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Kaolin foliar spray induces positive modifications in volatile compounds and fruit quality of Touriga-Nacional red wine

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ABSTRACT

Solar radiation and temperature play crucial roles in grapevine metabolic processes and are known to have a positive impact on grape berry composition; however, excessive exposure to these factors can be detrimental. Kaolin-based particle film technology has emerged as a valuable solution for mitigating the effects of heat and water stresses in vineyards. This work aimed to evaluate the effects of kaolin application on the phenolic composition, chromatic characteristics, oenological parameters, volatile compounds and sensory profile of red wine. The study was carried out in one growing season in the commercial vineyard "Quinta dos Aciprestes" in the Douro Superior sub-region. Twelve rows in triplicate of the Touriga-Nacional variety underwent foliar kaolin treatment (applied at the pre-veraison stage, at the manufacturer-recommended and -tested dosage of 5 % (w/v)), and another twelve rows comprised the non-treated control group. Kaolin increased the phenolic compound and tartaric acid concentrations (2.4 % and 20.8 % respectively), total acidity (2.4 %), the deep reddish colour of the berries, total and coloured anthocyanin (2.8 %), and total and polymeric pigments (3.6 %); meanwhile, a decrease was observed in pH (-1.4 %) and alcoholic degree (-4.8 %). No significant differences were observed in any sensory parameters between the wine from the kaolin-treated and control vines, but the tasters found the aroma of the former to be fruitier and more complex, with an agreeable acidic taste and persistence. It was possible to group the volatile compounds into two distinct groups based on the results of the Pearson's correlation matrix. This grouping corresponds to the sensory descriptors common to each of the respective volatiles. Overall, the results further support the potential efficacy of utilising kaolin to alleviate summer-related stress in grapevines.

KEYWORDS: Climate change mitigation, kaolin application, sensory profile, wine secondary metabolites, wine quality

INTRODUCTION

The fields of viticulture and winemaking offer numerous social, economic and environmental advantages, including the establishment of local identities, the generation of rural income, job creation and the promotion of tourism. The fundamental pillars of the industry are the sustainable production of grapes and the maintenance of high-quality standards. However, this is all at risk of being adversely impacted by climate change in the coming decades (Fraga *et al.*, 2016; Ollat *et al.*, 2017). The recurrence of the combined increase in mean air temperature and changes in rainfall patterns poses a risk to crop yield and quality. Extreme temperatures (>35 °C) during the growing season can severely effect leaf photosynthetic efficiency and berry metabolism (Chuine *et al.*, 2004), this effect being exacerbated under water deficit conditions (Roby *et al.*, 2004). As a result, the sector's economy is expected to be negatively impacted due to warmer and dryer climates, with Portuguese viticulture being challenged (Fraga *et al.*, 2016), namely the renowned Douro Demarcated Region (DDR).

The metabolite composition of grape leaf and berry, and thus wine quality, are negatively affected by high temperatures and water deficit: sugar levels increase, resulting in turn in an increase in wine alcohol percentage (Dinis *et al.*, 2020). For example, in Sauvignon Blanc, an increase in light intensity and high temperatures were found to affect the primary and secondary grape metabolites, responsible for changing the final wine sensory attributes (Scheiner *et al.*, 2010). To mitigate summer stresses, some winegrowers have invested in deficit irrigation strategies. However, given the high natural limitations in water resources, large scale water capitation systems and distribution are very costly and and environmentally unsustainable. In light of this, and in order to ensure economic sustainability and grape and wine quality, it is crucial to develop mitigation alternatives. Our research group has thus been trying to understand the complex physiological and biochemical grapevine responses to the application of exogenous compounds (Bernardo *et al.*, 2017; Dinis *et al.*, 2018; Brito *et al.*, 2019) and how they affect wine quality (Dinis *et al.*, 2020).

Kaolin (Al2Si2O5(OH)4) is a white inert clay mineral that reflects potentially damaging ultraviolet and infrared radiation, which is much higher than photosynthetically active radiation (Shellie and King, 2013). In grapevine, a film of kaolin particles applied to the vegetation has been found to lead to lower leaf temperature and higher protection of the photosystem II structure and function in leaves exposed to excessive solar radiation (Dinis *et al.*, 2016a; Dinis *et al.*, 2018). In grape berries, kaolin's effects on total soluble solid content have been found to be very inconsistent, depending on the grape variety and the number and timing of the applications (Lobos *et al.*, 2015; Kok and Bal, 2018; Luzio *et al.*, 2021). Meanwhile, most of the literature on the subject reports increased levels of anthocyanin and other flavonoids in berries from kaolin-treated vines, which are triggered by several molecular mechanisms involved in the synthesis of phenolics, such as the phenylpropanoid and flavonoid pathways (Conde *et al.*, 2016; Dinis *et al.*, 2016a; Bernardo *et al.*, 2022). In a study in which kaolin and leaf removal were simultaneously applied, the resulting Sauvignon Blanc white wine was perceived to have an abundance of tropical or fruity notes (Coniberti *et al.*, 2013). A white wine (Cerceal cv.) obtained from vines treatad with kaolin, was found to have a lower alcohol degree, higher total acidity and malic and tartaric content, as well as a higher content of esters with <C12, which are associated with fruity notes. In a three-season study conducted on Cabernet Sauvignon (Brillante *et al.*, 2016), while no significant differences in sensory wine attributes were observed, the wine from kaolin-treated plants was judged to be more attractive in appearance and was appreciated slightly more than the control. Compared to white varieties, knowledge is lacking regarding red wines produced from vines treated by kaolin and their oenological properties, primary and secondary metabolites, and sensory attributes (Coniberti *et al.*, 2013; Ferrari *et al.*, 2017; Dinis *et al.*, 2020). Despite efforts to characterise the impacts of kaolin application on red wine quality, more research is needed on its effects on the accumulation of secondary metabolites and the composition of volatiles in the wine, and its relationship with sensory attributes. In this context, we aimed to evaluate effects of foliar kaolin application to the red grapevine variety Touriga-Nacional cv. in the Douro Region. For this purpose, we studied several variables of wine attributes: alcoholic degree, acidity, volatile compounds, primary and secondary metabolism and sensory profiles.

