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Impact of season, rootstock, and cultivar on chemical composition and volatile profiles of 'Arinto' and 'Fernão Pires' white wines from a tropical climate

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ABSTRACT

New varieties and rootstocks adapted to tropical climates remain a challenge for improving wine production and quality, emphasising the need to optimise sensory characteristics and typicity. This study aimed to characterise the oenological potential of wines from the 'Arinto' and 'Fernão Pires' cultivars, grafted onto IAC 572 and 1103 Paulsen rootstocks, in a semiarid tropical region over four consecutive vintages (considering the first and second semesters). The impact of harvest season, rootstock, and cultivar on the chemical and volatile composition was evaluated to provide new insights into tropical wine typicity. Gas chromatography coupled with mass spectrometry (GC-MS) was used to quantify volatile compounds. The season of harvest was the most influential factor, followed by cultivar and rootstock. Wines produced from grapes harvested in the second semester of the year exhibited greater oenological potential, with higher concentrations of key volatile compounds, including ethyl esters, higher alcohol acetates, and terpenes. Principal Component Analysis (PCA) identified phenylethyl acetate, isoamyl acetate, linalool, ethyl propanoate, ethyl hexanoate, and ethyl octanoate as markers of wines from the second semester. Additionally, methyl geranate was detected for the first time, suggesting its potential as a typicity marker for these varieties. These findings reinforce the viability of 'Arinto' and 'Fernão Pires' for tropical wine production, providing valuable knowledge for viticultural strategies in emerging tropical winegrowing regions.

KEYWORDS: tropical wines, Arinto, Fernão Pires, harvest season, rootstock, GC-MS, volatile compounds

INTRODUCTION

São Francisco Valley is a winegrowing region located in northeastern Brazil, with production starting in 1985, where grapevines can be produced at least two to three times a year, due to year-round sunshine and irrigation. The intra-annual variability in this tropical semiarid climate allows for the production of wines with different quality and typicality, varying according to the harvest date. For grapes intended for winemaking, the harvest is expected to be carried out only after reaching technological maturity, which is determined by the ratio between the sugar content and titratable acidity, the aromatic maturity and the phenolic maturity. With high temperatures and typically intense solar radiation in the region, grapes tend to rapidly reach high levels of total soluble solids, while organic acids degrade, leading to an acceleration of technological maturity (Padilha *et al.*, 2019; Oliveira *et al.*, 2020). Depending on the harvest date, in the first or second semester, wineries can choose the oenological potential of the grapes to elaborate still and/or sparkling wines. Other countries are also producing tropical wines, such as India, Thailand, Myanmar, Venezuela, and others (Pereira *et al.*, 2016; Pereira, 2020).

Wine grape cultivars and rootstock evaluation in new vitivinicultural regions are important to diversify the production for improving wine quality and typicality, also expecting global warming and climatic change, focusing on sustainable viticulture (van Leeuwen *et al.*, 2020). The rootstock can impact vine growth by accelerating or delaying the technological ripening process of the berries, thereby affecting the composition of phenolic compounds, sugars, and acids in the grapes. Consequently, it influences the flavour, aroma, and mouthfeel of the wine, thus enabling improvements in wine quality, composition, and sensory attributes (Romero *et al.*, 2019; Zombardo *et al.*, 2020; Thutte *et al.*, 2024). To enhance the ability of grapes to produce high-quality wines, considering their chemical and sensory attributes as well as the overall quality of grapes and wines, it is essential to select the appropriate scion and rootstock combinations. These choices directly influence the phenotype, typicity, and aromatic profile of the wine (Gautier *et al.*, 2019).

'Fernão Pires' is the most widely planted white grape cultivar used for winemaking in Portugal, representing approximately 70 % of the vineyards destined for still and/or sparkling wines (Coelho *et al.*, 2007). 'Arinto' is also an important white grape used in the Alentejo winegrowing region to produce wines in Portugal. Wines elaborated with 'Fernão Pires' and 'Arinto' grapes present high quality and are highly recognised in Portugal, and could be an important socioeconomic alternative for improving wine typicality in the S*ão* Francisco Valley. To our knowledge, there are no studies on these products, which could contribute to enhancing the performance of the wineries in the region.

The global synergistic effect of numerous classes of volatile and non-volatile compounds, present in the wine matrix at different concentrations and appropriate amounts, directly influences the sensory perception of aroma, flavour, and overall quality. For this reason, despite its complexity, the determination of volatile organic compounds remains a key parameter in wine quality assessment, as many volatile compounds originate from grapes (varietal compounds) and can influence the fermentation process, leading to the formation of intermediate compounds that contribute to the development of the final aroma and flavour (Caruso *et al.*, 2011; Boroski *et al.*, 2017; Cerreti *et al.*, 2016; de Souza *et al.*, 2021). The final concentration of volatile compounds in wines depends on the climate, soil, yield, cultivar, grape maturation at harvest, winemaking process, yeasts, and other factors (Le Menn *et al.*, 2019; van Leeuwen *et al.*, 2020).

The most widely used tool for determining volatile compounds in wines is gas chromatography (GC), employing various methods such as GC with flame ionisation detection (GC-FID) for fatty acids, aldehydes, and higher alcohols, headspace solid-phase microextraction (HS-SPME) coupled with GC-mass spectrometry (GC-MS) for esters, and other methods (Antalick *et al.*, 2010; Sherman *et al.*, 2017; Ziegler *et al.*, 2020).

White wines from the São Francisco Valley are being elaborated using several cultivars, such as 'Chenin blanc', 'Viognier', 'Verdejo', 'Sauvignon blanc' and 'Muscat', for still and sparkling wines (Pereira, 2020). 'Arinto' and 'Fernão Pires' are still very little used for commercial wines. In this way, this study aimed to characterise the oenological potential of wines from the 'Arinto' and 'Fernão Pires' cultivars, grafted onto rootstocks IAC 572 and 1103 Paulsen in a semi-arid tropical region, produced over four consecutive vintages (considering the first and second semesters), evaluating the impact of harvest season, rootstock, and cultivar on the chemical and volatile composition. The chemical and volatile profiles of the wines were determined to assess new insights about the wine typicalities.

MATERIALS AND METHODS

1. Chemicals and standards

All solvents were HPLC grade. Absolute ethanol (purity > 99.8 %) and methanol was obtained from Merck (Darmstadt, Germany). Ultrapure water (18.2 MΩ cm) was obtained from distilled water by a Milli-Q Plus water system (Millipore, Saint-Quentin-en-Yvelines, France). Sodium chloride (99 %) and anhydrous sodium sulfate were supplied by VWR-Prolabo (Fontenay-sous-Bois, France).

2. The experimental design

The research was conducted in a commercial vineyard, located in a partner winery in Pernambuco State, Brazil (09° 2' S latitude and 40° 11' W longitude), at 350 m altitude above sea level, in a tropical semi-arid climate. The vines were planted in 2013 and trained in an overhead pergola system, with rows oriented in a North–South direction. The plot was randomised into five blocks, where 50 plants were marked in different lines and positions (ten plants in each block, at the beginning, middle and end of the vineyard), to avoid possible soil variability. 'Arinto' and 'Fernão Pires' were grafted onto two different rootstocks, 1103 Paulsen (*V. berlandieri* × *V. rupestris*), and IAC-572 (*V. riparia* × *V. rupestris* × *V. caribaea*). Four consecutive seasons were evaluated, the first harvest was made in March 2015 (first semester of the year: 2015_1), the second one in September 2015 (second semester of the year: 2015_2), the third one in January 2016 (first semester of the year: 2016_1), and the fourth and last one in August 2016 (second semester of the year: 2016_2). The grapes were harvested according to the company's decision, to elaborate still and/or sparkling wines, respecting the oenological potential of the grapes. The climate in the region is classified as BSwh, according to the Köeppen Climate Classification (Figure 1), and the soil is classified as yellow eutrophic argisol (soil taxonomy alfisol) (Oliveira *et al.*, 2020).

3. Winemaking

For each treatment, 40 kg of grapes were harvested from the 50 vines previously marked, by cultivar *vs* rootstock interaction, for winemaking in an experimental scale, in glass tanks of 20 L each. Grapes were destemmed, slightly crushed and pressed, and sulfited (50 mg.L^{-1} SO_2) and afterwards clarified at a temperature of 0 ± 2 °C for 48 h, racked and inoculated with the same commercially selected active dry yeast (*Saccharomyces cerevisiae* var. *Bayanus*—200 mg.L^{-1}), with SO_2 (30 mg.L^{-1}) to finally carry out a traditional vinification for white wines, after alcoholic fermentation at 18 ± 2°C for 30 days. The malolactic fermentation process did not occur. After clarification (in a cold room at 0 ± 2 °C for 48 h), wines were manually placed in 750 mL glass bottles, closed with agglomerated cork stoppers and stored at 18 ± 2 °C. The wines were sent to Lisbon, Portugal, by air transport, in boxes with thermal variation control, arriving the next day, and stored at 18 ± 2 °C until the chemical analysis which was performed at the University of Lisbon (ISA) in Portugal. Volatile compound analysis was carried out at the University of Bordeaux (Institut des sciences de la vigne et du vin (ISVV)) in France.

4. Chemical profiles of the grapes and wines

Total soluble solids (TSS), pH and titratable acidity (TA) were determined on grape samples at harvest. Wines were analysed six months after bottling (pH, total and volatile acidity, alcohol content, dry extract, free and total sulfur dioxide), according to the standardised methods of the International Organization of Vine and Wine (OIV, 2014). In addition, wines were analysed by spectrophotometry to determine total phenols (Ribéreau-Gayon, 1970), flavonoids and non-flavonoids (Kramling & Singleton, 1969), and colour intensity (OIV, 2014). All parameters were determined according to the methods proposed and described in the cited scientific literature.

