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ORIGINAL RESEARCH ARTICLES

Exploring the impact of elevated pH and short maceration on the physicochemical stability and phenolic composition of tropical red wines

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ABSTRACT

This study explored the impact of high pH and short maceration on red wines' physical and chemical traits and stability. Specifically, wines from Petit verdot, Merlot, Malbec and Tempranillo grapes, harvested in a tropical wine-producing region, were analysed. A consistent maceration period of 96 hours was applied in all vinification trials. After six months of bottling, parameters such as colour, acetaldehyde levels, higher alcohols and phenolic compounds were assessed. The findings highlighted significant impacts of grape pH on chemical stability, influenced by the phenolic profile of each grape variety. The short maceration period reduced phenolic compound extraction in high-pH musts, leading to decreased antioxidant potential and chemical stability. Critical indicators included colorimetric parameters, acetaldehyde and free SO_2 content. Acetaldehyde levels were strongly correlated with free SO_2 consumption and colour variations, signifying oxidative processes. Wines with higher concentrations of (+)-catechin, procyanidins and monomeric anthocyanins exhibited enhanced stability, while the presence of hydroxycinnamic acids was associated with oxidative changes. Caffeic acid emerged as a potential marker of oxidative stress, particularly in grapes from warmer climates. To improve the stability of wines made from high-pH grapes, extended maceration times or increased SO_2 dosages may be required.

KEYWORDS: spoilage indicators, phenolic compounds, acetaldehyde, GC-BID

INTRODUCTION

Wine deterioration may be explained as a set of undesirable changes that occur from grape harvest to wine bottle storage, making the product sensorially unpalatable (Zea *et al.,* 2015). Concerning the deterioration mechanisms, chemical and physical changes are related to each other, while microbiological spoilage occurs in different ways. Therefore, all physical changes have chemical agents as causers (Waterhouse & Elias, 2010).

Loss of colour is the main factor in physical deterioration and occurs due to the decrease of monomeric anthocyanins, which copigment with other anthocyanins and acetaldehyde (Forino *et al.,* 2020; Waterhouse *et al.,* 2016). On the other hand, the accumulation of acetaldehyde is the main indicator of wine chemical deterioration (Han *et al.,* 2019). This compound is produced from ethanol oxidation and reacts with phenolic compounds, which intensify colour losses and interfere with the aroma profile, adding an often-undesirable odour descriptor of ripe/rotten apple (Zea *et al.,* 2015). Furthermore, the loss of sulfur dioxide can also be an important method for monitoring chemical deterioration, as its consumption can be related to polyphenol oxidation (Waterhouse & Elias, 2010). Sulfur dioxide is the main preservative and antioxidant agent added to wines (Gabriele *et al.,* 2018).

In general, dissolved oxygen concentration, storage time and temperature are the main factors studied related to wine deterioration. However, as low oxygen levels are common during bottle storage, deterioration can occur due to intrinsic traits such as pH (Forino *et al.,* 2020), total acidity and alcoholic content, or techniques applied in winemaking, such as duration time of maceration (Alencar *et al.,* 2018). These factors can influence the quality of the wine since they interfere with the stability and extraction of phenolic compounds with antioxidant potential (Forino *et al.,* 2020). Therefore, these conditions can play important roles in chemical and physical deterioration, making it necessary to explain this correlation.

The control of pH is essential for red wines, as this parameter plays an important role in the chemical stability and sensory balance of the beverage. In general, typical pH values range from 3.3 to 3.7 in red wines (Jackson, 2020). Thus, pH values > 3.7 favour oxidative reactions in molecules of monomeric anthocyanins, which induce self-association and affect colour stability, causing the red wine to lose its colour and reach a tone close to brick red or tawny, with orange reflections. In addition, the pH value may be related to the sulfur dioxide effectiveness, as the higher the pH the lower the percentage of reactive SO_2 (free SO_2) capable of binding acetaldehyde and protecting wines from chemical oxidation and deterioration by microorganisms, making the addition of higher doses of potassium metabisulfite necessary when the wine show pH > 3.7. However, high levels of free sulfur can cause irritation and a burning sensation in the nose due to chemical and pungent aromas (Ribéreau-Gayon *et al.,* 2006a) and also allergic adverse reactions (OIV, 2021a).

Climate change has provided different chemical characteristics to the grapes and consequently to the wines, such as an increase in the pH and a reduction of acidity levels, which bring challenges to wine producers (van Leeuwen & Darriet, 2016). Nowadays, studies relating chemical properties and harvest seasons of the grapes are common in emergent wine-producing regions, especially in the regions that produce tropical wines (Oliveira *et al.,* 2018). These studies propose discovering the best harvest months to produce wines with lower pH and high acidity since these conditions reduce the chemical instability of the wine. In tropical wine-producing regions, common grapes show high pH values and low acidity and give red wines a low-ageing capacity, recommending their immediate consumption (2 to 3 years). Therefore, these studies may play an important role as a reference for traditional wine-producing regions to adapt to these changes, using some solutions already applied in tropical climate regions (Mozell & Thachn, 2014).

Maceration is a necessary technique that must be put in contact with grape skins and seeds, being always applied to red wine winemaking (Ribéreau-Gayon *et al.,* 2006b). This process aims to extract minerals, polysaccharides, volatile compounds (and precursors), pigments (anthocyanins), tannins and other interesting phenolic compounds, all of which are important to the chemical and sensory quality of wines (Jackson, 2020). The maceration time influences this extraction, since when below five days (120 hours), it does not provide the maximum obtention of anthocyanins (Jackson, 2020). Then, short maceration time may not be enough to extract enough other phenolic compounds to red wines, such as procyanidins, catechins and flavonols, also providing low antioxidant activity to the beverage (Alencar *et al.,* 2018) and smaller effect in cardiovascular disease's prevention, which is also related to the reducing wine's shelf life (Waterhouse *et al.,* 2016). Therefore, the low antioxidant activity would make major applications of sulfites necessary, which also explains why white and rosé wines receive higher amounts of preservatives (Gabriele *et al.,* 2018).