MATERIALS AND METHODS

1. Wine samples

The experiment was performed in 2017 in the Douro Region (in the Douro Superior sub-region, the hottest and driest one). We chose the *Vitis vinifera* L. red variety Touriga-Nacional due to its ability to ripen under intense heat and because it is representative of Portuguese red wines in terms of quality and typicity. In the commercial vineyard "Quinta dos Aciprestes" (41° 12′ N; 7° 29′ W; 150 m above sea level), the selected rows were divided into two groups (Figure 1): twelve rows in triplicate (i.e., a total of 36 rows) comprised the kaolin-treated group, while the other 12, not receiving any treatment and also in triplicate, comprised the control group. Kaolin particle film (Surround® WP, Engelhard Corporation, Iselin, NJ, USA) was applied to the koalin-treated group at the pre-veraison stage (early July). It was prepared in an aqueous solution at the manufacturer-recommended dosage of 5 % (w/v), supplemented with 0.1 % (v/v) Tween ® 20 (Sigma-Aldrich, St. Louis, MO, USA, CAS number 9005-64-5) to improve adherence. After preparation, it was immediately applied to the leaves following standard operating procedures that had been adjusted for agricultural use. The berries were harvested manually to prevent the grapes from the different treatments getting mixed together during transport and in the cellar. Wine was produced from these two groups of berries following a commercial winemaking process for DOC Douro. The harvested grapes were crushed to release their

FIGURE 1. Experimental plot (between the red lines) in the commercial vineyard "Quinta dos Aciprestes" (*Real Companhia Velha* Company) located in the Douro Demarcated Region (Douro Superior sub-region).

juice and pulp, and the juice, skins and seeds were transferred to fermentation vessels to which commercial wine yeasts (*S. cerevisiae*) were added. The yeast strain was chosen and the fermentation carried out according to enterprise practices in the same way for the koalin treatment and the control. After fermentation, the wine was transferred to stainless steel tanks for ageing and to undergo stabilisation processes.

2. Oenological parameters

The wine samples were analysed for oenological parameters (i.e., pH, total acidity and alcoholic degree), following the the OIV methodologies (OIV, 2003). The tartaric acid concentration in the wines was measured enzymatically (Miura One, TDI S.A.).

3. Colour and chromatic characterisation and content of anthocyanins and pigments

Wine colour intensity was determined from the sum of absorbance at 620, 520 and 420 nm (1 mm cell), and tonality as the ratio of absorbance at 420 and 520 nm (OIV, 2009). The absorption spectra of the wine samples were scanned over the 380–780 nm range using 1 mm quartz cells. Data were collected to determine L^* (lightness), a^* (measure of redness), and b^* (measure of yellowness) coordinates using the CIELab method (OIV, 2009). The Chroma $[C^* = [(a^*)^2 + (b^*)^2]^{1/2}]$ and hue-angle [H = tang_1(b^*/a^*)] values were also determined. Additionally, total and coloured anthocyanin and total and polymeric pigment contents were determined according to the method proposed by Somers and Evans (1977).

4. Biochemical analysis

The aluminium (Al) concentration was determined by atomic absorption spectrometry in a graphite furnace (Unicam 939 AA spectrometer, GF90 furnace) according to Dinis *et al.* (2020). Each run of samples was preceded by calibration using aqueous mixed standards prepared in HNO_3 (1.0 M). For this purpose, five different dilutions of standard (0 - 0.050 ppm) were used, as well as the blank; the range of concentrations were selected based on the expected concentrations. Total phenolic content was determined from the absorbance at 280 nm, according to Kramling and Singleton (1969). The results were expressed as gallic acid equivalents employing calibration curves and using gallic acid as the standard. All the analyses were performed in triplicate.

5. Analysis of the wine for volatile compounds

Briefly, 4 mL of the wine samples, 50 µL of internal standard acetyl valeryl (21.41 mg/L), and 1.2 g of sodium chloride (≥99.5 %, Sigma-Aldrich, St. Louis, MO, USA) were placed in vials (12 mL) and thermostated at 40.0 ± 0.1 °C. Then, the volatile compounds were extracted using a 50/30 µm fused silica fibre coating with divinylbenzene/carboxen/poly(dimethylsiloxane) (DVB/CAR/PDMS) (Supelco Inc, Bellefonte, PA, USA), which was inserted into the headspace and continuously stirred at 250 rpm for 30 min. Three independent aliquots were analysed per bottle, with a total of 3 bottles per type of wine (kaolin and control types).