5. Volatile compound analyses in wines

Wines were analysed using different methods developed and validated by the laboratory of Institut des sciences de la vigne et du vin (ISVV), at the University of Bordeaux, France, obtained by gas chromatography (GC), using five different methods according to each class of compounds analysed. The determination of the volatile compounds was performed in duplicate for all samples, with the displayed

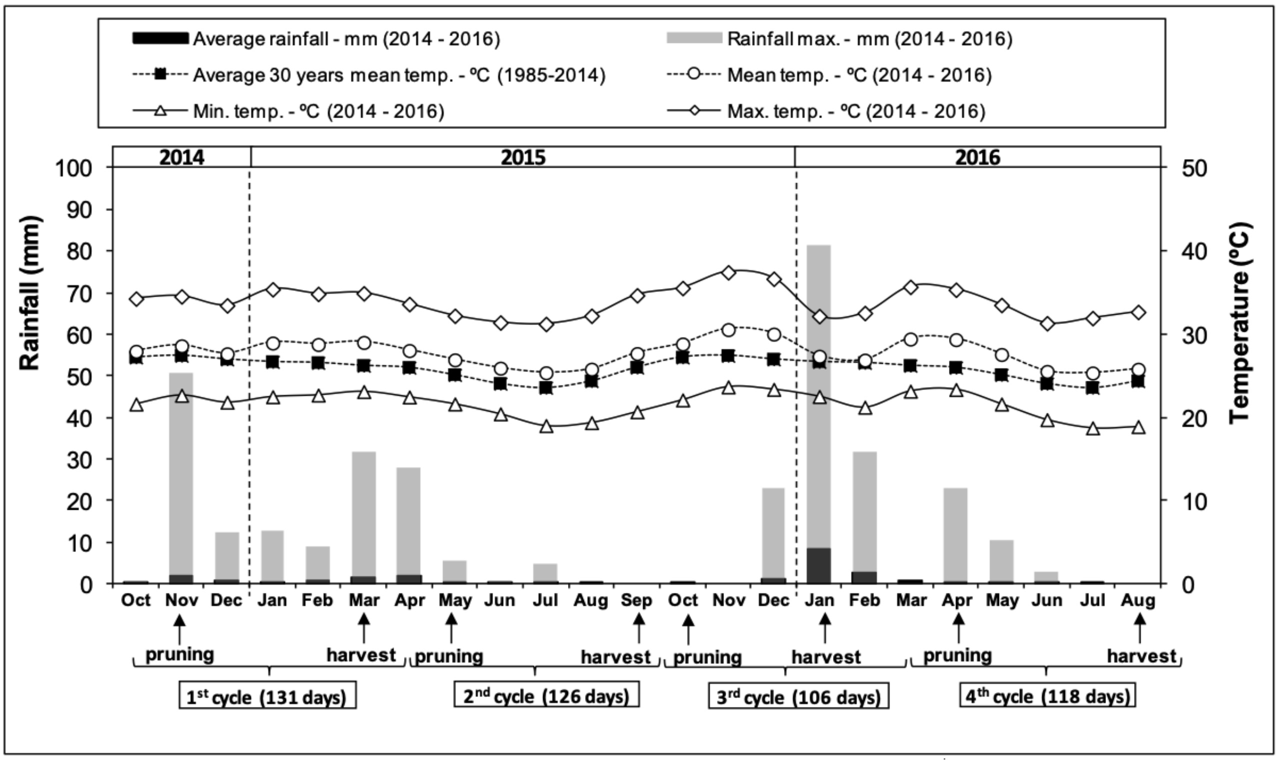

FIGURE 1. Meteorological data during the four harvest seasons in a tropical semi-arid climate of Brazil.

values corresponding to the mean of the two results obtained by comparing the retention times and/or mass spectra (NIST database mass spectra) with those of pure standards, and the quantification was based on the calibration curves obtained from linearity experiments for each analyte and standard (Antalick *et al.*, 2010).

5.1. Apolar esters (HS-SPME - GC/MS)

The HS-SPME–GC-MS method was used to quantify 32 apolar esters, including fatty acid ethyl esters, higher alcohol acetates, branched acid ethyl esters, isoamyl esters, methyl esters, ethyl cinnamates and minor esters (Antalick *et al.*, 2010). In accordance with this method, 20 µL of internal standard solution was added to 25 mL of wine. The content of 10 mL of wine sample was added to a 20 mL standard headspace vial previously filled with 3.5 g of sodium chloride and the vial was tightly sealed with a PTFE-lined cap. After this, the solution was homogenised using a vortex mixer. The carrier gas was helium N55 with a column-head pressure of 8 psi. The temperature of the oven was programmed at 40 °C for 5 min, then raised to 220 °C at 3 °C/min, and then kept for 30 min. The mass spectrometer was operated in the electron ionisation at 70 eV with selected-ion-monitoring (SIM) mode. A mixture of ethyl-d_5 butyrate, ethyl-d_5 hexanoate, ethyl-d_5 octanoate, and ethyl-d_5 cinnamate in 20 $mg.L^{-1}$ in ethanol was used as the internal standard.

5.2. Ethyl acetate, ethanal, methanol and higher alcohols (direct injection by GC/FID analysis)

Ethyl acetate, ethanal, methanol and higher alcohols were quantified using a modified version of the official OIV method (OIV-MA-AS315-02A; OIV, 2014). According to this method, 5 mL of wine was spiked with 50 µL of internal standard solution. The vials were filled with this solution for direct injection into a gas chromatograph Agilent 6890N coupled to a flame ionisation detector (FID). The column was a CP-Wax 57 CB (50 m × 0.25 mm × 0.2 µm, Varian). Quantification was performed using a calibration curve obtained from a 12 % hydroalcoholic solution. 4-Methylpentan-2-ol at 10 $g.L^{-1}$ in 50 % hydroalcoholic solution was used as an internal standard.

5.3. Volatile fatty acids (liquid–liquid extraction and GC/FID)

Hexanoic, octanoic, decanoic and dodecanoic acids were quantified by the method previously developed by Bertrand (1981). A total of 50 mL of wine previously dosed with 200 µL of internal standard solution was successively extracted with 4 mL and twice with 2 mL of a diethyl ether/isohexane mix (1:1, *v/v*). The organic phases were collected and injected into a gas chromatograph HP5890 coupled to a flame ionisation detector (FID). The column was an FFAP type (BP 21, 50 m × 0.25 mm × 0.2 µm, SGE). Measurements were carried out following the protocol described by Bertrand (1981). Quantification was performed with calibration curves obtained from white wines. Octan-3-ol at 20 $mg.L^{-1}$ in an alcoholic solution was used as an internal standard.

5.4. Additional volatile compounds (liquid-liquid extraction and GC/MS analysis)

The GC-MS method was used to identify the polar esters (ethyl lactate, ethyl succinates and ethyl hydroxyl esters), the three branched acids (isobutyric acid, isovaleric acid and 2-methylbutyric acid), and linalool in the wines, according to Antalick *et al.* (2010). For the liquid–liquid extractions with dichloromethane, samples of 50 mL of wine were successively extracted with 4 mL and twice with 2 mL of solvent. GC analysis was performed on an HP 5890 GC system coupled to an HP 5972 quadrupole mass spectrometer and equipped with an automatic Gerstel MPS2 sampler. The identification of the esters in wines was performed by comparing retention times and mass spectra with those of pure esters standards. For the quantitative study, 20 µL of a stock solution of internal standards with ethyl-d_5 butyrate, ethyl-d_5 hexanoate, ethyl-d_5 octanoate and ethyl-d_5 cinnamate at 200 $mg.L^{-1}$ each in absolute ethanol was added to 25 mL of the samples and 10 mL of this solution was taken for analysis (Antalick *et al.*, 2010).

6. Statistical analysis

The chemical analyses of grapes and wines were made in triplicate, whereas the volatile analyses in wines are expressed by means of duplicates. The significance was examined using one-way analysis of variance (ANOVA) and multivariate statistical analysis (Principal Component Analysis (PCA)) were performed on the data. The means were separated, and a multiple comparison test was conducted using the Tukey test to evaluate the differences between each level of treatment, adopting a 95 % confidence level, as used in the statistical analysis. All statistical analyses were performed by using the R software package (R Core Team, 2020).

RESULTS AND DISCUSSION

1. Chemical composition of the grape must

The classical parameters and oenological potential of the grapes, expressed in total soluble solids, pH and total acidity of the musts at harvest, showed significant differences according to seasons, rootstocks, cultivar, and interactions (Table 1).

The factors rootstock, harvest date and the interaction between rootstock and harvest date were significant for all chemical parameters, for both cultivars. The average of the rootstock per semester and the semester presented significant variability for TSS in both cultivars, while total acidity was significant per semester and the interaction between rootstock and semester, for ‘Arinto’, but only per semester for ‘Fernão Pires’ (Table 1). The cultivar effect was significant for pH and total acidity, but not for TSS. There is a substantial influence, particularly of the rootstock, harvest date, and their interaction, on all parameters. However, when considering the semester, only the total soluble solids content and, to some extent, total acidity, showed significant differences. Tecchio *et al.* (2022) showed that there was no significant difference between rootstocks for soluble solids of Syrah and Sauvignon Blanc (average values of 16.57 and 18.81°Brix, respectively). The differences were mainly due to the harvest season, influenced by the climate with the intra-annual variability (Oliveira *et al.*, 2020; Pereira *et al.*, 2020). In the São Francisco Valley, the first semester has distributed rainfall, while the second semester sees lower rainfall and extended periods of low relative humidity. This change affects grape production dynamics, requiring adaptive management strategies to address seasonal climate variations (Pereira *et al.*, 2020; Mirás-Avalos & Araujo, 2021). Climatic data are available in Figure 1.

