et al.* 2011c). In both cultivars, pH was significantly higher in the wines obtained from over-ripe grapes. These data can explain why the color intensity decrease in the wine obtained from over-ripe grapes in the case of 'Tempranillo' but do not explain why in the case of 'Merlot' happens the opposite. In this case it can be hypothesized that the over-ripening of the 'Merlot' grapes could have favored the release of more copigments. Finally, the total polysaccharide concentration (Tab. 4) tended to increase in the overripe control wines of both cultivars but these differences were not significant. These data agree with previously published results (Gil *et al*. 2012).

In general, all the treatments reduced the sugar content of the must and the ethanol content of the wine very effectively with an average decrease of 0.9 degrees, thus making it possible to obtain an ethanol content similar to that of the corresponding normal control wine. The transformation ratio of sugar in ethanol was very similar in all the experimental groups with a minimal value of 16.80 $g \cdot L^{-1}$ for obtaining 1 % of ethanol and a maximal value of 17.10 with an average value of 16.95. These values are close to that established by OIV (16.83). However, the different treatments did not always correct wine acidity effectively and sometimes they affected the wine composition negatively because other wine components were probably diluted.

Specifically, adding acidified water caused a significant increase in pH and significant decrease in titratable acidity in both cultivars. In addition, adding water also significant-

Table 4

Total polysaccharide concentration of the different wines

Parameter	Cultivar	Normal grapes		Overripe grapes			
		Control wine	Control wine	Addition of acidified water	Substitution of acidified water	Addition of acidified must	Substitution of acidified must
Total polysaccharides ($mg \cdot L^{-1}$)	M	525 ± 75 A	602 ± 77 A	633 ± 34 A	611 ± 35 A	563 ± 40 A	590 ± 18 A
	T	1072 ± 61 B	1165 ± 50 B	883 ± 79 A	908 ± 58 A	783 ± 60 A	879 ± 61 A

All data are expressed as the average values of 3 replicates ± standard deviation. M: 'Merlot'; T: 'Tempranillo'. Different letters indicate the existence of statistical differences ($p < 0.05$).

ly decreased TPI and the total anthocyanin concentration measured by spectrophotometry; however, no significant differences were detected in the different types of anthocyanins measured by HPLC (Tab. 2). The color intensity also decreased significantly in 'Merlot' wines although no differences were found in 'Tempranillo' wines. However, adding acidified water did not affect the proanthocyanidin composition (Tab. 3) and only significantly decreased the total polysaccharide concentration of 'Tempranillo' wines (Tab. 4). These results show that adding acidified water causes a general decrease in the concentration of most wine components.

Substituting a portion of the original grape must with acidified water generated very similar results to those obtained by simply adding acidified water. Similar reductions in ethanol content and titratable acidity, and increases in wine pH in both cultivars were obtained. A general decrease in other wine components was also observed although these differences were in general less intense than when acidified water was added. For example, the decreases in the TPI and the total anthocyanins were somewhat lower and the color intensity of the 'Merlot' wine was not affected by this treatment.

Adding acidified must effectively reduced the wine ethanol content and also significantly reduced wine pH, unlike the two acidified water treatments. This treatment also caused a significant decrease in the TPI in both cultivars but it seems to affect other parameters to a lesser extent, such as total anthocyanin concentration and color intensity in the case of 'Merlot'. Moreover, the total proanthocyanidin concentration was even significantly higher than in the overripe control wine, which indicates that substituting a portion of the original must with the acidified must favors proanthocyanidin extraction during winemaking. This could be due to the decrease in pH. In contrast, the total polysaccharide concentration was significantly lower than in the overripe control 'Tempranillo' wine at a similar level as that in the acidified water treatments.