Next, the SPME fibre was manually inserted into the injection port (250 °C) of the equipment GC × GC-ToFMS LECO Pegasus 4D (LECO, St. Joseph, MI, USA). The inlet was lined with a 0.75-mm I.D. glass liner and splitless injection mode was used (30 s). The GC × GC-ToFMS system comprised an Agilent GC 7890A gas chromatograph (Agilent Technologies, Inc., Wilmington, DE), with a dual stage jet cryogenic modulator (licensed from Zoex) and a secondary oven. A DB-FFAP column (nitroterephthalic-acid-modified polyethylene glycol, 26 m × 0.25 mm I.D., 0.25-µm film thickness, J&W Scientific Inc., Folsom, CA, USA) and an Equity-5 column (5 % diphenyl/95 % dimethyl siloxane, 0.79 m × 0.25 mm I.D., 0.25-µm film thickness, Supelco, Inc., Bellefonte, PA, USA) were used for first and second dimensions respectively. Helium was used as the carrier gas at a constant flow rate of 2.50 mL/min. The following temperature programmes were used: the primary oven temperature increased from 40 °C (1 min) to 150 °C at 6 °C/min, and then increased to 230 °C (1 min) at 20 °C/min. The secondary oven temperature programme was the same as the primary oven temperature programme offset by 5 °C (higher). Both the MS transfer line and MS source temperatures were 250 °C. The modulation period was 3 s, with the modulator offset by 15 °C above the primary oven temperature programme, with hot and cold pulses in periods of 0.80 and 0.70 s respectively. The ToF analyser was operated at a spectrum storage rate of 125 spectra/s, with a mass spectrometer running in the EI mode at 70 eV and detector voltage of − 1497 V, using an m/z range of 35–350. Automated data processing software ChromaTOF® (LECO) was used to process total ion chromatograms at a signal-to-noise threshold of 100. For identification purposes, the mass spectrum and retention times of the analytes were compared with the standards when available. The mass spectrum of each peak was compared to those existing in mass spectral libraries: an in-house library of standards and two commercial databases (Wiley 275 and US National Institute of Science and Technology (NIST) V.2.0–Mainlib and Replib). Moreover, a manual analysis of mass spectra was performed using additional information; i.e., a linear retention index (RI) value, experimentally obtained using a van Den Dool and Kratz RI equation (van Den Dool and Kratz, 1963). To determine the RI, a C8-C20 n-alkanes series was used (solvent n-hexane was used as C6 standard), comparing the values with those reported in existing literature for chromatographic columns similar to a first dimension column. Quantification was performed using the internal standard method, and the concentration of each volatile component was expressed in µg/L acetyl valeryl equivalents.

6. Sensory analysis of wine samples

A sensory evaluation of the (one-year-old) wines was performed via a QDA test (Quantitative Descriptive Analysis), using a list of selected descriptors. The descriptors were scored from 1 to 5, where 1 corresponded to the sensory characteristic not being perceived and 5 to it being intense. The evaluations were carried out at the UTAD laboratory by a panel of twelve tasters, of which ten belonged to the Tasting Panel of DeBA/ECVA-UTAD (Departamento de Biologia e Ambiente/Escola de Ciências da Vida e do Ambiente- Universidade de Trás-os-Montes e Alto Douro, and had been trained in the sensory evaluation of food and beverages. The panel comprised 70 % women with an average age of 46.7 years old, and 30 % men with an average age of 45.7 years old. Each panelist conducted the evaluations between 10:00 and 12:00 in individual booths under white lights (ISO 8589, 2007) and under controlled temperature (20 °C ± 2 °C) and relative humidity (60 ± 20 %) conditions. Recommended glassware was used according to ISO 3591 (1977). The glasses were filled with a volume of 50 mL of wine in all the tasting sessions for the purposes of repeatability. The samples were presented randomly (ISO 6658, 2017) and codified with a 3-digit alpha-numeric code.

7. Statistical analysis

The statistical analyses were performed using SPSS 20.0 software. After testing for ANOVA assumptions (homogeneity of variances with Levene's mean test and normality with the Kolmogorov–Smirnov test), statistical differences among treatments were evaluated by one-way factorial ANOVA. Differences were considered significant when $p < 0.05$; specifically, *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$. The absence of superscript indicates no significant difference between treatments. Values are presented as mean ± standard deviation (SD). Regarding volatile compounds, a hierarchical cluster analysis (HCA) combined with the heatmap visualisation and Principal Component Analysis (PCA) were applied to the dataset using the MetaboAnalyst 3.0 (web software, The Metabolomics Innovation Centre (TMIC), Edmonton, AB, Canada). The area of each variable was auto-scaled. The significance of the differences between the kaolin-treated and control samples in the detected compounds was tested using a two-sided Mann–Whitney test (using the SPSS software 20.0 (IBM, New York, NY, USA).

The Pearson correlation coefficient was used to measure the correlation between the oenological attributes and the volatile compounds that showed values higher than the detection limit found in the literature, as well as to identify the significant differences between the two analysed types of wines.

RESULTS AND DISCUSSION

1. Weather conditions

Weather data (Figure 2) were recorded by a weather station located 100 meters from the experimental site. 2017 was a very hot year, with 36 days of maximum temperatures above 35 ºC. February was the month with the highest rainfall (about 10 mm); in June, July and August the average temperatures were above 25ºC and precipitation levels were close to zero (4.2, 6.8 and 0 mm respectively).