Another factor explaining the high differences in grape maturity is the main objective of the harvest. Wineries in São Francisco Valley can choose if the grapes will be harvested to make still wines or sparkling wines. Grapes with low sugar content and high acidity are mainly used for sparkling wines, as observed with results from the first semester, while grapes with high sugar content and low acidity for still white wines are observed in the results from the second semester. The decision to harvest earlier or later depends on the climatic conditions, the market demand, the wine type and the supply in the winery (Pereira, 2020).

2. Chemical composition of the wines

The varietal wines from Arinto and Fernão Pires were analysed using classical chemical methods, to evaluate the impact of grape variety, rootstock, and harvest date over four consecutive vintages. All wines were fermented to dryness, and the results are summarised in Table 2. Previous studies have shown that rootstocks and harvest dates in São Francisco Valley impacted grape/must and wine composition (Oliveira *et al.*, 2019a; Oliveira *et al.*, 2019b; Tecchio *et al.*, 2022). Vilanova *et al.* (2021) showed that the chemical composition of Albariño white musts presented a higher influence of the vintage than the rootstock. Ethanol content exhibited significant differences between wines harvested in the first and second semesters. For both Arinto and Fernão Pires, wines from the second semester generally showed higher ethanol concentrations, with the most notable differences occurring in the 2015_2 and 2016_1 vintages. Specifically, Arinto wines from P1103 and IAC572 rootstocks displayed the highest ethanol content in the 2015_2 vintage, while the lowest ethanol levels were observed for P1103 in the

TABLE 1. Physicochemical characteristics of 'Arinto' and 'Fernão Pires' grapes harvested from two different rootstocks and harvest dates (seasons) in San Francisco Valley.

Rootstock	**Harvest**	**Physicochemical composition**					
		Arinto			Fernão Pires		
		TSS (°Brix)	pH	Total acidity (g.L^{-1})	TSS (°Brix)	pH	Total acidity (g.L^{-1})
Paulsen 1103	2015_1	10.1 ± 0.1^{c}	3.33 ± 0.01^{b}	10.6 ± 0.0^{c}	20.4 ± 0.1^{e}	3.31 ± 0.01^{d}	10.0 ± 0.1^{a}
	2015_2	25.0 ± 0.2^{a}	3.46 ± 0.02^{a}	8.3 ± 0.0^{d}	25.3 ± 0.0^{b}	3.61 ± 0.02^{b}	5.4 ± 0.0^{f}
	2016_1	14.2 ± 0.0^{b}	3.33 ± 0.00^{b}	10.5 ± 0.1^{c}	13.4 ± 0.0^{f}	3.68 ± 0.01^{a}	8.1 ± 0.2^{b}
	2016_2	24.7 ± 0.1^{a}	3.44 ± 0.01^{a}	7.2 ± 0.0^{f}	24.8 ± 0.2^{c}	3.41 ± 0.01^{c}	5.7 ± 0.1^{e}
IAC 572	2015_1	15.3 ± 0.4^{b}	3.16 ± 0.01^{c}	14.3 ± 0.1^{a}	12.2 ± 0.0^{g}	3.34 ± 0.03^{d}	9.9 ± 0.1^{a}
	2015_2	25.7 ± 1.1^{a}	3.46 ± 0.03^{b}	7.8 ± 0.0^{e}	27.0 ± 0.0^{a}	3.70 ± 0.02^{b}	5.0 ± 0.0^{g}
	2016_1	14.9 ± 0.0^{b}	3.14 ± 0.00^{c}	11.4 ± 0.1^{b}	12.4 ± 0.0^{g}	3.81 ± 0.00^{a}	7.2 ± 0.1^{c}
	2016_2	24.7 ± 0.1^{a}	3.60 ± 0.00^{a}	5.3 ± 0.3^{g}	24.5 ± 0.1^{d}	3.38 ± 0.01^{c}	6.7 ± 1.0^{d}
Rootstock (R) per Harvest	Sig.	***	***	***	***	***	**
Harvest date (H)	Sig.	***	***	***	***	***	***
R x H	Sig.	***	***	***	***	***	***
Rootstock (R) per Semester	Sig.	**	*n.s.*	*n.s.*	*	*n.s.*	*n.s.*
Semester (S)	Sig.	***	*n.s.*	***	***	*n.s.*	***
R x S	Sig.	*	*n.s.*	***	**	*n.s.*	*n.s*
Cultivar	Sig.		TSS (°Brix)			*n.s.*	
			pH			***	
			Total acidity (g.L^{-1})			***	

Data expressed as the mean ± standard deviation (n = 3). Different letters in the same column for 'Arinto' and for 'Fernão Pires' indicate significant difference according to Tukey's test ($p < 0.05$) for each cultivar separately; *n.s.* (not significant); * (significant differences at a 95 % confidence level); ** (significant differences at a 99.9 % confidence level); *** (significant differences at a 99.99 % confidence level); Harvest corresponding to: 2015_1 (first harvest in 2015); 2015_2 (second harvest in 2015); 2016_1 (first harvest in 2016); 2016_2 (second harvest in 2016). TSS: total soluble solids. Total titratable acidity expressed in g.L^{-1}of tartaric acid.

TABLE 2. Physicochemical characteristics of 'Arinto' and 'Fernão Pires' wines from different rootstocks and harvest dates (seasons).

Cultivar	Rootstock	Harvest	Physicochemical composition					Color and global phenolic composition			
			Alcohol content (°GL)	pH	TTA (g.L^{-1})	VA (g.L^{-1})	Dry extract (g.L^{-1})	Color index	Total phenols (mg.L^{-1})	Flavonoids (mg.L^{-1})	Non-flavonoids (mg.L^{-1})
Arinto	Paulsen 1103	2015_1	7.0 ± 0.2f	3.67 ± 0.01^{a}	8.3 ± 0.0^{d}	0.53 ± 0.00^{d}	31.7 ± 0.1^{a}	0.112 ± 0.000^{a}	285.3 ± 0.0^{de}	183.5 ± 0.3^{cd}	101.8 ± 0.3^{e}
		2015_2	15.3 ± 0.0^{a}	3.57 ± 0.00^{c}	6.7 ± 0.1f	0.83 ± 0.00^{c}	27.6 ± 0.0^{b}	0.107 ± 0.003^{a}	289.7 ± 1.9^{cd}	184.8 ±2.0^{cd}	104.9 ± 0.2^{d}
		2016_1	7.2 ± 0.0f	3.23 ± 0.01f	10.7 ± 0.0^{a}	0.35 ± 0.01^{a}	25.5 ± 0.0^{cd}	0.058 ± 0.004^{c}	278.9 ± 5.6^{de}	126.3 ± 5.8^{e}	152.5 ± 0.7^{a}
		2016_2	14.5 ± 0.0^{b}	3.37 ± 0.00^{e}	8.5 ± 0.1^{c}	0.50 ± 0.01^{e}	25.0 ± 0.1^{cd}	0.019 ± 0.001^{d}	278.9 ± 14.1^{de}	172.9 ± 13.0^{cd}	105.9 ± 1.1^{d}
	IAC 572	2015_1	10.4 ± 0.2^{d}	3.50 ± 0.00^{d}	7.2 ± 0.1^{e}	0.35 ± 0.01g	24.8 ± 0.1^{d}	0.089 ± 0.001^{b}	305.8 ± 9.9^{c}	191.4 ± 9.0^{c}	114.5 ± 1.4^{d}
		2015_2	15.2 ± 0.0^{a}	3.62 ± 0.01^{b}	6.0 ± 0.0g	0.82 ± 0.01^{c}	27.3 ± 0.0^{b}	0.111 ± 0.003^{a}	268.1 ± 3.7^{e}	169.5 ± 4.3^{d}	98.6 ± 0.6f
		2016_1	7.6 ± 0.1^{e}	3.37 ± 0.01^{e}	10.5 ± 0.0^{b}	0.94 ± 0.01^{b}	26.4 ± 0.4^{bc}	0.055 ± 0.003^{c}	352.2 ± 3.7^{b}	224.3 ± 3.3^{d}	127.9 ± 1.3^{d}
		2016_2	14.5 ± 0.2^{b}	3.49 ± 0.00^{d}	7.2 ± 0.0^{e}	0.46 ± 0.01f	22.6 ± 1.3^{e}	0.014 ± 0.000^{d}	423.4 ± 4.9^{a}	319.2 ± 5.0^{a}	104.2 ± 0.7^{de}
Rootstock (R)/ Harvest (H)	by Harvest	Sig.	***	***	***	***	***	***	n.s.	***	***
		Sig.	***	***	***	***	***	***	***	***	***
R x H		Sig.	***	***	***	***	***	***	***	***	***
Rootstock (R)/ Semester (S)	by Semester	Sig.	n.s.	n.s.	n.s.	n.s.	*	n.s.	**	*	n.s.
		Sig.	***	ns	***	n.s.	n.s.	n.s.	n.s.	***	**
R x S		Sig.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	***	n.s.
Fernão Pires	Paulsen 1103	2015_1	9.7 ± 0.0^{e}	3.58 ± 0.00^{d}	7.7 ± 0.1^{a}	0.58 ± 0.00f	28.2 ± 0.1^{b}	0.134 ± 0.000^{a}	500.0 ± 9.9^{b}	327.8 ± 10.1^{b}	172.2 ± 0.2^{b}
		2015_2	14.2 ± 0.0^{d}	3.95 ± 0.01^{b}	6.4 ± 0.1^{d}	0.88 ± 0.00^{a}	24.5 ± 0.2^{e}	0.118 ± 0.001^{c}	289.7 ±8.1^{c}	191.6 ±8.1^{c}	98.1 ± 0.5g
		2016_1	6.7 ± 0.0g	3.61 ± 0.01^{d}	7.7 ± 0.0^{a}	0.75 ± 0.01^{c}	22.5 ± 0.0f	0.039 ± 0.001^{e}	289.7 ± 1.9^{c}	178.5 ± 1.3^{c}	111.1 ± 0.8^{d}
		2016_2	14.5 ± 0.1^{c}	3.83 ± 0.00^{c}	7.7 ± 0.0^{a}	0.60 ± 0.00^{e}	43.4 ± 0.1^{a}	0.023 ± 0.002f	290.7 ± 3.7^{c}	175.4 ± 5.0^{c}	115.3 ± 1.3^{c}
	IAC 572	2015_1	9.1 ± 0.0f	3.54 ± 0.00^{e}	6.9 ± 0.1^{c}	0.60 ± 0.00^{e}	25.9 ± 0.2^{c}	0.124 ± 0.000^{b}	594.9 ± 4.9^{a}	394.9 ± 4.3^{a}	200.01 ± 0.7^{a}
		2015_2	14.7 ± 0.0^{b}	4.16 ± 0.01^{a}	5.6 ± 0.0^{e}	0.86 ± 0.01^{b}	25.2 ± 0.1^{d}	0.103 ± 0.004^{d}	256.2 ± 5.6^{d}	154.0 ± 6.7^{d}	102.17 ± 1.2f
		2016_1	6.3 ± 0.0h	3.83 ± 0.03^{c}	7.3 ± 0.1^{b}	0.70 ± 0.01^{d}	23.9 ± 0.5^{e}	0.043 ± 0.003^{e}	259.4 ± 3.2^{d}	147.1 ± 3.9^{d}	112.31 ± 1.5^{d}
		2016_2	15.0 ± 0.2^{a}	3.92 ± 0.00^{b}	5.7 ± 0.1^{e}	0.46 ± 0.00g	22.9 ± 0.2f	0.012 ± 0.002g	260.5 ± 1.9^{d}	154.0 ± 1.4^{d}	106.48 ± 0.5^{e}
Rootstock (R)/ Harvest (H)	by Harvest	Sig.	***	***	***	***	***	***	n.s.	*	***
		Sig.	***	***	***	***	***	***	***	***	***
R x H		Sig.	***	***	***	***	***	***	***	***	***
Rootstock (R)/ Semester (S)	by Semester	Sig.	n.s.	*	***	n.s.	*	n.s.	n.s.	n.s.	n.s.
		Sig.	***	***	***	n.s.	n.s.	n.s.	**	**	**
R x S		Sig.	n.s.	n.s.	**	n.s.	*	n.s.	n.s.	n.s.	n.s.
Cultivar		Sig.	n.s.	***	***	n.s.	n.s.	n.s.	n.s.	n.s.	*