Finally, substituting a portion of the original must with acidified must was probably the more interesting treatment since it reduced the wine ethanol content and pH to a similar extent as adding acidified must, but the dilution of the other wine components was quite low or even inexistent. The TPI and the total anthocyanin concentration were not affected in 'Tempranillo' wines and were even significantly increased in 'Merlot' wines. Similar results were observed in the anthocyanins analyzed by HPLC. In addition, the color intensity and the total proanthocyanidin concentration were significantly higher in both cultivars. It must be highlighted that the percentage of prodelphinidins was significantly higher in 'Tempranillo' wines, which indicates a higher extraction of skin tannins (Souquet *et al*. 1996, Gil *et al*. 2012). In contrast, the polysaccharide concentration of 'Tempranillo' wines was significantly lower than in the non-treated wine similarly to all the other treatments.

Fig. 2 shows the results of the descriptive sensory analysis of the different 'Merlot' (Fig. 2A) and 'Tempranillo' (Fig. 2B) wines. Spider web graphics are used to compare first the normal control wine with the overripe control wine, second the overripe control wine with wines with added or

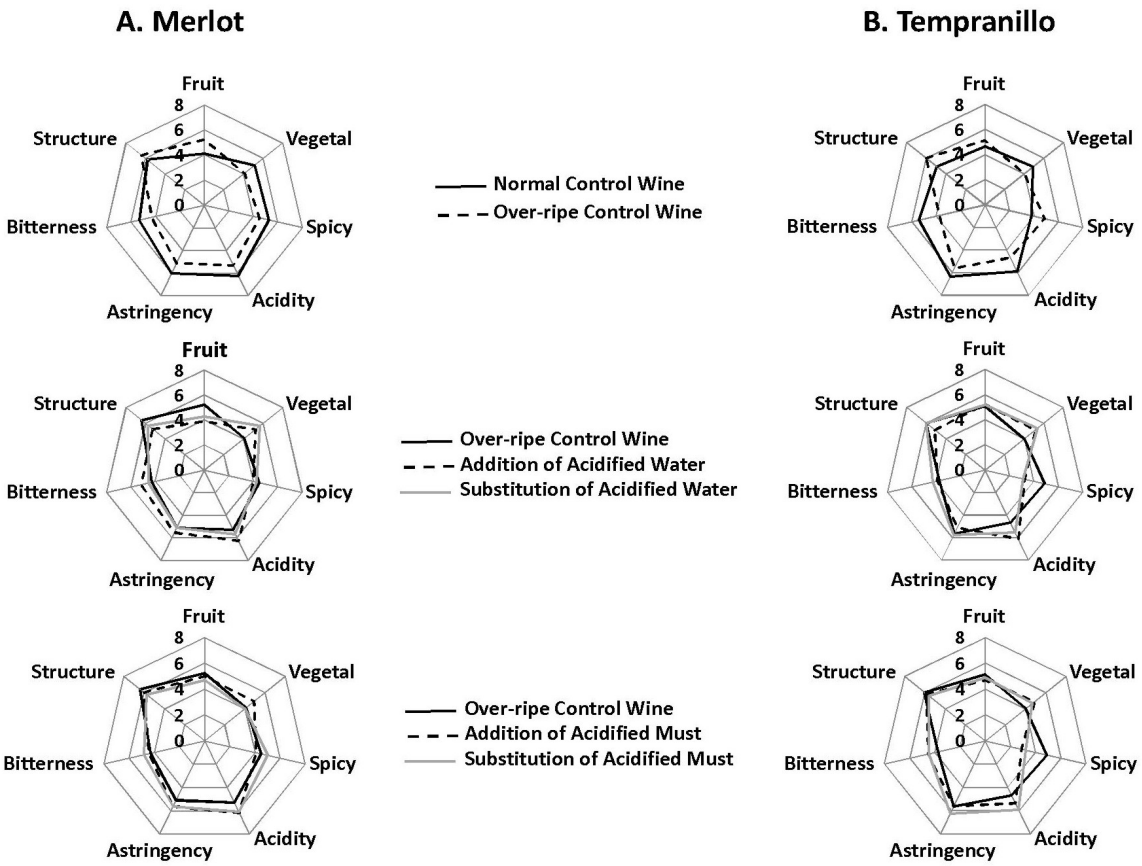

Fig. 2: Descriptive sensory analysis of the different wines.

substituted acidified water, and third the overripe control wine with wines with added or substituted acidified must.