In this approach, indicators of physical and chemical deterioration of red wines were investigated, aiming to explain how these processes may be influenced by the high pH values of the grapes in the harvest and the short maceration time applied during the winemaking. Thus, without interference, four red grape cultivars with different phenolic potential were studied for winemaking with a short maceration time. Phenolic compounds, antioxidant activity and higher alcohols were determined to help understand the influence pathways of these factors over red wine deterioration.

MATERIALS AND METHODS

1. Red wine trial

For this experiment, four red grape cultivars from the same vineyard and with different phenolic potential were selected: Petit verdot, Merlot, Malbec and Tempranillo. Pruning of vines was carried out in August 2020, and grapes were harvested in December 2020 (cycle of around 120 days) from

Bebedouro experimental vineyard in Petrolina, Pernambuco, Brazil (latitude: 9º 9' S, longitude 40º 22' W, altitude 365.5 m).

Grapes received oenological inputs to start the fermentation process (Table S1). It is important to mention that metabisulfite applications were carried out with coherent dosages to red wines, as per the manufacturer's indications. Must pH and total acidity did not suffer any oenological intervention during the winemaking. Each grape cultivar must (Malbec, Merlot, Petit verdot and Tempranillo) was then divided into 20 L glass bottles capped with cylindrical glass airlock valves, totalling 8 experimental samples (two for each grape cultivar).

The winemaking followed the vinification described by Alves *et al.* (2022). The alcoholic fermentation (AF) was carried out under a controlled temperature for approximately 10 days. During the AF, a short maceration time of four days concomitant with AF was performed on all samples, before pressing. The spontaneous malolactic fermentation was carried out under a controlled temperature (18 ± 2 ºC). The stabilisation was conducted with cold storage (0 ºC) for 20 days and tartrate stabiliser addition. Free SO_2 content was adjusted to reach 65 mg L^{-1}. Wines were bottled (750 mL) and stored in the cellar at 18 ºC for six months until the assays were accomplished. Vinification parameters are shown in Table S1 (Supplementary material).

2. Physicochemical and instrumental colour evaluations

Titratable acidity (TA) was expressed in g L^{-1} of tartaric acid, volatile acidity (VA) was expressed in g L^{-1} of acetic acid, sulfur dioxide by Ripper titrimetric method expressing results in mg L^{-1} of free SO_2 (OIV, 2021b). Total reducing sugars were analysed according to the Lane–Eynon titratable method and expressed in g L^{-1}. The instrumental colour was performed by the CIELab and CIEL*C*h systems, using a colorimeter (ColorQuest-XE, HunterLab, Virginia, USA). Colour intensity (CI) and tonality were determined by the wine sample reading in the 420, 520 and 620 nm wavelengths, using a UV-Vis spectrophotometer (Thermo Fisher Scientific Oy Ratastie 2, FI-01620 Vantaa, Finland).

3. Monomeric, polymeric and copigmented anthocyanins

Monomeric, polymeric and copigmented anthocyanins and their respective percent of distribution in the wine samples were determined according to Cliff *et al.* (2007), using acetaldehyde 20 % and SO_2 5 % (w/v) solutions, and performing readings at 520 nm wavelength. Measurements were carried out using a spectrophotometer (Shimadzu Corporation, UV 1800, Japan).

4. Acetaldehyde and higher alcohol determinations

Acetaldehyde, 3-methyl-1-butanol and 1-propanol were determined using gas chromatography with barrier discharge ionisation detection (GC-BID) by injection of 1 µL of wine sample spiked with 1-pentanol as the internal standard (Ribani *et al.,* 2004). A GC-2010 Plus (Shimadzu Corporation, Kyoto, Japan) with a Carbowax (30 m × 0.25 mm ID × 25 µm) capillary column (Shimadzu, USA) was used. Helium 5.0 (99.999 %) was used as carrier gas at a constant flow of 1.8 mL min^{-1} in the column and 50 mL min^{-1} in the detector, using purifiers VICI (Valco Instruments Co. Inc). The injector was kept at 200 ºC with a split ratio of 35. BID temperature was maintained at 300 ºC. The column temperature program was 40 ºC for 3 min, raised to 65 ºC and then to 200 ºC for 10 min (total 20.70 minutes). Calibration curves ranging from 15 to 250 mg L^{-1} to acetaldehyde and 100 to 800 mg L^{-1} to alcohols (3-methyl-1-butanol and 1-propanol) were used. The internal standard 1-pentanol 100 mg/L was applied in calibration curves and injections. Acetaldehyde, 3-methyl-1-butanol, 1-propanol and 1-pentanol standards were obtained from Sigma-Aldrich (USA).

5. Quantification of phenolic compounds by High-performance liquid chromatography (HPLC-DAD-FD)

The phenolic compounds were quantified by High-Performance Liquid Chromatography (HPLC-DAD-FD), according to methods validated under the same analytical conditions (Natividade *et al.,* 2013). The chromatograph (Waters model Alliance e2695, USA) was attached simultaneously to the detectors Diodes Array Detectors—DAD (280, 320, 360 and 520 nm) and Fluorescence—FD (280 nm excitation and 320 nm emission). The Gemini-NX C18 column (150 mm × 4.60 mm × 3 µm) and the Gemini-NX C18 pre-column (4.0 mm × 3.0 mm) (Phenomenex®, Torrance, USA) were used to separate the 28 phenolic compounds that were quantified in the wines: malvidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, pelargonidin-3-*O*-glucoside (anthocyanins), gallic, caffeic, *trans*-caftaric, chlorogenic, ρ-cumaric, ferulic acids (phenolic acids), quercetin 3-β-D-glucoside, rutin, myricetin, kaempferol-3-*O*-glucoside, isorhamnetin-3-*O*-glucoside (flavonols), *trans*-resveratrol, *cis*-resveratrol, piceatannol (stilbenes), (+) -catechin, (-)-epicatechin, (-)-epigallocatechin gallate, (-)-epicatechin gallate, procyanidins A2, B1 and B2 (flavonols and tannins). Employing gradient elution, the mobile phase consisted of a 0.85 % solution of orthophosphoric acid (Fluka, Switzerland) in ultrapure water (Purelab Option Q Elga System, USA) as phase A and acetonitrile HPLC grade (J. T. Baker, USA) as phase B, totalling 60 minutes of running. The oven temperature was maintained at 40 °C and the flow at 0.5 mL min^{-1}. The wine was injected without dilution in the equipment, after filtration in a 13 mm diameter nylon membrane and 0.45 µm pore size (Phenomenex®, USA), using 10 µL/sample as the injection volume.