Data expressed as mean ± standard deviation (n = 3). Different letters in the same column for 'Arinto' and 'Fernão Pires' wines indicate significant differences according to Tukey's test ($p < 0.05$). *n.s.* (not significant); * (significant at a 95 % confidence level); ** (significant at a 99.9 % confidence level); *** (significant at a 99.99 % confidence level); Harvest date corresponding to: 2015_1 (first harvest in 2015); 2015_2 (second harvest in 2015); 2016_1 (first harvest in 2016); 2016_2 (second harvest in 2016). Total titratable acidity (TTA) expressed in g.L^{-1} of tartaric acid; Volatile acidity (VA) expressed in g.L^{-1} of acetic acid; colour index (A_{420} nm); total phenols, flavonoids and non-flavonoids expressed as mg.L^{-1} of gallic acid.

2016_1 vintage. For Fernão Pires, the highest ethanol content was recorded in the IAC572 rootstock during the 2016_2 vintage, while the lowest was observed in the 2016_1 vintage. The pH values of the wines showed significant variation across rootstocks and vintages. For Arinto, the highest and lowest pH values were observed in wines from P1103 rootstocks, while for Fernão Pires, the extremes occurred in wines from IAC572 rootstocks. For Arinto, the highest pH was recorded in the 2015_1 vintage and the lowest in 2016_1. In Fernão Pires, the highest pH occurred in 2015_2, while the lowest was observed in 2015_1. Total acidity exhibited marked differences across the harvests. For Arinto, the highest total acidity was found in the 2016_1 vintage, likely due to incomplete maturation during that harvest. The 2016_1 vintage exhibited unusually high acidity, possibly due to climatic conditions during that period, which influenced grape ripening. In contrast, the lowest acidity for Arinto was observed in the 2015_2 vintage. For Fernão Pires, total acidity was generally higher in wines from the P1103 rootstock, particularly in the 2015_1, 2016_1, and 2016_2 vintages. The IAC572 rootstock showed the lowest acidity in second-semester vintages, suggesting varying rootstock adaptability to climatic conditions. While volatile acidity and dry extract varied across harvests and rootstocks, all values remained within acceptable standards for white wines. These results indicate a modest impact of rootstocks on these parameters, with harvest timing and climate playing a significant role in the wine's profile. Significant differences in phenolic content and colour were observed across rootstocks, harvest dates, and their interactions. However, total phenol content did not significantly differ across rootstocks for Arinto or Fernão Pires, with other phenolic and colour parameters highlighting the influence of both rootstock and harvest timing on the wine's profile. These findings are consistent with previous studies (Oliveira *et al.*, 2019a; Oliveira *et al.*, 2019b), which emphasised the impact of both rootstock and harvest date in determining the composition of grapes and wines, particularly in regions like the São Francisco Valley.

3. Volatile profile of the wines

Fifty-six volatile compounds were identified and quantified in the wines (Table 3). From these, 51 compounds were identified in 'Arinto' wines and 53 in 'Fernão Pires' wines. In a study on the aromatic profile of 20 white grapevine varieties, Díaz-Fernández *et al.* (2023) reported that the 'Fernão Pires' grape was closer to the muscat reference varieties, being probably the most aromatic variety among those studied. The dominating monoterpene alcohols, particularly from Muscat varieties, are linalool, geraniol, nerol, citronellol, and α-terpineol (Mateo & Jiménez, 2000). According to Silva *et al.* (2024), Arinto wines generally express aromas with moderate intensity of citrus fruits and, sometimes, some mineral notes. The volatiles belong to several groups, higher alcohol acetates, ethyl esters of branched aliphatic acid, aromatic esters, methyl and ethyl minor esters, terpenes, higher alcohol, acids, and miscellaneous compounds. Major esters and alcohols were the most concentrated of the volatiles, while esters were the group with the highest number of compounds identified and quantified.

3.1. Major esters

Nine compounds were identified and quantified in this group, the predominant ester compounds responsible for aromatic characteristics (Table 3). The most prominent compounds were ethyl acetate, ethyl lactate and diethyl succinate, and concentrations varied according to the variety, harvest season and rootstock. The acetate esters are formed by the interaction between the acetic acid and higher alcohols, having a great participation in the aroma profile of white wines (Englezos *et al.*, 2016). Lactate esters, such as ethyl lactate, are formed by association with the lactic acid production pathway, contributing to creamy notes in wines (Arcena *et al.*, 2019). The ethyl acetate was more concentrated in the first semester of 2016 for 'Arinto' wines in both rootstocks, while in 'Fernão Pires' wines the highest values were observed in the second semester of both harvest seasons and rootstocks. This compound is related to tree fruit aroma and solvent, depending on the concentration (Petronilho *et al.*, 2020). All wines presented a concentration of ethyl acetate below 150,000 µg.L^{-1}, having preferentially fruity aroma (pineapple, apple). Above this amount, ethyl acetate is known to have an unpleasant nail polish, with vinegar aroma (Englezos *et al.*, 2016). These authors reported that in 'Chardonnay', 'Muscat', 'Riesling' and 'Sauvignon blanc' wines, the concentrations of ethyl acetate ranged from 3650 µg.L^{-1} to 8488 µg.L^{-1}. The compounds diethyl succinate (flowery and fruity), ethyl decanoate (notes of sweet, waxy, soap, fruity), ethyl dodecanoate (except for 'Fernão Pires' on IAC 572 rootstock, with notes of waxy, soap), ethyl hexanoate (fruity, pineapple, apple), ethyl octanoate (sweet, fruity, apple skin), and ethyl propanoate (sweet, ripe strawberry, solvent) showed the same behaviour for both cultivars, rootstocks and seasons, higher in all wines elaborated with grapes harvested in the second semester (Table 3). The sum of all major esters had different behaviour according to the cultivar. For 'Arinto', in 2015 the wines from the second semester of the year presented the highest concentration for both rootstocks, while in 2016, the highest values were observed in the first semester. For 'Fernão Pires' wines, there was a trend of higher concentrations in the second harvest for both seasons and rootstocks (Table 3). We can suggest that cv. 'Fernão Pires' was more stimulated by drought and the medium temperatures observed in September and August, in the second and fourth harvests, respectively (Figure 1) than Arinto, promoting high concentrations of the major esters. The concentration of the sum of all chemical families is available in Figure 2. Aroma concentration is strongly dependent on the climatic and genetic constitution of each cultivar, influencing the biosynthesis of the volatiles (van Leeuwen *et al.*, 2020). In 'Tempranillo Blanco' wines, previous studies have shown that the main ethyl esters determined were ethyl hexanoate and ethyl octanoate, confirming the effect of the cultivar on wine profile (Ayestarán *et al.*, 2018). In São Francisco Valley the grape harvests between May and September are most indicated and preferred to produce more concentrated and high-quality wines, as compared to those that occurred between November and March (Pereira, 2020). In this case, there are low-medium temperatures during the night (18–