2. Effects of kaolin particle film on red wine oenological parameters

One of the most significant impacts of climate change is the production of wines with higher alcoholic degrees and lower acidity, mainly in warm regions (Mira de Orduña, 2010) like the Douro.

Therefore, the evaluation of these parameters is very important. The results of the analyses for the main oenological parameters in the wines from koalin-treated plants are shown in Figure 3 and in Supplementary Table 1.

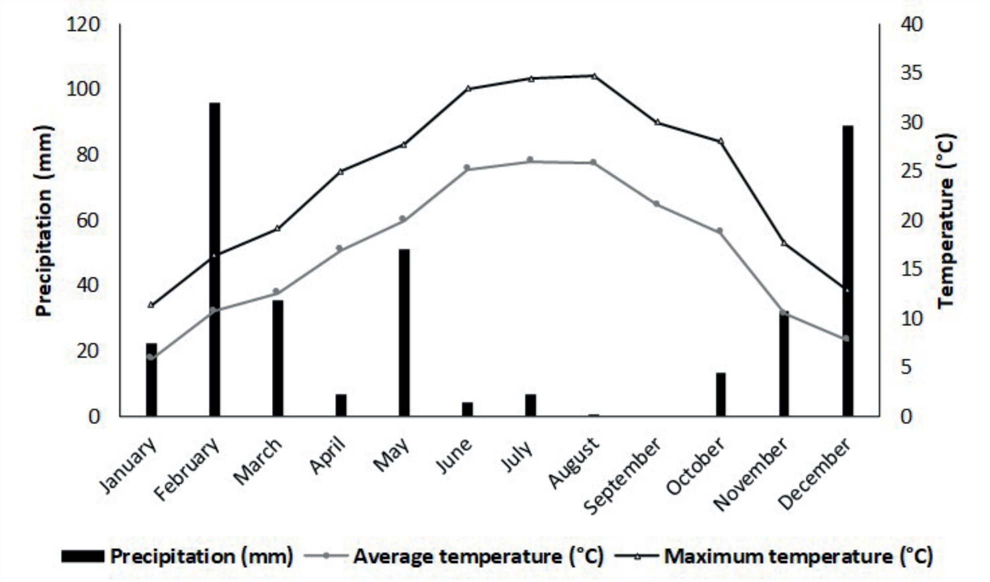

FIGURE 2. Monthly climatic conditions (total precipitation and average temperatures) of the experimental site during the year of 2017.

Kaolin treatment resulted in a wine with significantly higher total acidity (+2.41 %) and tartaric acid (+ 20.8 %) and lower pH (-1.42 %) and alcoholic degree (-4.81 %) than the control wine, and thus it was more balanced. A similar increase in total acidity has been found in previous works (Ou *et al.*, 2010; Frioni *et al.*, 2019; Valentini *et al.*, 2022; Teker, 2023). In a previous study by Dinis *et al.* (2020), the wine produced using berries from vines treated with kaolin also showed a lower alcoholic degree and higher tartaric acid levels and, consequently, higher total acidity. The reduction in alcohol content can be attributed to the shading effect that kaolin has on grapevine berries, which reduces water loss from the fruit and can also delay the maturation process (Coniberti *et al.*, 2013).

In the present study, the higher levels of tartaric acid in the wines made from kaolin-treated berries, and thus their higher total acidity, can be attributed to the a reduced degradation of the acids due to the healthy leaves protecting the berries from the sun as well as to the shading effects of koalin (Dinis *et al.*, 2020). Due to the higher acid concentration of the wine from the koalin-treated berries, its pH was lower. Higher concentrations of organic acids, particularly tartaric acid, are desirable in grapevine varieties that thrive in warm climates, such as the region under study and other areas highly vulnerable to climate change. As a result, the application of kaolin shows potential for producing well-balanced wines, while decreasing the need for extensive and expensive must or wine acidification processes.

3. Kaolin effect on the chromatic characteristics of red wine

In addition to CIELAB, C and H colour space parameters are used by some industry professionals since they correlate well with how the human eye perceives colour. The colour of red wine is an essential sensory attribute for customers, who tend to prefer deep red (González-Neves *et al.*, 2014). The colour of red wine will depend on various factors, including the initial monomeric anthocyanin profile, the presence of acetaldehyde, pyruvic acid and other yeast metabolites, and the formation of stable polymeric pigments (Cheynier *et al.*, 2006; Hayasaka *et al.*, 2007). In the present study, the effects of kaolin on wine colour were evaluated; the results are shown in Table 1. Accordingly, applying kaolin had the effect of increasing the a^* parameter and simultaneously decreasing the b^* parameter. In CIELAB, a^* represents the green-red axis. An increase in a^* indicates a shift towards the red spectrum, resulting in a more reddish coloration of the wine. Meanwhile, b^* represents the blue-yellow axis and its decrease indicates a shift towards the blue spectrum, giving a bluish tint to the wine colour. L^* values were lower in the wine from koalin-treated berries, which also showed the highest anthocyanin content: i.e., they were darker. The lower L^* and higher a^* values observed in the wines from berries treated with kaolin indicate the darker colour of the grape skin (Qin *et al.*, 2022). Additionally, a decrease was observed in the chroma (C^*ab) and the lightness (L^*) values. There were relevant differences in hue angle (h_{ab}), which were higher in the wine from the control vines. All the samples had hue values of between 60° and 120°, which correspond to colours that are intermediate between yellow and green. The significant reduction in chroma (-2.13 %) and hue angle (-0.65 %) observed in the wine from kaolin-treated berries corresponds to a slight increase in the deep reddish colour. The total and coloured anthocyanins and total and polymeric pigments of the wines from the control and kaolin-treated vines are shown in Figure 4: a significant increase in all these parameters as a result of the kaolin application can be observed. Brillante *et al.* (2016) and Dinis *et al.* (2016a) also observed an increase in anthocyanin content after kaolin application. According to the authors, kaolin increases shade, leading to the downregulation of gene expression in the anthocyanin biosynthesis pathway (Jeong *et al.*, 2004; Koyama and Goto-Yamamoto, 2008; Conde *et al.*, 2016).