The ferulic acid standard was obtained from ChemService (West Chester, USA). The caffeic, *trans*-caftaric, ρ-coumaric, chlorogenic and gallic acids plus the standards of piceatannol and viniferin were acquired from Sigma-Aldrich (USA); the (-)-epicatechin gallate, (-)-epigallocatechin gallate, (+)-catechin, (-)-epicatechin, procyanidin A2, procyanidin

B1, procyanidin B2, kaempferol-3-*O*-glucoside, quercetin 3-β-D-glucoside, isorhamnetin-3-*O*-glucoside, rutin, malvidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, pelargonidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside and *trans*-resveratrol standards were obtained from Extrasynthese (France); and the *cis*-resveratrol was acquired from Cayman Chemical (Michigan, USA).

6. Statistical analysis

Each replicate of vinification was analysed using three different bottles. All data were evaluated by the analyses of variance (one-way ANOVA) and Tukey test ($p \leq 0.05$), applying Shapiro–Wilk and Levene tests to normality and homogeneity, respectively; both using the R Studio Desktop program (1.4.1106 version, Boston, USA). Correlation among the deterioration indicators was carried out using the Spearman coefficient and Principal Component Analysis (PCA), using Pearson's correlation matrix, was performed to confirm the relations among analysis and the wines, both using XLStat (Addinsoft Inc., Anglesey, UK, 2015).

RESULTS AND DISCUSSION

1. Deterioration mechanisms in red wines and spoilage indicators

Figure 1 presents the physical and chemical indicators evaluated in this study to characterise the stability and composition of wines produced with high-pH grapes and short maceration times. Volatile acidity (VA), often used as an indicator of microbial activity, was included to confirm the absence of microbiological spoilage, as bacteria can produce acetic acid from ethanol oxidation (Jackson, 2020). All samples displayed VA levels within the acceptable range defined by the International Organization of Vine and Wine (OIV, 2021b), staying well below the maximum limit of 1.2 g L^{-1}. These results confirm that no microbiological activity influenced the wine samples, enabling a focused evaluation of their chemical attributes.

Colorimetric parameters such as lightness (L), redness (a*) and yellowness (b*) were assessed to evaluate the physical conditions of the samples. As shown in Figure 1B and

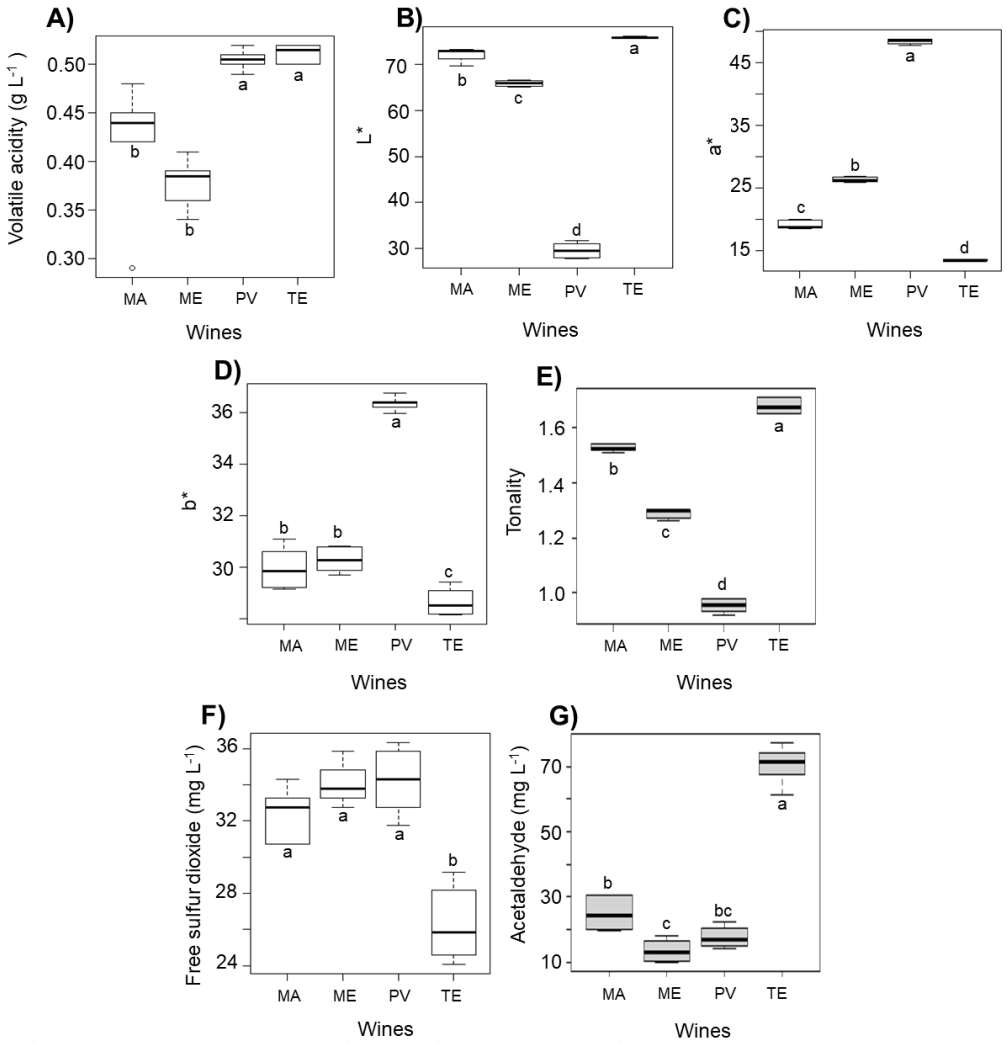


FIGURE 1. Spoilage indicators to Petit verdot, Merlot, Malbec and Tempranillo tropical red wines, produced with high pH grape values and short maceration time (96 h) during winemaking.