TABLE 3. Concentrations of individual volatile compounds in 'Arinto' and 'Fernão Pires' wines produced from different rootstocks and harvest date (seasons). (part 1/3)

Compound (µg.L⁻¹)	Arinto								Fernão Pires							
	1103 Paulsen				IAC 572				1103 Paulsen				IAC 572			
	2015_1	2015_2	2016_1	2016_2	2015_1	2015_2	2016_1	2016_2	2015_1	2015_2	2016_1	2016_2	2015_1	2015_2	2016_1	2016_2
Major esters																
Ethyl acetate	60.547	81.014	124.679	82.660	28.034	86.395	145.822	76.202	49.477	89.166	19.315	87.975	47.620	71.856	17.741	66.243
Ethyl lactate	21.804	16.752	7.731	14.439	32.702	18.983	13.758	20.600	17.961	24.174	9.944	15.361	22.362	17.306	5.131	13.384
Diethyl succinate	3.487	4.278	1.020	4.366	2.601	4.387	3.085	3.705	1.721	4.982	713	3.861	1.761	3.417	2.760	2.227
Ethyl butyrate	504.46	412.69	125.16	478.60	376.67	399.80	119.08	480.49	250.43	434.84	211.46	468.18	234.42	448.86	195.37	470.36
Ethyl decanoate	239.02	288.81	168.09	304.17	185.78	323.94	152.27	342.37	131.04	246.14	233.97	386.99	127.41	226.60	275.41	340.85
Ethyl dodecanoate	4.12	4.86	1.19	6.49	3.00	7.58	1.20	6.64	2.25	3.25	10.53	16.24	3.16	2.53	12.31	6.23
Ethyl hexanoate	889.97	1,017.69	305.78	1,023.00	790.99	971.34	289.73	1,061.02	589.40	907.71	478.69	968.97	564.41	862.13	451.24	991.80
Ethyl octanoate	661.91	671.67	366.50	661.62	641.74	674.52	345.44	732.97	503.97	621.60	531.22	678.10	525.86	658.66	586.25	744.15
Ethyl propanoate	361.04	409.25	270.40	389.18	259.05	422.66	275.26	383.04	244.24	429.34	121.66	382.79	205.92	396.32	84.89	324.28
Sum	*88,498.52*	*104,848.97*	*134,667.12*	*104,328.06*	*65,594.23*	*112,564.84*	*163,847.98*	*103,513.53*	*70,880.33*	*120,964.88*	*31,559.53*	*110,098.27*	*73,404.18*	*95,174.10*	*27,237.47*	*84,731.67*
Higher alcohol acetates																
Isoamyl acetate	662.55	715.54	192.78	380.37	422.51	815.54	179.74	722.75	161.84	974.34	626.24	566.82	320.64	2.010.46	662.14	1.492.07
Isobutyl acetate	36.64	36.84	50.21	28.56	19.52	38.71	68.91	35.30	21.72	53.61	34.45	37.81	17.87	74.76	36.49	59.24
Butyl acetate	2.77	3.34	2.54	1.85	1.85	3.46	2.10	2.71	1.78	4.39	3.12	2.12	1.36	7.25	4.35	4.74
Hexyl acetate	16.80	22.26	6.47	17.09	14.15	22.90	4.63	25.25	5.92	63.50	74.58	27.63	9.42	59.84	43.68	78.78
Octyl acetate	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.
Propyl acetate	60.46	58.43	76.08	46.48	33.71	62.63	78.75	57.95	46.57	86.29	49.64	57.55	39.11	135.95	62.21	104.91
Sum	*779.22*	*836.41*	*328.08*	*474.35*	*491.74*	*943.24*	*334.13*	*843.96*	*237.83*	*1,182.13*	*788.03*	*691.93*	*388.40*	*2,288.26*	*808.87*	*1,739.74*
Ethyl esters of branched aliphatic acids																
Ethyl isobutyrate	181.44	185.95	423.27	186.63	161.19	170.81	406.27	155.96	136.61	147.40	69.27	137.02	107.59	103.36	34.59	89.74
Ethyl isovalerate	22.72	28.39	58.95	26.97	21.04	25.44	52.19	20.42	20.32	20.95	7.41	18.78	17.78	13.81	4.06	11.09
Ethyl 2-methylbutyrate	12.10	14.46	16.23	15.35	11.70	12.49	12.47	10.99	8.20	9.28	3.47	9.13	7.59	6.15	1.93	5.74

L.D.: limit of detection (concentration for signal/noise = 3); L.Q.: limit of quantification (concentration for signal/noise = 10) (Antalick *et al.*, 2010). Rootstock (IAC572 and Paulsen P1103); Harvest corresponding to: 2015_1 (first harvest in 2015); 2015_2 (second harvest in 2015); 2016_1 (first harvest in 2016); 2016_2 (second harvest in 2016). Rootstock (IAC 572 and 1103 Paulsen).

TABLE 3. Concentrations of individual volatile compounds in ‘Arinto’ and ‘Fernão Pires’ wines produced from different rootstocks and harvest date (seasons). (part 2/3)

Sum	*216.26*	*228.80*	*498.45*	*228.95*	*193.93*	*208.74*	*470.93*	*187.37*	*165.13*	*177.63*	*80.15*	*164.93*	*132.96*	*123.32*	*40.58*	*106.57*
Aromatic esters																
Ethyl phenylacetate	12.78	2.46	59.17	3.86	5.69	2.00	68.54	3.36	15.94	2.11	3.38	5.90	17.38	2.31	1.31	3.45
Ethyl cinnamate	1.07	0.62	0.82	0.37	0.30	0.19	0.59	0.28	0.55	0.24	0.24	0.65	0.55	0.25	0.11	0.20
Ethyl dihydrocinnamate	0,38	0.24	0.11	0.14	0.08	0.12	0.11	0.13	0.09	0.13	0.04	0.15	0.10	0.16	0.04	0.17
Phenylethyl acetate	59.11	96.75	9.83	46.89	27.75	107.80	12.28	80.24	18.01	159.46	18.95	67.29	24.07	359.99	13.02	215.15
Sum	*73.34*	*100.07*	*69.93*	*51.26*	*33.82*	*110.11*	*81.52*	*84.01*	*34.59*	*161.94*	*22.61*	*73.99*	*42.1*	*362.71*	*14.48*	*218.97*
Minor esters																
Methyl butyrate	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.
Methyl hexanoate	1.02	0.87	0.77	0.90	1.31	0.77	0.72	1.03	0.78	0.61	0.57	0.65	0.68	0.39	0.54	0.64
Methyl octanoate	0.97	0.59	0.98	0.60	1.65	0.52	0.88	0.71	0.92	0.39	0.79	0.43	0.82	0.24	0.72	0.35
Methyl decanoate	0.19	0.14	0.42	0.17	0.26	0.14	0.40	0.18	0.17	0.09	0.29	0.16	0.12	0.04	0.27	0.10
Isoamyl butyrate	0.54	0.46	0.13	0.45	0.49	0.43	0.80	0.46	0.28	0.47	0.27	0.49	0.25	0.42	0.23	0.43
Isoamyl hexanoate	1.27	1.41	0.28	1.26	1.36	1.44	0.27	1.38	0.85	1.38	0.89	1.22	0.81	1.11	1.00	1.29
Isoamyl octanoate	2.94	2.97	0.47	2.66	2.40	3.42	0.41	3.05	1.47	3.00	2.66	2.07	1.39	1.96	4.46	2.54
Ethyl trans-2-hexenoate	1.18	1.42	1.99	0.95	1.20	1.32	1.82	0.73	0.73	1.46	1.58	0.70	0.99	0.79	0.91	0.83
Isobutyl hexanoate	0.15	0.12	0.04	0.13	0.14	0.12	0.05	0.13	0.08	0.12	0.08	0.16	0.07	0.10	0.05	0.12
Methyl geranate	3.44	2.02	0.05	0.03	0.90	2.18	0.02	0.05	1.84	2.16	0.23	0.03	1.86	0.74	0.17	0.18
Ethyl valerate	1.05	0.99	1.69	0.93	1.18	0.89	1.51	0.87	0.98	0.92	0.46	0.92	0.74	0.68	0.37	0.63
Ethyl heptanoate	0.61	0.62	0.41	0.41	0.41	0.67	0.37	0.35	0.37	1.03	0.15	0.61	0.32	0.62	0.13	0.31
Ethyl nonanoate	0.35	0.50	0.37	0.36	0.25	0.45	0.36	0.36	0.22	0.45	0.28	0.52	0.20	0.33	0.27	0.28
Sum	*13.71*	*12.11*	*7.60*	*8.85*	*11.55*	*12.35*	*7.61*	*9.30*	*8.69*	*12.08*	*8.25*	*7.96*	*8.25*	*7.42*	*9.12*	*7.70*
Sum of odorant esters	89.58	106.02	135.57	105.09	66.32	113.83	164.74	104.63	71.32	122.49	32.45	111.03	73.97	97.95	28.11	86.80
Terpenes																
Linalool	< L.Q.	5.53	< L.D.	< L.Q.	< L.D.	9.93	< L.Q.	< L.D.	13.45	160.73	18.26	67.75	10.48	92.44	17.24	101.43

L.D.: limit of detection (concentration for signal/noise = 3); L.Q.: limit of quantification (concentration for signal/noise = 10) (Antalick *et al.*, 2010). Rootstock (IAC572 and Paulsen P1103); Harvest corresponding to: 2015_1 (first harvest in 2015); 2015_2 (second harvest in 2015); 2016_1 (first harvest in 2016); 2016_2 (second harvest in 2016). Rootstock (IAC 572 and 1103 Paulsen).