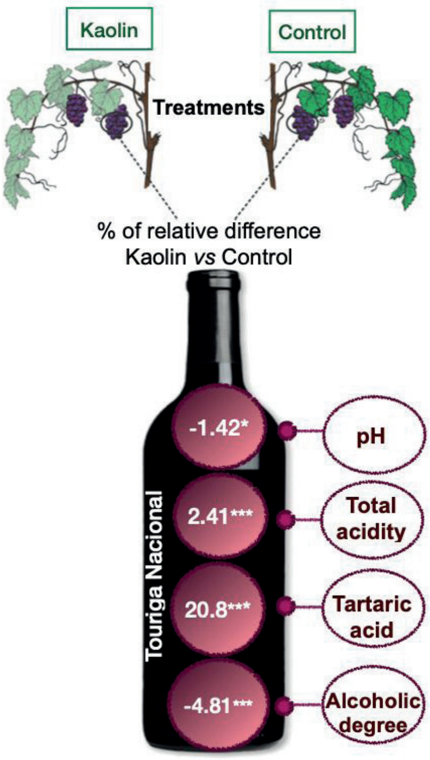

FIGURE 3. Main oenological parameters analysed in the 'Touriga Nacional' cv. wines: pH, total acidity, tartaric acid content and alcoholic degree. The results are expressed as a percentage change (increases and decreases) in the parameters of wine from kaolin-treated vines compared to wine from untreated vines (control). Significant differences were considered when $p < 0.05$, more specifically when *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$. The absence of superscript indicates no significant difference between treatments, according to the one-way factorial ANOVA.

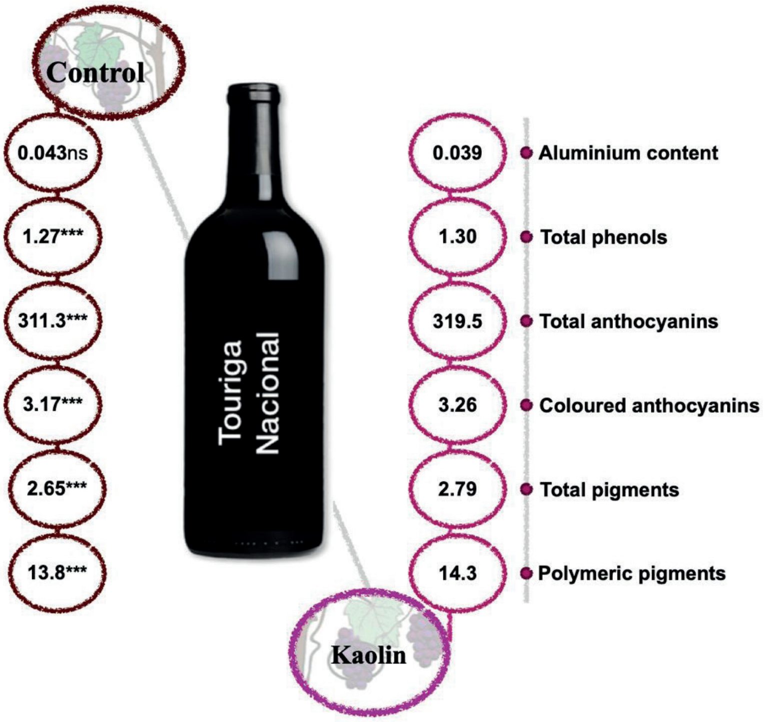

FIGURE 4. Biochemical parameters analysed in the 'Touriga Nacional' cv. wines: aluminium content, total phenols, total and coloured anthocyanins, and total and polymeric pigments. The results are expressed as mean values of the parameters obtained in wines from kaolin-treated and untreated vines (control). Significant differences were considered when $p < 0.05$, more specifically when *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$. The absence of superscript indicates no significant difference between treatments, according to the one-way factorial ANOVA.

TABLE 1. Chromatic parameters: colour intensity, tonality, lightness (*L**), *a**, *b**, chroma (*C**) and hue angle (*H*) of the 'Touriga Nacional' cv. wines from kaolin-treated and untreated vines (control). The results are expressed as mean values ± standard deviation.

Treatment	Intensity colour (A.U.)	Tonality (A.U.)	L*	a*	b*	C*	H*
Control	11.1 ± 0.006***	0.745 ± 0.001***	11.8 ± 0.047	43.2 ± 0.072***	42.3 ± 0.070	61.0 ± 0.100	0.766 ± 0.001
Kaolin	11.4 ± 0.006	0.761 ± 0.001	11.2 ± 0.062***	44.4 ± 0.075	41.2 ± 0.132***	59.7 ± 0.130***	0.761 ± 0.002**
significance	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.006

* Significant differences were considered when $p < 0.05$, more specifically when *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$. Absence of superscript indicates no significant difference between treatments, according to the one-way factorial ANOVA.