MA = Malbec; ME = Merlot; PV = Petit verdot; TE = Tempranillo.

TABLE 1. Spearman correlation among the deterioration indicators in tropical red wines produced with high pH grape values and short maceration time (96 hours) during the winemaking.

Variables	L*	a*	b*	Acetaldehyde	Volatile acidity	Free SO_2	Tonality
L*	1						
a*	–1.000	1					
b*	–1.000	1.000	1				
Acetaldehyde	0.800	–0.800	–0.800	1			
Volatile acidity	0.400	–0.400	–0.400	0.800	1		
Free SO_2	–1.000	1.000	1.000	–0.800	–0.400	1	
Tonality	1.000	–1.000	–1.000	0.800	0.400	–1.000	1

Correlation: weak ($r \geq 0.5$), moderate ($0.5 \leq r \leq 0.8$), strong ($0.8 \leq r < 1.0$), perfect ($r = 1.0$).

Figure 1C, Tempranillo and Malbec wines presented higher L* values (75.89 and 72.16, respectively) and lower a* values (13.4 and 19.08, respectively), which reflect greater lightness and reduced red pigmentation. These patterns are consistent with oxidative changes that affect anthocyanins and tannins, compounds essential for maintaining the characteristic red hue of wines (Waterhouse *et al.,* 2016). Petit verdot, on the other hand, showed the most intense colour, with lower L* and higher a*, suggesting greater pigment preservation. While b* values varied less between samples, their uniformly high levels indicate a tendency toward yellow or orange tones, likely linked to pH and oxidation effects (Esteban *et al.,* 2019). Tonality (Figure 1E) further supports this observation, with Tempranillo and Malbec showing higher 420/520 nm ratios, which are indicative of shifts in colour stability due to oxidative processes.

Sulfur dioxide (SO_2) and acetaldehyde concentrations were also analysed to understand their roles in chemical dynamics. As shown in Figure 1F, all samples experienced SO_2 reductions from the initial adjustment (65 mg L^{-1}), reflecting the possibility of ongoing oxidative reactions. SO_2 acts as a primary antioxidant, reacting with compounds like acetaldehyde to limit oxidation. Among the samples, Tempranillo showed the highest SO_2 reduction (60 %), indicating a possible heightened oxidative activity compared to Petit verdot, Malbec and Merlot. Similarly, acetaldehyde concentrations were highest in Tempranillo (70.52 mg L^{-1}), followed by Malbec (24.88 mg L^{-1}), Petit verdot (17.59 mg L^{-1}) and Merlot (13.48 mg L^{-1}). These elevated levels of acetaldehyde, a by-product of ethanol oxidation, highlight the possibility of the extent of oxidative changes, which can influence both colour stability and phenolic composition (Han *et al.,* 2019).

Table 1 provides further insights through statistical correlations among the chemical indicators. The negative correlation between acetaldehyde and SO_2 ($r = –0.8$) demonstrates their inverse relationship, where oxidative reactions consume SO_2 while producing acetaldehyde. Additionally, colorimetric parameters (L*, a* and tonality) showed strong correlations with these chemical markers, reinforcing their significance in characterising oxidative changes in the wines. These results underscore the role of acetaldehyde as a crucial marker for understanding chemical stability in tropical red wines.

Bueno *et al*. (2018) quantified acetaldehyde in red wines (vintages from 2009 to 2014) with oxygen exposure to force oxidation, showing values ranging from 11.3 to 31.8 mg L^{-1}. Picariello *et al*. (2017) found less than 30 mg L^{-1} of this compound in experimental red wines treated with hydrogen peroxide and oxygen to simulate two years of aging, and analyzed after 30 days; while Han *et al*. (2019) quantified at most 17.69 mg L^{-1} in wines with 12 months of storage with forced oxidation using oxygen ingress. These data reinforce a large amount of acetaldehyde found in the Tempranillo wine, suggesting the high oxidation degree of this wine in this approach.

Then, this section highlights the chemical characterisation of tropical red wines, emphasising the influence of high pH and short maceration on spoilage indicators and their relationships with colorimetric attributes. The findings provide valuable insights into the chemical behaviour of wines produced under tropical conditions, offering practical relevance for winemaking practices.

2. Influence of the pH value on red wine deterioration

The pH results are shown in Table 2. Tempranillo wine presented the highest pH value (4.08), followed by Petit verdot (3.98), Malbec (3.83) and Merlot (3.78). These values are considered high since typical pH ranges for red wines are between 3.3 and 3.7 (Jackson, 2020; Leão & Soares, 2009; Oliveira *et al.,* 2018; Waterhouse *et al.,* 2016). High pH levels can influence the stability and chemical composition of wines, particularly by affecting the pigmentation and phenolic content. For example, anthocyanins, which are key compounds for wine colour, become less stable at high pH, leading to reduced colour intensity and changes in the red wine hue (Jackson, 2020). Since colour is a primary attribute perceived by consumers, these changes can impact not only the visual appeal but also the olfactory and gustatory perceptions of the wine.

According to Tables 2 and 3, Tempranillo wine exhibited the lowest colour intensity (1.52) and total anthocyanin content (5.32 mg L^{-1}), while also presenting the highest

TABLE 2. Classical physicochemical analyses of Petit verdot, Merlot, Malbec and Tempranillo musts and respective tropical red wines produced with high pH grape values and short maceration time (96 hours) during the winemaking.