TABLE 3. Concentrations of individual volatile compounds in ‘Arinto’ and ‘Fernão Pires’ wines produced from different rootstocks and harvest date (seasons). (part 3/3)

Terpineol	11.58	14.24	5.68	13.52	8.90	21.93	9.03	8.69	65.29	192.19	42.11	198.80	58.27	64.14	18.93	70.70
β-citronellol	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.Q.	< L.D.	< L.D.	< L.D.	< L.Q.	< L.D.	< L.Q.
Nerol	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	5.95	< L.D.	< L.Q.	< L.D.	< L.Q.	< L.D.	< L.Q.
Geraniol	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	20.25	< L.Q.	10.23	< L.D.	13.78	< L.Q.	14.77
Sum	*11.58*	*19.77*	*5.68*	*13.52*	*8.90*	*31.86*	*9.03*	*8.69*	*78.74*	*379.12*	*60.37*	*276.78*	*68.75*	*170.36*	*36.17*	*186.90*
Higher alcohols																
Propanol	38.25	29.38	21.45	23.04	37.35	25.29	20.05	24.91	32.04	29.08	31.41	22.17	29.42	38.44	31.19	33.59
Isobutanol	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.	< L.D.
Butanol	1.32	1.48	0.81	0.86	1.17	1.34	0.72	0.97	0.74	1.11	0.73	0.76	0.92	1.47	0.74	1.01
2-methylbutanol	6.28	17.78	6.85	15.94	12.90	16.22	6.58	14.94	8.14	16.43	7.52	13.44	7.49	18.17	7.69	17.88
3-methylbutanol	115.00	118.79	43.11	91.69	89.65	109.92	40.85	96.43	68.54	116.16	56.76	88.08	62.98	125.84	62.64	107.10
2-methylpropanol	22.58	19.22	14.48	17.17	17.32	17.58	18.68	17.35	12.44	18.99	12.06	18.55	10.75	21.19	8.60	20.38
2-phenylethanol	12.59	13.09	5.17	8.96	11.88	13.36	8.88	12.35	6.00	16.35	4.66	12.19	7.65	14.82	3.77	13.30
Sum	*196.02*	*199.74*	*91.87*	*157.66*	*170.27*	*183.71*	*95.76*	*166.95*	*127.90*	*198.12*	*113.14*	*155.19*	*119.21*	*219.93*	*114.63*	*193.26*
Acids																
Butyric	4.41	2.67	1.45	2.44	5.25	2.83	2.45	3.60	2.13	3.94	2.51	3.82	3.32	3.37	2.17	3.79
Isobutyric	2.86	2.20	7.00	1.58	3.57	2.25	1.60	2.35	2.00	3.54	1.84	2.15	2.39	2.83	1.27	2.65
Isovaleric	1.43	1.44	2.93	1.04	1.61	1.58	0.99	1.39	1.02	2.32	0.82	1.45	1.36	2.08	0.73	1.88
Hexanoic	12.01	10.01	6.30	9.95	15.04	9.85	9.82	11.33	9.84	10.52	12.64	11.23	12.25	9.13	11.31	11.38
Octanoic	17.22	13.29	11.25	15.81	21.80	14.79	16.99	14.97	17.88	12.44	25.65	12.76	18.93	11.78	22.00	14.92
Decanoic	2.77	2.54	4.60	3.63	3.37	3.42	3.84	3.21	2.72	2.19	8.61	4.01	2.87	2.42	6.58	3.85
Dodecanoic	0.17	0.04	0.04	0.06	0.05	0.05	0.06	0.05	0.03	0.02	0.16	0.09	0.04	0.03	0.17	0.06
Sum	*40.87*	*32.19*	*33.57*	*34.51*	*50.69*	*34.77*	*35.75*	*36.90*	*35.62*	*34.97*	*52.23*	*35.51*	*41.16*	*31.64*	*44.23*	*38.53*
Miscellaneous compounds																
Methanol	39.46	35.22	58.22	25.56	41.39	23.02	57.71	27.93	31.83	20.11	17.20	19.15	27.26	17.64	17.57	20.59
Ethanal	19.21	30.68	18.99	10.32	10.68	25.56	14.13	12.58	12.13	26.90	9.52	14.92	5.00	28.16	11.76	18.69

L.D.: limit of detection (concentration for signal/noise = 3); L.Q.: limit of quantification (concentration for signal/noise = 10) (Antalick *et al.*, 2010). Rootstock (IAC572 and Paulsen P1103); Harvest corresponding to: 2015_1 (first harvest in 2015); 2015_2 (second harvest in 2015); 2016_1 (first harvest in 2016); 2016_2 (second harvest in 2016). Rootstock (IAC 572 and 1103 Paulsen).

20 °C) and medium-high temperatures throughout the day (28–33 °C). The temperatures between November and March are higher (24–28 °C during nights, and 35–42 °C during days) (Figure 1). These climatic conditions can explain the lowest concentrations of the major esters in this period, by reduction of the biosynthesis (Schwab *et al.*, 2008). The same conditions of the second semester influenced and promoted an increase in the phenolic concentrations in grapes and wines in previous studies, confirming our hypothesis for volatiles (Padilha *et al.*, 2019; Oliveira *et al.*, 2019a; Oliveira *et al.*, 2019b).

3.2. Higher alcohol acetates

Six higher alcohol acetates were identified and quantified (Table 3). The most abundant compounds were isoamyl acetate (aroma of banana) and propyl acetate (fruity, solvent). Isoamyl acetate, hexyl acetate, and the sum of all compounds were higher in all wines elaborated from the second semester in both rootstocks (except for 'Fernão Pires' wines from 2016 on 1103 Paulsen), due to the best maturation of the grapes in the second and fourth harvests, as explained before according to the climatic conditions (Figure 1). The other compounds had different responses according to the cultivar, rootstock and harvest season, including propyl acetate, the second most concentrated in the wines. The concentration of isoamyl acetate ranged from 179.74 µg.L^{-1} in 2016_1 (IAC 572) to 815.54 µg.L^{-1} in 2015_2 (IAC 572) in 'Arinto' wines, while in 'Fernão Pires', varied between 161.84 µg.L^{-1} in 2015_1 (1103 Paulsen) and 2010.46 µg.L^{-1} in 2015_2 (IAC 572) (Table 3). Previous studies showed that the concentrations of isoamyl acetate in Portuguese white wines were 1150 µg.L^{-1} in 'Arinto', and 2060 µg.L^{-1} in 'Fernão Pires' (Barros *et al.*, 2012). Ziegler *et al.* (2020) reported concentrations of hexyl acetate in 'Riesling' wines from Germany between 23.8 µg.L^{-1} and 89.0 µg.L^{-1}, which presents a very cold and different climate as compared to São Francisco Valley. Differences in concentration are highly influenced by the age of the vines, climate, soil, winemaking and other factors, which play a very important role in biosynthetic pathways for volatile formation (Schwab *et al.*, 2008).

3.3. Ethyl esters of branched aliphatic acids, and aromatic esters

Three compounds were identified and quantified (Table 3). Ethyl isobutyrate was the more concentrated in this group, presenting aromatic descriptions of fruits, such as strawberry and kiwi, with sweet but also solvent in high concentrations. In 'Arinto' wines, this compound was higher in the second semester of 2015 in both rootstocks and conversely, in 2016, wines presented the highest amounts in the first semester for both rootstocks. The cv. 'Fernão Pires' presented different behaviour. The wines from the second semester for both rootstocks in 2015 and 2016 presented the highest amounts for 1103 Paulsen. For IAC 572, only wines from 2016_2 presented the highest concentrations of ethyl isobutyrate. In this case, the genetic effect observed for cv. 'Fernão Pires' was the main factor explaining the differences observed (van Leeuwen *et al.*, 2020). Different responses were observed for the other two volatile compounds, ethyl isovalerate (fruity, cheese) and ethyl 2-methylbutyrate (fruity, kiwi).

Four compounds were identified as aromatic esters (Table 3), and two different behaviours were observed. The first one was the compounds ethyl phenylacetate (flowery notes, rose, winy) and ethyl cinnamate (fruity notes, such as cherry, fig, and flowery), which presented the highest concentrations