Additionally, kaolin has a temperature-lowering effect. A study by Coniberti *et al.* (2013) demonstrated that kaolin spraying results in a temperature decrease of approximately 5°C in Sauvignon blanc berries. Temperature is a critical factor in berry anthocyanin biosynthesis since the optimal temperature range for this process is around 30°C. When temperatures exceed 35°C, anthocyanin accumulation ceases (Spayd *et al.*, 2002) and can even lead to anthocyanin degradation (Mori *et al.*, 2007; Azuma *et al.*, 2012; Carbonell-Bejerano *et al.*, 2013). In fact, in this study, the average maximum temperatures were 33.3, 34.4 and 34.6 °C in June, July and August respectively (Figure 2), which can affect anthocyanin biosynthesis. Therefore, by reducing berry temperature, kaolin helped maintain the optimal temperature range for anthocyanin biosynthesis, resulting in enhanced accumulation of these pigments, and ultimately intensifying grape and wine colour. Furthermore, Uhlig (1998) has reported significant damage and tissue death in over 40 % of the clusters on the defoliated sides of the vines when high temperatures prevailed. These conditions initially impact the chlorophyll pigments, causing a decline in yellow carotenoid pigments and in turn a browning of the lesions (Greer *et al.*, 2003). Thus, the reduction in leaf and berry temperature caused by kaolin application, especially in hot years like 2017, may explain the increase in the pigments observed in this work.

4. Effects of kaolin particle film features on red wine biochemical composition

The effects of kaolin particle film on Al concentration and total phenolic compounds of both red wines are shown in Figure 4. The most common of the numerous concerns that winemakers have regarding kaolin application is the amount of aluminium (Al) that can be found in the grapes and vines, since kaolin is an aluminium silicate. However, the results of the present work indicate that the berries and musts did not assimilate the powdered Al, since the concentrations of Al detected in the wine from kaolin-treated vines were even lower than those found in the control wine, although no significant differences were observed. Similar results were found in work by Dinis *et al.* (2020). According to these authors, one plausible explanation for this is that the pH of kaolin is altered as it settles in the soil, making it less acidic, and, as a consequence, Al solubility decreases and its absorption by the vines is minimised.

In the present study, the foliar kaolin application significantly increased the concentration of total phenols. These results correspond with those of previous studies, in which kaolin application also increased the content of the phenolic compounds (mainly phenolics) in the wine (Conde *et al.*, 2016; Dinis *et al.*, 2016a). In response to kaolin application, phenylpropanoid is generally stimulated at gene expression and/or protein activity levels (Conde *et al.*, 2016). This stimulation is expected to have a positive impact on the quality of the berries and wines, as well as on the protection of the vines against abiotic stressors (Dinis *et al.*, 2020). Kaolin particle film has been found to reduce the amount of radiation reaching plant tissues, leading to a decrease in canopy temperature and to relief from heat stress and sunburn (Dinis *et al.*, 2016b). This mechanism, along with its positive impact on photosynthesis, may have contributed to the higher concentrations of phenolics and anthocyanins in the wines from the vines treated with kaolin reported in this study; these comprise the phenolic compounds usually produced in leaves when under stress, but which, due to the leaves being in more comfortable conditions as a result of the koalin, have been translocated to the berry, since they no longer need to be mobilised in response to the stressful summer conditions.

5. Effects of kaolin on volatile compounds

The effects of kaolin on the volatile compounds in the wines were evaluated by applying a clustering analysis (Figure 5). The heatmap is a graphical representation of the chromatographic results obtained for the volatile components (Table 2 and Supplementary Table 2), which allows a rapid visual evaluation of the wines' volatile profiles to be carried out. The different shades of the chromatic scale of the heatmap indicate the relative amounts of each volatile component (dark blue to dark red = minimum to maximum). The dendrogram is an exploratory tool that reveals two clusters (with Euclidean distance of 60) corresponding to the two types of wines (Figure 5A); i.e., the control and the wine produced from kaolin-treated grapes. Table 2 contains the 95 volatile components detected in both types of wines distributed over 6 chemical families: acids, alcohols, esters, norisoprenoids, phenols and terpenic compounds. Their odour thresholds and aroma properties are also reported. Furthermore, the influence of kaolin treatment on the volatile profiles of the wines is evident in the Principal Component Analysis (PCA – Figure 5B), where PC1 and PC2 account for 58.5 % of the variability observed in the dataset. Two distinct clusters corresponding to the wines under investigation are