Musts				
Parameters[1]	Petit verdot	Merlot	Malbec	Tempranillo
pH	3.56 ± 0.01 d	3.82 ± 0.01 b	3.72 ± 0.01 c	3.89 ± 0.01 a
Total soluble solids (°Brix)	21.87 ± 0.07 b	22.29 ± 0.10 a	20.38 ± 0.06 c	19.62 ± 0.11 d
Titratable acidity (g L^{-1})	6.65 ± 0.09 a	4.55 ± 0.09 c	5.30 ± 0.31 b	4.90 ± 0.09 bc
Volatile acidity (g L^{-1})	0.01 ± 0.01 a	0.01 ± 0.01 a	0.01 ± 0.01 a	0.01 ± 0.01 a
Density (g cm^{-3})	1.091 ± 0.01 b	1.093 ± 0.01 a	1.085 ± 0.01 c	1.081 ± 0.01 d
Total reducing sugars (g L^{-1})	180.00 ± 0.01 c	214.98 ± 1.19 a	190.18 ± 3.37 b	189.09 ± 1.59 b
Wines				
pH	3.98 ± 0.01 b	3.78 ± 0.01 d	3.83 ± 0.04 c	4.08 ± 0.01 a
Titratable acidity (g L^{-1})	4.98 ± 0.06 a	4.23 ± 0.11 c	4.53 ± 0.11 b	4.15 ± 0.08 c
Total reducing sugars (g L^{-1})	1.54 ± 0.01 b	2.23 ± 0.15 a	2.15 ± 0.06 a	1.50 ± 0.02 b
Alcohol (v/v %)	11.13 ± 0.21 b	12.24 ± 0.13 a	10.1 ± 0.11 d	10.35 ± 0.07 c
Colour intensity (420, 520 and 620nm)	5.85 ± 0.32 a	1.99 ± 0.08 c	2.28 ± 0.1 b	1.52 ± 0.06 d

[1] Means followed by different letters in the same lines differ significantly according to the Tukey means test ($p \leq 0.05$).

pH value (4.08). This pattern aligns with the relationship between high pH and anthocyanin instability. In contrast, Petit verdot, despite its high pH (3.98), showed significantly higher colour intensity (5.85) and anthocyanin content (58.96 mg L^{-1}). This result suggests that the phenolic profile of the grape cultivar can mitigate some of the effects of high pH. Forino *et al*. (2020) explain that anthocyanin extraction during maceration is reduced as must pH increases, which may partly account for these differences.

Colour intensity is another critical physical parameter that reflects the chemical stability of red wines. Petit verdot stood out with the highest colour intensity, while Tempranillo showed the lowest. As Jackson (2020) notes, wines undergoing short maceration times may experience colour loss during fermentation, even if anthocyanin levels remain constant. This behaviour is consistent with CIELab and tonality analyses (Figure 1). Moreover, Petit verdot had a notably distinct anthocyanin profile, with a higher percentage of monomeric anthocyanins (53 %) compared to the other samples (Figure S2). Forino *et al*. (2020) reported that high pH induces more orange hues in red wines due to lower absorbance at 520 nm, which is particularly evident in wines with lower monomeric anthocyanin content, such as Tempranillo.

Cliff *et al*. (2007) observed that younger wines generally have higher monomeric anthocyanin concentrations. Their analysis of 173 commercial red wines found that older vintages exhibited greater polymeric anthocyanin content, reaching 69.3 % in older wines compared to 39.5 % in younger ones. In this study, Tempranillo and Malbec wines showed the highest polymeric anthocyanin indices (60 % and 55 %, respectively), suggesting that polymerisation is more advanced in these samples. This supports the idea that high pH contributes to anthocyanin polymerisation or binding with other molecules, such as acetaldehyde, impacting wine colour and stability.

High pH values also affect the chemical quality of red wines by reducing the effectiveness of free sulfur dioxide (SO_2). Wines at pH 4.0 require 10 times higher free SO_2 levels than wines at pH 3.0 to maintain equivalent stability (Butzke, 2018). Figure 1 shows Tempranillo as the sample with the lowest free SO_2 concentration (26.28 mg L^{-1}), confirming the higher consumption of SO_2 at elevated pH levels. Petit verdot, despite its high pH, maintained a higher free SO_2 concentration (34.22 mg L^{-1}), likely due to its greater phenolic compound concentration (Table 3). Ćurko *et al*. (2021) reported that proper SO_2 dosages delay phenolic oxidation, preserving bioactive compounds and antioxidant activity during wine storage (Gabriele *et al.,* 2018).

The relationship between pH and other chemical components is further illustrated by the Principal Component Analysis (PCA, Figure 2). Tempranillo wine, with the highest pH (4.08), also showed the highest acetaldehyde (70.52 mg L^{-1}) and 1-propanol (383.09 mg L^{-1}) levels. In contrast, Merlot, which had the lowest pH (3.78), exhibited lower acetaldehyde (13.48 mg L^{-1}) and 1-propanol (49.46 mg L^{-1}) levels. This result confirms a direct relation between acetaldehyde and 1-propanol concentrations previously reported by Muñoz *et al*. (2006). Thus, this behavior may be related to free SO_2 losses, as the sulfur dioxide antioxidant activity may limit the reactivity of acetaldehyde (Waterhouse *et al.,* 2016).

Interestingly, the behaviour of 3-methyl-1-butanol differed from that of acetaldehyde and 1-propanol. Merlot showed the highest concentration of 3-methyl-1-butanol (746.40 mg L^{-1}), followed by Petit verdot, Malbec and Tempranillo (630.21, 483.17 and 395.52 mg L^{-1}, respectively). At high concentrations, higher alcohols like 3-methyl-1-butanol can contribute undesirable pungent aromas and act as substrates for oxidation, intensifying "oxidised aroma" notes (Waterhouse *et al.,* 2016).

Ethanol is the main alcohol present in wines and it was clustered with the 3-methyl-1-butanol and free SO_2 in the PCA (Figure 2). This alcohol may play an important role in preventing chemical spoilage or chemical reactions provided by microorganisms, due to its properties of dehydration, hydrophobicity, hydrophilicity and disinfectant (Ribéreau-Gayon *et al.,* 2006a). The Merlot wine was the sample with the highest alcohol content (12.24 %) and it must also present the highest total reducing sugars (214.98 g L^{-1}), according to Table 2. Interestingly, this sample

TABLE 3. Phenolic compounds of the Petit verdot, Merlot, Malbec and Tempranillo tropical red wines, produced with high pH grape values and short maceration time (96 hours) during the winemaking.