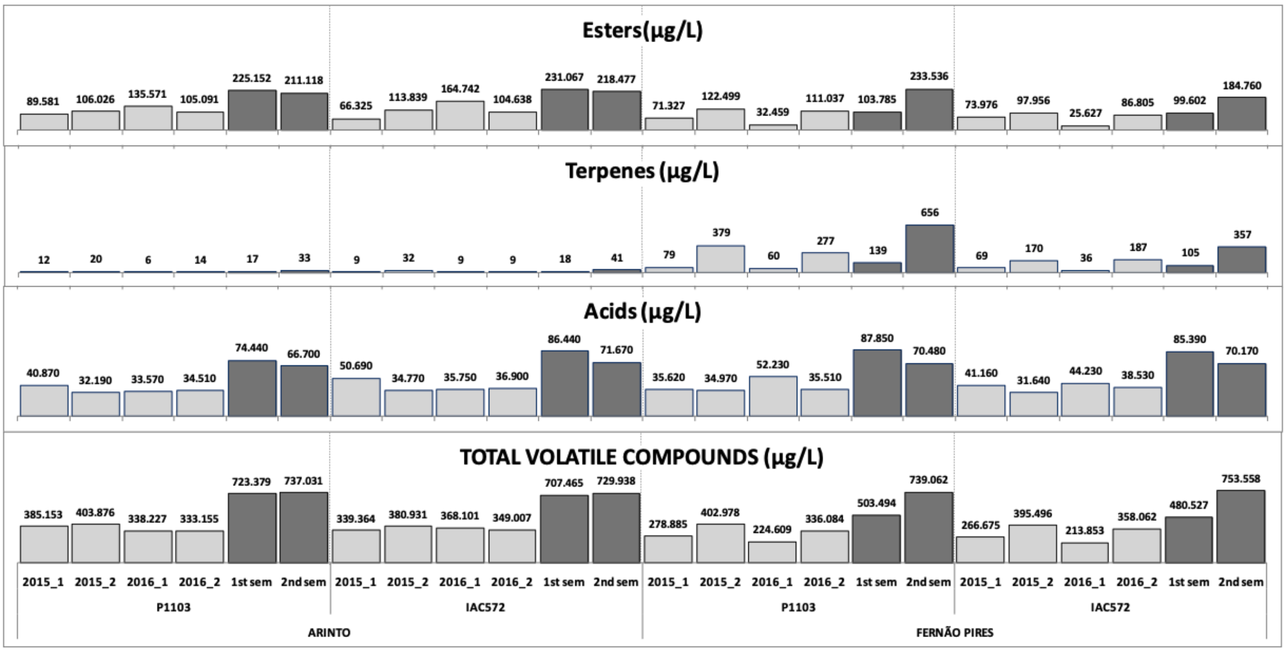

FIGURE 2. Sum of concentrations of all individual volatile compounds by chemical families, in 'Arinto' and 'Fernão Pires' wines, produced over four consecutive harvest seasons, by semester. Harvests correspond to: 2015_1 (first harvest in 2015); 2015_2 (second harvest in 2015); 2016_1 (first harvest in 2016); 2016_2 (second harvest in 2016). Vines were grafted onto two different rootstocks (1103 Paulsen and IAC 572).

in wines from 'Arinto' in both rootstocks, over all four harvest seasons. The highest amounts of ethyl phenylacetate were observed in the third harvest season (2016_1) in both rootstocks. The second behaviour was observed for the compounds ethyl dihydrocinnamate (fruity, pineapple, almond), and phenylethyl acetate (flowery, mimosa, fruity, olive, honey). Except for Arinto wines from 2015_1 and 2015_2 in both rootstocks, in all other wines, these compounds also presented the highest amounts in 2015_2 and 2016_2 (Table 3). 'Arinto' wines from Portugal presented 413 µg.L^{-1} of phenylethyl acetate, higher than the results obtained in the present study, which variation can be related to the age of the vines and climatic conditions (Barros *et al.*, 2012). Previous studies referred to the variation in concentration of phenylethyl acetate between 4.8 µg.L^{-1} and 45.8 µg.L^{-1} in 'Riesling' wines (Ziegler *et al.*, 2020). The sum of all aromatic esters also presented similar responses as compared to the two metabolites described before. In this case, except for the 2016 'Arinto' wines from 1103 Paulsen, for the other wines the sum was higher during the second semester for Arinto and Fernão Pires wines for both vintages.

3.4. Minor esters and terpenes

In this group of volatiles, thirteen compounds were identified (Table 3). The most abundant compounds determined were isoamyl octanoate (fruity, such as pear, wax and soap) and methyl geranate (fruity, pear). It is important to highlight that methyl geranate was identified and quantified in 'Arinto' and 'Fernão Pires' wines, and the concentrations ranged from 0.02 µg.L^{-1} in 'Arinto' (2016_1 in the rootstock IAC 572) to 3.44 µg.L^{-1} in 'Arinto' wines (2015_1 in the rootstock 1103 Paulsen) (Table 3). This compound was identified first in red wines, but it was referred to be more concentrated in white wines, such as 'Gewürztraminer'. In the present study, methyl geranate presented higher concentrations in both white wines as compared to the red wines described from many terroirs in France (Antalick *et al.*, 2010). Englezos *et al.* (2016) showed higher concentrations of methyl octanoate, methyl decanoate, and ethyl nonanoate than results obtained in the present study, using different yeasts. We can suppose that the interaction of different climatic conditions, age of the vines, rootstock, soil management and winemaking can explain these differences (van Leeuwen *et al.*, 2020).

Five metabolites were identified in the group of terpenes (Table 3). The most important terpenes quantified in the wines were terpineol (flowery, oil, anise, mint), presented in all wines, and linalool (flowery, muscat, lavender), mostly in 'Fernão Pires' wines. Except for 'Arinto' wines from 2016_1 and 2016_2 in the rootstock IAC 572, in all other wines, this metabolite and the sum of all terpenes presented the highest amounts in 2015_2 (second harvest season) and 2016_2 (fourth harvest season) (Table 3). Linalool was determined in all 'Fernão Pires' wines. Terpenes present in wines are highly dependent on grape cultivar and climatic conditions, which can explain the high differences found in this study between 'Arinto' and 'Fernão Pires' (Song *et al.*, 2018). Terpineol was evaluated in Portuguese white wines, and the concentrations were 22.4 µg.L^{-1} of terpineol, and 24.9 µg.L^{-1} of linalool in 'Fernão Pires', but they were not detected in 'Arinto' wines (Barros *et al.*, 2012). Terpineol and linalool were also quantified in in skin and pulp of 'Arinto' grapes studied in Portugal (Cabrita *et al.*, 2006). In 'Fernão Pires' grapes cultivated in Portugal, the amount of linalool was higher (66 %) than other terpenes (Coelho *et al.*, 2007). These compounds are very sensitive and can be quickly transformed, promoting a reduction and even loss of their intensity and participation in the olfactory wine profile (Schwab *et al.*, 2008). Previous studies showed that monoterpene interactions impact aroma perception, but that also, the ratios of the enantiomers are crucial to aroma perception when low and medium concentrations of other monoterpenes are present (Chigo-Hernandez *et al.*, 2022).

3.5. Higher alcohols, acids, and miscellaneous compounds

Seven volatile compounds were identified and quantified as higher alcohols (Table 3). The most abundant compounds observed were 3-methylbutanol and propanol, with descriptive notes of herbaceous, with strong and pungent tastes and smells. These compounds are synthesised by decarboxylation and reduction of α-keto acids produced as intermediates of amino acids synthesis and catabolism (Ayestarán *et al.*, 2018). Except for propanol and 2-methylpropanol, which presented variations, all other higher alcohols and the sum of all metabolites presented the highest amounts in the wines produced with grapes harvested in the second semester (2015_2 and 2016_2), corresponding to the second and fourth harvests, for both cultivars and rootstocks. It is possible to highlight that the vigour of the rootstocks had different behaviour according to the cultivar. In 'Arinto' wines, the lowest concentration of 3-methylbutanol was observed in the rootstock IAC 572, while the highest amounts was observed in the rootstock 1103 Paulsen (lower vigour than IAC 572). Conversely, in 'Fernão Pires' wines, the lowest concentration was observed in the rootstock 1103 Paulsen, while the highest amount was observed in the rootstock IAC 572. This behaviour is supposedly due to the scion/cultivar and rootstock compatibility, which is highly variable and dependent on the genetic and climatic adaptation of each cultivar to a specific terroir (van Leeuwen *et al.*, 2020).

The metabolite 2-phenylethanol is an important higher alcohol, whose olfactory notes are described as flowery, rose, and sweet (Rocha *et al.*, 2006). The rootstock effect was different for 2-phenylethanol when compared to 3-methylbutanol. This behaviour reinforces the specific response between cultivar/rootstock effects for each metabolite, explained by different metabolic pathways in the formation of volatiles in grapes and wines (Slaghenaufi & Ugliano, 2018). 2-phenylethanol is a volatile compound formed from the amino acid phenylalanine, and the 2-phenylacetaldehyde is its immediate precursor in the biosynthesis, which occurs preferentially in berry skin rather than in the juice or pulp (Slegers *et al.*, 2015). The sum of all higher alcohols in the wines of 'Arinto' and 'Fernão Pires' did not exceed 350 mg.L^{-1}, which is the limit recommended for all white wines, providing positive olfactory notes and complexity. Concentrations above this value are not desirable as they bring negative notes, such as soap, solvent and waxy, which are unfavourable to wine quality (Ayestarán *et al.*, 2018).

For acids, seven metabolites were identified and quantified (Table 3). The most abundant metabolites in the wines were octanoic acid and hexanoic acids, and the concentrations varied according to the harvest season, cultivar and rootstock. In 'Arinto' wines, the highest amounts of acids were observed with the rootstock IAC 572, while in 'Fernão Pires' wines, the highest values were observed with the rootstock 1103 Paulsen (Table 3).

Finally, two other compounds were determined in the wines, methanol and ethanal (or acetaldehyde), which are considered defects in wines (van Leeuwen *et al.*, 2020).

Methanol is a volatile harmful component produced from the demethylation of pectin by pectinolytic enzymes that are naturally present in fruits such as grapes during alcohol fermentation of wine (Lee *et al.*, 2022). In the present study, the amounts varied depending on the harvest season and the cultivar, and the concentrations were below the limit authorised by OIV (400 mg.L^{-1}) (Table 3).

The acetaldehyde (*i.e.*, ethanal) is a potent negative volatile metabolite found in wines, foods and other beverages, which produces olfactory notes of green, grassy, herbaceous, or green apple-like off-flavour. Normally these notes are present when the grapes were harvested green, a few days before the best maturation to deliver the positive aroma that valorises the wine typicality for a given cultivar. In this study, the concentrations of both defects changed also depending on the harvest season, rootstock and cultivar, and the concentrations are below the threshold in wines, which is between 100–125 mg.L^{-1} (Liu & Pilone, 2000).