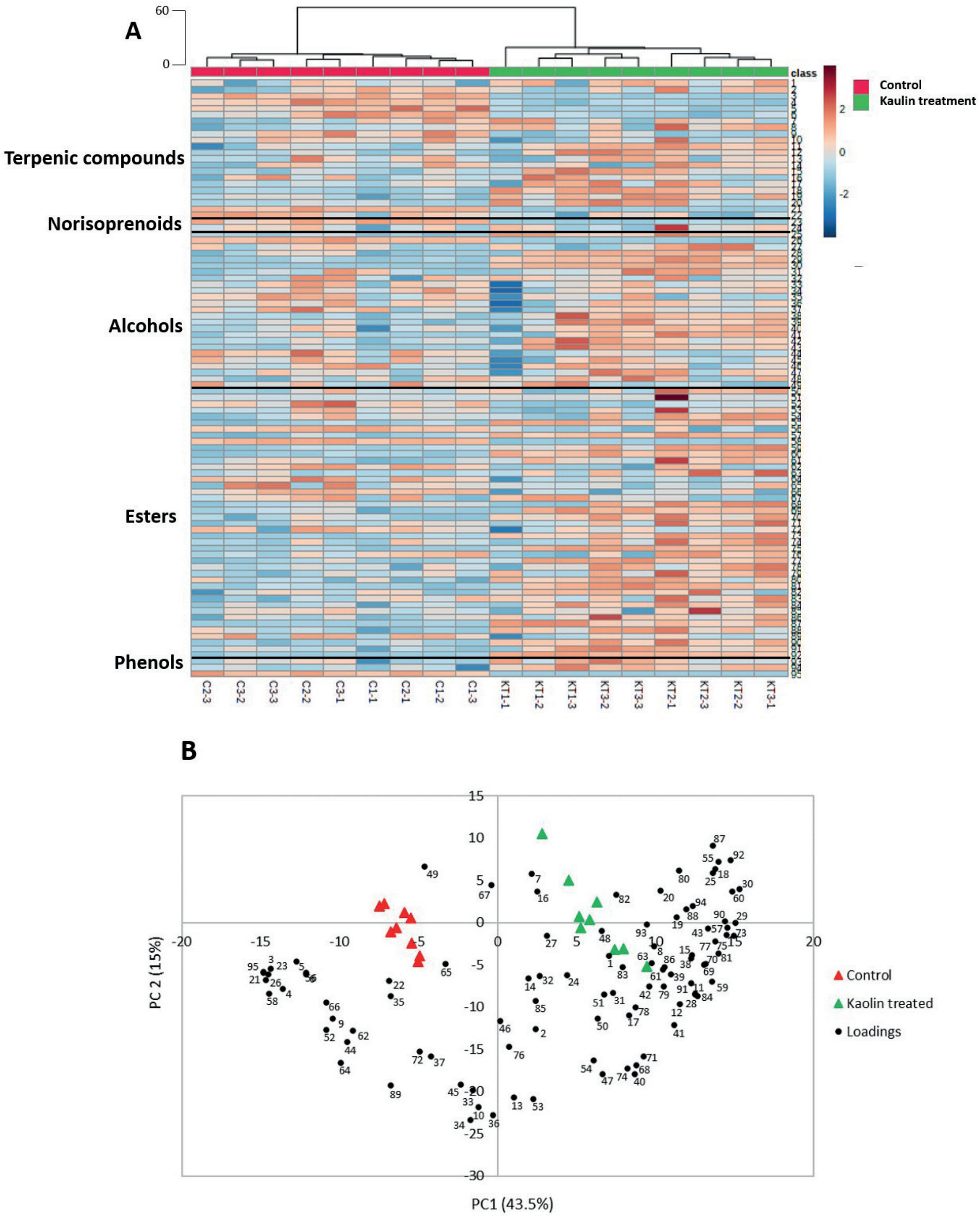

FIGURE 5. Statistical processing of the 95 volatile components from Touriga Nacional wines under study, referred to as control and kaolin-treated grapevines, which reveals the differences between the classes of wines: A) Heatmap and dendrogram representation, in which the content of each compound is indicated using different colours (dark blue to to dark red = minimum to maximum). A dendrogram for the HCA results using Ward's cluster algorithm on the data set is also included; B) PC1×PC2 biplot illustration, which represents 58,5 % of the dataset variability. Peak number assignment in Table S2.

discernible and dispersed through PC1 (represents 43.5 % of the data set variability). The wine produced from the kaolin-treated grapes, located at PC1 positive, is characterised by a higher number of volatiles. Both the statistical analyses (Figures 5 A and B) reveal that the wine produced from kaolin-treated grapes contained a higher amount of the majority of esters, alcohols and volatile phenols than the control wine. In fact, significant differences in 63 % of the detected components (corresponding to 60 components) were found between the two types of wines (differences corresponding to $p < 0.05$), and, of these, 41 (43 %) showed an increase with the application of kaolin, and, therefore, 19 compounds (20 %) showed a decrease. Previous studies have demonstrated that kaolin treatment has an influence on both the primary and secondary metabolites of grapes (Conde *et al.,* 2016; Conde *et al.,* 2018; Dinis *et al.* 2016). Given that the volatile composition of wine arises from a complex combination of factors, notably including the metabolic processes of the grapes themselves, the kaolin treatment can be expected to yield discernible variations in the resulting wine product, as can be observed in Figure 5.

Of all the aroma compounds found in wine, terpenic compounds receive the most attention and are the most studied due to their floral, rose, citrus, pine and mint aromas (Black *et al.*, 2015). In this work, kaolin led to a significant increase in six terpenic compounds and a decrease in seven of them (Table 2).

The wine from treated vines exhibited higher ester content than the control wine, with significant increases in 23 compounds (Table 2), while the conrol wine showed significant increases in only seven ester compounds. Esters are compounds that exhibit aromas and flavours reminiscent fruits, such as bananas, strawberries, pineapple, raspberries and cherries, and which can even be citrusy and floral (Lytra *et al.*, 2013; Cameleyro *et al.*, 2017; Luo *et al.*, 2022). Over 160 esters have been identified in wine, many of which exist in concentrations below human sensory perception. Furthermore, esters can interact with one another, resulting in an entirely distinct aroma when combined, and a single ester can significantly impact another's intensity, adding complexity to the sensory experience (de la Fuente Blanco *et al.*, 2023).