	Wines			
Phenolic compounds[1]	Petit verdot	Merlot	Malbec	Tempranillo
Flavanols and condensed tannin[2,3]				
(+)-Catechin	11.52 ± 1.19 a	6.54 ± 0.46 b	1.44 ± 0.07 d	3.67 ± 0.55 c
(-)-Epicatechin	4.06 ± 0.38 a	2.6 ± 0.21 b	0.88 ± 0.07 c	1.07 ± 0.18 c
(-)-Epicatechin gallate	0.57 ± 0.15 b	0.6 ± 0.02 b	0.93 ± 0.09 a	0.90 ± 0.04 a
(-)-Epigallocatechin gallate	2.37 ± 0.1 b	3.18 ± 0.07 b	4.37 ± 0.11 a	3.65 ± 0.2 a
Procyanidin A2	1.26 ± 0.12 a	1.02 ± 0.05 b	0.8 ± 0.01 c	0.73 ± 0.02 c
Procyanidin B1	12.18 ± 0.49 a	7.06 ± 0.53 b	5.94 ± 0.16 c	1.68 ± 0.08 d
Procyanidin B2	3.28 ± 0.45 a	1.84 ± 0.08 c	2.29 ± 0.17 b	ND
Total	35.233 ± 2.53 a	22.85 ± 1.09 b	16.58 ± 0.22 c	11.70 ± 0.96 d
Flavonols[2,3]				
Kaempferol-3-*O*-glucoside	1.56 ± 0.18 b	2.12 ± 0.03 a	0.54 ± 0.04 c	1.61 ± 0.25 b
Quercetin 3-β-D-glucoside	8.92 ± 0.63 b	15.82 ± 0.93 a	1.87 ± 0.11 d	5.69 ± 0.88 c
Isorhamnetin-3-*O*-glucoside	8.65 ± 0.72 a	6.25 ± 0.11 a	2.34 ± 0.13 c	1.42 ± 0.12 d
Myricetin	0.55 ± 0.06 a	0.47 ± 0.01 b	0.45 ± 0.01 b	0.45 ± 0.01 b
Rutin	2.39 ± 0.18 a	0.62 ± 0.09 b	0.63 ± 0.03 b	0.58 ± 0.02 b
Total	22.07 ± 1.71 b	25.27 ± 1.02 a	5.85 ± 0.23 d	9.75 ± 1.22 c
Monomeric anthocyanins[2,3]				
Malvidin-3-*O*-glucoside	55.45 ± 2.54 a	14.85 ± 1.55 b	12.2 ± 0.25 c	4.76 ± 0.82 d
Pelargonidin-3-*O*-glucoside	1.74 ± 0.11 a	0.47 ± 0.05 c	0.66 ± 0.02 b	0.33 ± 0.02 d
Delphinidin-3-*O*-glucoside	0.44 ± 0.04 a	ND	ND	ND
Petunidin-3-*O*-glucoside	0.95 ± 0.04 a	0.38 ± 0.02 b	0.31 ± 0.01 c	0.23 ± 0.01 d
Peonidin-3-*O*-glucoside	0.38 ± 0.03 a	0.40 ± 0.05 a	0.03 ± 0.01 b	ND
Total	58.96 ± 2.74 a	16.11 ± 1.64 b	13.21 ± 0.24 c	5.32 ± 0.83 d
Phenolic acids[2,3]				
Gallic acid	7.47 ± 0.9 a	1.31 ± 0.06 b	1.23 ± 0.02 b	1.73 ± 0.06 b
Ferulic acid	0.7 ± 0.02 a	0.46 ± 0.06 c	0.52 ± 0.03 b	0.49 ± 0.03 bc
ρ-Coumaric acid	4.81 ± 0.37 b	5.15 ± 0.2 b	9.05 ± 0.24 a	8.97 ± 0.35 a
Caffeic acid	14.3 ± 0.75 b	8.45 ± 0.1 d	10.99 ± 0.22 c	33.04 ± 0.19 a
trans-caftaric acid	495.03 ± 32.6 a	96.74 ± 3.94 c	71.36 ± 1.1 c	297.51 ± 1.99 b
Chlorogenic acid	0.73 ± 0.08 b	0.91 ± 0.04 a	0.75 ± 0.03 b	0.90 ± 0.1 a
Total	523.05 ± 34.65 a	113.02 ± 3.86 c	93.89 ± 1.53 c	342.64 ± 2.08 b
Stilbenes[2,3]				
trans-resveratrol	0.26 ± 0.01 b	0.26 ± 0.01 b	0.27 ± 0.01 ab	0.27 ± 0.01 a
cis-resveratrol	0.89 ± 0.02 b	0.57 ± 0.01 d	1.04 ± 0.05 a	0.7 ± 0.08 c
Piceatannol	0.7 ± 0.01 a	0.46 ± 0.01 c	0.52 ± 0.01 b	0.49 ± 0.01 bc
Total	1.44 ± 0.03 b	1.11 ± 0.02 d	1.59 ± 0.05 a	1.25 ± 0.07 c

[1] Means followed by different letters in the same line differ significantly according to the Tukey means test ($p \leq 0.05$). [2] ND = Not detected, below the method's quantification limit. [3] Phenolic compounds quantified by HPLC-DAD-FD and results expressed in mg L-1.

had the lowest pH value. On the other hand, the Tempranillo wine did not fit this behaviour, as it had a lower alcohol percentage (10.35 %) than Petit verdot wine (11.13 %) even with a must richer in sugar (189.09 g L^{-1} facing 180 g L^{-1}, respectively). These samples had the highest pH value. Then, pH may have influenced alcohol production, which implies that high pH may have decreased the yeast fermentation performance in the AF, with ethanol as the main result of this process. High pH levels may reduce the activity of the yeasts *Saccharomyces* ssp. as had been reported by Forino *et al.* (2020).

FIGURE 2. Principal Component Analysis obtained by the colour and physicochemical analysis, including spoilage indicators and higher alcohols, quantified in the tropical red wines Petit verdot, Merlot, Malbec and Tempranillo, produced with high pH grape values and short traditional maceration time (96 h) during the winemaking.