3.6. Principal component analysis of wines

PCA was applied to different results to evaluate which set of parameters was most effective in discriminating between wines according to the treatments. Firstly, PCA was applied to chemical attributes, and wines were mostly separated according to the harvest date, and the first two principal components (PC) explained 65.3 % of the total variability (Figure 3A). PC1 explained 40.1 %, and in the positive axis are located wines from the second semester of 2015 and 2016 (second and fourth harvests), mainly characterised by alcohol degree and pH, while in the negative axis of PC1

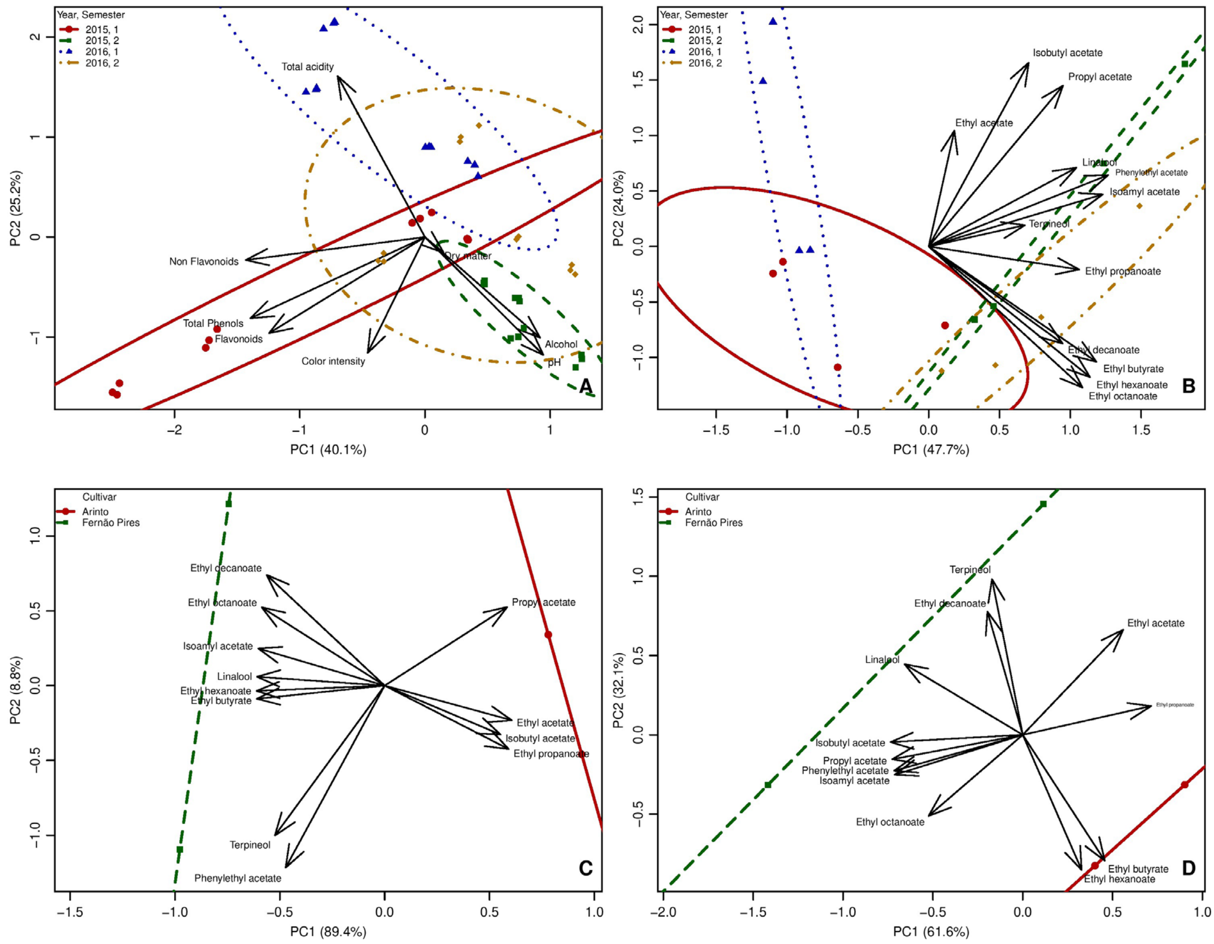

FIGURE 3. Principal component analyses (PCA) from physicochemical analysis of Arinto and Fernão Pires white wines (A), and from volatile metabolites, according to the four consecutive harvest dates (seasons) and two rootstocks (B), and evaluated in individual seasons, in 2016_1 (C) and 2016_2 (D).

are the wines from the first semester of 2015 and 2016 (first and third harvests), mainly characterised by phenolics, colour and density. PC2 explained 25.2 % of the variability, and differences were variable according to the variety and rootstock. Volatile compounds were more distinguishable than chemical analysis, because PCA obtained from the most representative volatile compounds, explained 71.7 % of total variability (Figure 3B). PC1 explained 47.7 %, and also separated all wines from the second semesters, located in the positive axis, characterised by the highest concentrations of the metabolites phenylethyl acetate, isoamyl acetate, linalool, ethyl propanoate, ethyl hexanoate, and ethyl octanoate, as confirmed by Table 3. These compounds can be considered as markers of the wines from the second semester, obtained from grapes harvested from the best weather for maturation (Figure 1). Wines from the first semester of 2015 and 2016 are located in the negative axis of PC1, characterised by volatile acids (Table 3). PC2 explained 24.0 % of the variability, and differences were due to the variety, followed by rootstock. Previous studies confirmed that volatile composition is highly influenced by harvest season (Šuklje *et al.*, 2018).

When we evaluated individual harvest dates (season), the effect of the cultivar was higher than rootstock. PC1 × PC2 accounted for 98.2 % of the variability in the first season of 2016, PC1 explained 89.4 % of the variability, and 'Arinto' wines were placed in the positive axis, which loadings characterising these wines were propyl acetate, ethyl acetate, isobutyl acetate, and ethyl propanoate (Figure 3C). 'Fernão Pires' wines are located in the negative axis of PC1, and the volatiles were ethyl decanoate, ethyl octanoate, phenylethyl acetate and terpineol. PC2 explained 8.8 % of the variability and separated the rootstocks. Evaluating the second season of 2016, the results were slightly different. PC1 × PC2 explained 93.7 % of the variability, PC1 accounted for 61.6 % of the variability and separated the cultivars, 'Arinto' wines are located in the positive axis, characterised by the metabolites ethyl propanoate, ethyl butyrate and ethyl hexanoate, while 'Fernão Pires' wine from IAC 572 rootstock was placed in the negative axis of PC1, which loadings were phenylethyl acetate, isoamyl acetate, propyl acetate, and isobuthyl acetate. PC2 explained 32.1 % of the variability and separated in the positive axis 'Fernão Pires' wine from 1103 Paulsen, which presented the highest concentration of terpineol and ethyl decanoate (Figure 3D). The effect of rootstock was also evaluated and results showed that, in 2016, wines were differently separated, according to major ethyl esters. PC1 × PC2 accounted for 99.9 % of the variability in the first season of 2016, PC1 explained 97.3 % of the variability, and wines from IAC 572 and from 1103 Paulsen were placed together in the positive axis, which loadings characterising these wines were ethyl propanoate and ethyl acetate (Figure 3E). The second season of 2016 was different, separating wines from rootstocks. PC2 explained 43.2 % of the variability, and wines from 1103 Paulsen were placed in the positive axis, with loadings characterising these wines were ethyl decanoate, ethyl acetate and ethyl propanoate, while in the negative axis were wines from IAC 572 rootstock, characterised by ethyl octanoate, ethyl hexanoate and ethyl butyrate (Figure 3F). Rocha *et al.* (2006) described that the most important compounds discriminating between 'Fernão Pires' and 'Arinto' wines were the esters and acids. Terpineol is synthesised in the grape berry by the α-terpinoel synthase, one of the first monoterpene synthases identified and functionally characterised in grapes (Lin *et al.*, 2019). We can point out that in the dry season with moderate temperatures and high solar radiation (second semester), the rootstock with the lowest vigour promoted the synthesis of terpineol for 'Fernão Pires', but not for 'Arinto', showing that the scion and rootstock interaction is highly dependent on the climatic and genetic factors. Coelho *et al.* (2007) suggested that linalol, α-terpineol, and geraniol can be used as markers of the maturation for 'Fernão Pires' in grapes during the maturation. Other studies showed differences in the rootstocks on wine volatile composition, mainly due to the vigour and influence on the compound biosynthesis (Wang *et al.*, 2019). Results obtained in previous studies showed that the climate was the main factor influencing the phenolic and volatile composition of white and red grapes and wines (Dutra *et al.*, 2018; Gutiérrez-Gamboa *et al.*, 2018; Oliveira *et al.*, 2019b).

CONCLUSION

The present study demonstrated that the chemical composition and volatile profile of wines from the 'Arinto' and 'Fernão Pires' cultivars in a tropical semi-arid climate were strongly influenced by the season of harvest, followed by the effects of cultivar and rootstock. Wines produced from grapes harvested in the second semester exhibited greater oenological potential, with higher concentrations of volatile compounds responsible for aromatic complexity, such as ethyl esters and higher alcohol acetates.

The use of GC/FID and GC/MS analyses confirmed that compounds such as phenylethyl acetate, isoamyl acetate, linalool, ethyl propanoate, ethyl hexanoate, and ethyl octanoate were key in differentiating second-semester wines, highlighting the influence of climate on the synthesis of these metabolites. Additionally, the first-time detection of methyl geranate in 'Arinto' and 'Fernão Pires' wines suggests its potential as a marker of typicity for these varieties in tropical regions.

PCA analysis highlighted that seasonality was the main distinguishing factor for the wines, while rootstock had a secondary but still relevant impact on the modulation of chemical and aromatic composition. The results reinforce the viability of both cultivars for tropical wine production, opening new possibilities for the diversification of viticulture in the São Francisco Valley and other similar climatic regions.

Future studies could explore the interaction between phenolic maturation and aromatic profile, as well as the influence of different management strategies to optimise varietal expression and the sensory quality of tropical wines.

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