The comparison between overripe control wines and normal control wines showed the expected results. In general the panelists considered that the overripe control wines for both cultivars were less vegetal, acidic, astringent and bitter than the normal control wines. They also found that overripe control 'Merlot' wine was more fruity and less spicy, and the 'Tempranillo' wine was more spicy and more structured. In general these differences can be associated with the different maturity stages of the grapes and similar results have been previously reported (Gil *et al*. 2012, Casassa *et al*. 2013).

In general, the panelists considered the wines obtained by adding or substituting acidified water to be more vegetal and acidic in both cultivars. Moreover, the 'Tempranillo' wine was considered less spicy and the 'Merlot' wine was considered less fruity and structured. In addition, the wines obtained by adding water were considered less structured for 'Tempranillo' grapes and more bitter for 'Merlot' grapes.

Finally, the wines obtained by adding or substituting acidified must seem to be less affected at a sensory level than wines obtained by the other treatments. Specifically, wines obtained by adding acidified must were considered more vegetal and acidic than overripe control wines but to a lesser extent than the equivalent wines treated with acidified water. The 'Tempranillo' wines obtained with this treatment were considered noticeably less spicy. In contrast, wines obtained by substituting a portion of the original must with acidified must were more similar to the overripe control wine, although they were considered more acidic and in 'Tempranillo' wines less spicy.

The panelists were also required to classify the wines by order of preference and the results are shown in Fig. 3. The classification by order of preference was very similar for both cultivars. The preferred wines were those obtained by substituting a portion of the original must with acidified water or acidified must, followed by the overripe control wine and then the wine obtained by adding acidified must. Finally, the normal control wine and especially the wine obtained by adding acidified water were classed as the least preferred wines.

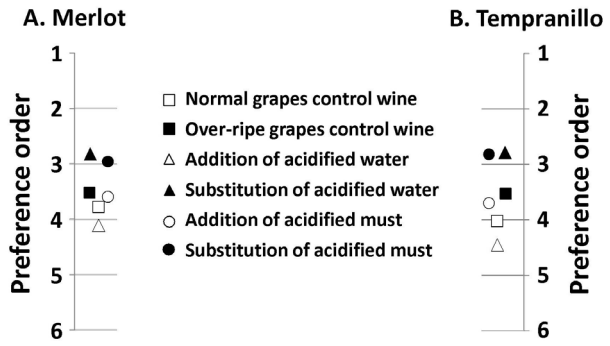

Fig. 3: Preference sensory analysis of the different wines.

Conclusions

It can be concluded that all the studied treatments are useful for reducing the ethanol content of wines elaborated with overripe grapes. However, adding or substituting with acidified water has the considerable drawback of increasing pH and decreasing titratable acidity and other wine components, which affects the wine sensory appreciation, especially when water is added. Moreover, adding or substituting with water is not authorized by the OIV or most wine producing countries, and can be analytically detected. In addition, adding water implies an increase in wine production, which is also a problem in the context of the global overproduction of wine and it is not acceptable for most wine consumers who seek authenticity. In contrast, adding or substituting with low ethanol white must acidified by cationic exchange reduces ethanol content and also pH, does not dilute the wine as much, does not increase the wine volume produced, is not an unauthorized practice and would probably be well accepted by consumers. Furthermore, using acidified must, especially for substitutions, does not affect, and in fact can even improve, the sensory quality of the wine.

Acknowledgements

The financial support of CAP (Comisión Académica de Posgrado de la Universidad De la República), ANII (Agencia Nacional de Investigación e Innovación, beca MOV_ CA_2015_1_107599), CISC (Comisión Sectorial de Investigación Científica, beca de Movilidad, 2017).

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Received September 12, 2019