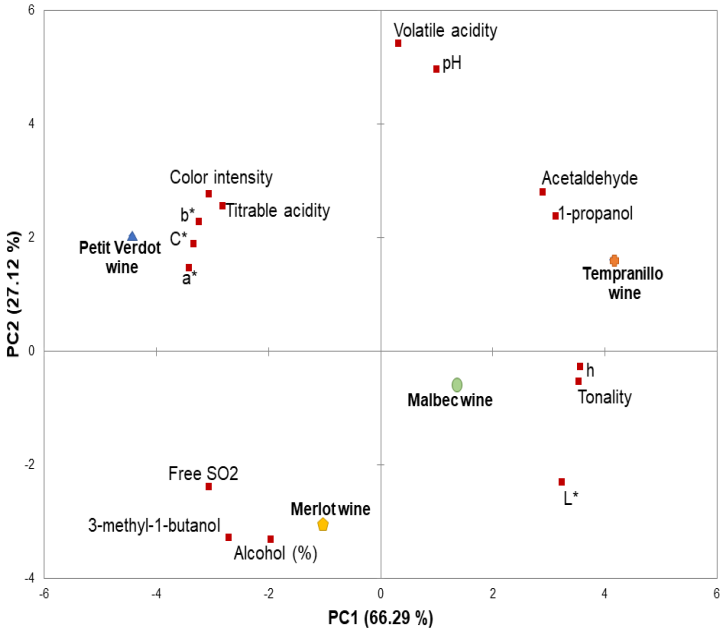
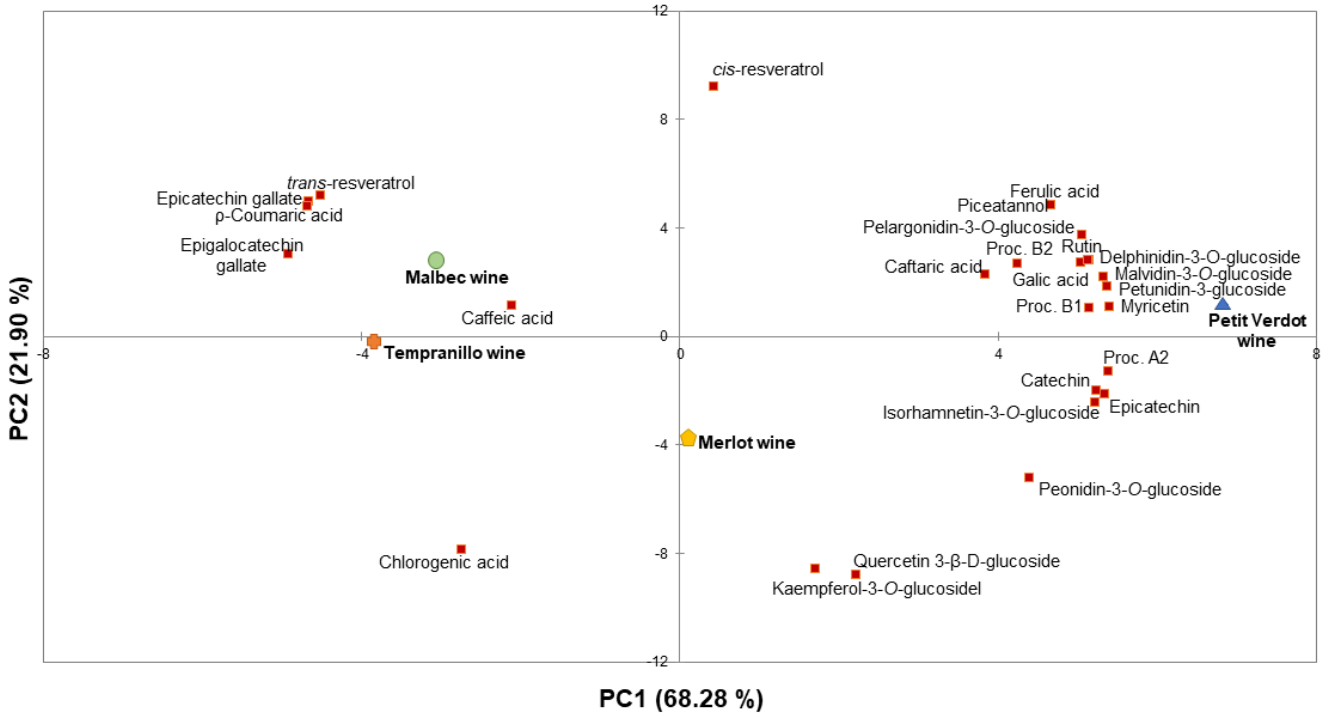


FIGURE 3. Principal Component Analysis obtained by the quantification of the phenolic compounds using the HPLC-DAD-FD method in the tropical red wines Petit verdot, Merlot, Malbec and Tempranillo, produced with high pH grape values and short maceration time during the winemaking (96 h).



3. Phenolic compounds and short maceration time during red wine winemaking

The 96-hour maceration period was selected to address the unique challenges of high-pH wines produced in warm climates, where phenolic ripeness and technological ripeness often do not align (Ribéreau-Gayon *et al.,* 2006b). Shorter maceration times are known to moderate phenolic extraction, which helps maintain balance in wines with elevated pH (Jackson, 2020). Additionally, Alencar *et al.* (2018) note that short maceration periods are particularly useful in tropical winemaking, preserving freshness and minimising oxidative reactions. This timeframe was chosen to examine how these factors influence phenolic composition and wine stability.

The results of phenolic compound analyses are presented in Table 3. Tempranillo and Malbec wines exhibited the lowest concentrations of total flavanols (condensed tannins), flavonols and anthocyanins, according to the HPLC quantification. Dutra *et al*. (2023) highlighted the importance of compounds like procyanidin B2 and (+)-catechin due to their high antioxidant capacity and bioaccessibility. These compounds were less abundant in Tempranillo and Malbec wines (Table 3), which may have influenced their lower chemical stability, in line with other results presented in this study.

The extraction of key sensory compounds, such as anthocyanins and condensed tannins, occurs primarily during maceration when the liquid is in contact with grape skins and seeds. The short maceration time applied in this study (4 days) likely contributed to the lower phenolic content observed in all wine samples. For example, Barbará *et al*. (2019) demonstrated that tropical Syrah wines with longer maceration times (10, 20 and 30 days) had significantly higher procyanidin B2 (17.48 mg L^{-1}) and (+)-catechin (18.40 mg L^{-1}) levels after 30 days of maceration.

Phenolic losses can also occur through reactive processes, such as their interaction with acetaldehyde, a key oxidation by-product (Waterhouse *et al.,* 2016). According to Figure 1, Tempranillo wine exhibited the lowest free SO_2 levels and the highest acetaldehyde concentrations, correlating with the lower phenolic compound levels observed in Table 3. This relationship highlights the role of phenolic compounds in wine oxidation resistance, as these compounds contribute to antioxidant activity by stabilising free radicals and preventing oxidative damage. This protective mechanism aligns with findings by Gabriele *et al*. (2018), who reported higher gallic acid, resveratrol and caffeic acid levels in wines with appropriate SO_2 dosages, which enhanced their antioxidant capacity.

Figure 3 illustrates the clustering of phenolic compounds, where Petit verdot stood out with higher levels of (+)-catechin, procyanidin B2 and (-)-epicatechin. Conversely, (-)-epicatechin gallate and (-)-epigallocatechin gallate were more abundant in Tempranillo and Malbec wines. The total flavanol content followed the expected trend, with Petit verdot having the highest levels, followed by Merlot, Malbec and Tempranillo. These compounds, particularly procyanidin B1 and (+)-catechin, likely contributed to the higher oxidative stability observed in Petit verdot wines.

Flavonols also exhibited a consistent pattern, with Petit verdot showing the highest concentrations of isorhamnetin-3-O-glucoside (8.65 mg L^{-1}), myricetin (0.55 mg L^{-1}) and rutin (2.39 mg L^{-1}). Merlot stood out with the highest total flavonol content, primarily due to its elevated quercetin 3-β-D-glucoside (15.82 mg L^{-1}) and kaempferol-3-O-glucoside (2.12 mg L^{-1}) levels. Despite the susceptibility of flavonols to oxidation, their correlation with storage time or chemical changes in wine has been reported as weak (Agazzi *et al.,* 2018).

Anthocyanin levels, presented in Table 3, confirmed malvidin-3-O-glucoside as the predominant anthocyanin in all samples. Petit verdot had the highest concentrations of malvidin-3-O-glucoside, pelargonidin-3-O-glucoside and petunidin-3-O-glucoside, and was the only wine where delphinidin-3-O-glucoside was detected. Merlot also showed notable levels, particularly of peonidin-3-O-glucoside. In contrast, Tempranillo wine had the lowest levels of monomeric anthocyanins, with no detectable delphinidin-3-O-glucoside or peonidin-3-O-glucoside. The higher pH of Tempranillo may have promoted anthocyanin polymerisation, reducing their monomeric forms and altering wine colour. Forino *et al*. (2020) reported that increased pH can shift anthocyanin structures, leading to changes in hue and promoting polymerisation.

Phenolic acids displayed distinct behaviours compared to other phenolics. Petit verdot had the highest concentrations of gallic acid (7.47 mg L^{-1}), ferulic acid (0.70 mg L^{-1}) and trans-caftaric acid (495.03 mg L^{-1}). Trans-caftaric acid, a key non-flavonoid compound in red wines, plays a critical role in phenolic metabolism (Jackson, 2020). Tempranillo had the second-highest phenolic acid content, particularly caffeic, ρ-coumaric and chlorogenic acids, which are associated with chemical changes in wines. Caffeic and ρ-coumaric acids have been implicated in anthocyanin acylation, potentially affecting colour stability (Cheynier *et al.,* 2010). Furthermore, the higher levels of ρ-coumaric acid in Tempranillo and Malbec wines may reflect hydrolysis processes, which have been linked to ageing and oxidative stress in wines (Agazzi *et al.,* 2018).

Stilbenes showed heterogeneous patterns, with the highest trans-resveratrol levels found in Tempranillo and Malbec (0.27 mg L^{-1} each). Malbec also had the highest cis-resveratrol levels (1.04 mg L^{-1}), while Petit verdot stood out with the highest piceatannol concentration (0.70 mg L^{-1}). Although stilbene levels were lower than typical values (~7 mg L^{-1}) (Waterhouse *et al.,* 2016), these compounds still contribute to the antioxidant profile of the wines.

CONCLUSION

The influence of pH grape value and short maceration time during winemaking on the chemical stability of red wines was investigated, using Petit verdot, Merlot, Malbec and Tempranillo cultivars. The results demonstrated that pH had significant implications for the chemical parameters of red wines, but its effects were modulated by the phenolic profile of each grape variety, which influenced reaction pathways and stability. The short maceration time (96 hours) limited the extraction of phenolic compounds in high-pH musts, reducing the antioxidant potential of the wines and affecting their chemical stability. Colorimetric parameters, tonality, acetaldehyde and free SO_2 content emerged as key indicators of chemical changes in these wines. Acetaldehyde levels showed a strong correlation with free SO_2 consumption, colour changes and higher pH values, reinforcing its role as a marker of oxidative processes.

The phenolic profile was shown to play a crucial role in the oxidative resistance of red wines. Wines rich in (+)-catechin,

procyanidins and monomeric anthocyanins exhibited greater chemical stability, while hydroxycinnamic acids, such as ρ-coumaric and caffeic acids, were associated with oxidative changes. The presence of excess caffeic acid may serve as a marker of oxidative stress in wines and could be linked to grapes from warmer regions. This highlights the importance of further studies on the relationship between phenolic acids and climate change. Shortening maceration time does not appear to be a viable strategy for high-pH musts, as phenolic extraction is essential for improving the stability of red wines. Producing red wines from high-pH grapes may require extended maceration times or higher SO_2 dosages to enhance the shelf life and overall stability of these wines.

Further studies are necessary to explore the long-term chemical behaviour of red wines under varying conditions, to better understand the reaction pathways and the complexity of oxidative processes.

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