In the present study, ethyl octanoate ranged from 13.8 to 20.0 mg/L and was the most abundant ester to be found. These results indicate that ethyl octanoate played a vital role in the aromatic composition and appearance of the samples. In previous studies, the OAV (odour activity value) of ethyl octanoate reached a concentration of more than 4000 (Jiang *et al.*, 2013).

The same tendency was observed in alcohol compounds, with kaolin application resulting in a significant increase in the concentration of 9 compounds, while a decrease was observed in only three volatile components. The alcohol found in the highest quantity was phenyl ethyl alcohol, but there were no significant differences between the two analysed wines. Of the alcohols that showed significant differences between the two analysed wines, 1-octanol was found in the highest concentrations in this study, and was associated with a fresh, orange-rose aroma (Yue *et al.*, 2015).

The concentrations of three phenol components found in these wines showed significant differences between the two type of wines under study. However, guaiacol and phenol concentrations were higher in the wine from treated vines, while 4-ethylguaiacol concentrations was higher in the control wine. These three phenol components give wines a medicinal and sweet aroma, depending on their detection threshold (Milheiro *et al.*, 2019).

6. Effects of kaolin on the wine sensory profile

The effects of kaolin application on several parameters of the wine sensory profiles are shown in the form of a spider diagram in Figure 6. Although, the results of statistical processing showed no significant differences between the two types of wines in terms of any of the analysed sensory parameters (Figure 6), the tasters perceived slight differences between the wines. The wines from kaolin-treated vines were fruitier and more complex in terms of aroma and also had a more mineral and spicy flavour. In the mouth, they had a pleasant acidic taste and persistence. In work carried out by Ou *et al.* (2010), trained panellists detected a significant influence of kaolin application on wines made from vines grown under 35 % of their Estimated Transpirational Requirements ($P < 0.05$), indicating the effect of an interaction between the kaolin and vine water status. In this study, kaolin application significantly increased the fresh fruit aroma of the wines and decreased the spicy flavour and bitter taste. In other work by Coniberti *et al.* (2013), leaf removal and kaolin application were associated with riper and fruity notes. In a more recent study by Brillante *et al.* (2016) wines from kaolin-treated vines were not perceived as being significantly different from the control; however, wines from vines treated with pinole (another film-forming antitranspirant applied to plants to reduce water loss), had a fruitier aroma.

7. Correlations between oenological attributes and volatile compounds

The correlations between oenological parameters and volatile compounds are shown in Figure 7. This figure visually represents the Pearson's correlation matrix between parameters at a 5 % confidence level using a red-to-blue colour scale, with the colours ranging from red $p = +1$ to blue $p = -1$. Despite this correlation analysis being reliable and mostly based on sufficient data, the fact that each coefficient was obtained from two groups (six observations) means that the results should be treated with caution. Considering this weakness, the heat map shows that most of the identified volatile compounds (13 out of 20) were positively correlated with the biochemical and oenological parameters (ranging from 0.559 to 0.996), except in the cases of pH and alcoholic degree. Interestingly, these 13 compounds have a very similar profile in terms of correlations. The remaining 7 compounds also showed similar behaviour

TABLE 2. Volatile components identified by HS-SPME/GC×GC-ToFMS from Touriga Nacional wines under study: control and kaolin-treated grapevine wines. Results are expressed as concentration μg/L, per equivalent of acetyl valeryl (internal standard). Statistical analysis was performed using one-way factorial ANOVA, and p values are included. Significant differences are considered when $p < 0.05$, more specifically when *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$. Absence of superscript indicates no significant difference between treatments. Reported odour descriptors and respective odour thresholds have also been added. # information not found in the literature for similar matrices. (part 1/4).

Compound	CAS	Concentration (μg/L)		*p*-values	Odor descriptor	Odor threshold (μg/L)	References
		Control wine	**Kaolin treated grapevines wine**				
			Terpenic compounds				
β-Myrcene	123-35-3	27.8 ± 2.17	29.8 ± 2.22	0.067	Herbaceous, wood	–#	El-Sayed (2016)
α-Terpinene	99-86-5	9.87 ± 1.45	10.0 ± 1.32	0.811	Lemon, wood	–	Burdock (2004)
Limonene	5989-54-8	173.3 ± 15.14	94.5 ± 5.55***	0.000	Lemon, orange, floral	15	Cejudo-Bastante *et al.* (2016); Wang *et al.* (2016); Liu *et al.* (2022)
trans-β-Ocimene	3779-61-1	12.0 ± 0.657	9.67 ± 0.38***	0.000	-	34	Tamura *et al.* (2001)
γ-Terpinene	99-85-4	18.6 ± 2.26	13.9 ± 0.645***	0.000	Woody, citrus	1000	Niu *et al.* (2020)
β-cis-Ocimene	3338-55-4	17.0 ± 2.75	11.5 ± 1.22***	0.000	Warm, floral, sweet odor	–	https://foodb.ca/compounds/FDB001462
Terpinolene	586-62-9	20.7 ± 2.98	21.8 ± 2.99	0.458	Pine, citric, sweet	–	Perestrelo *et al.* (2016)
α-Terpinolene	586-62-9	164.5 ± 15.4**	195.2 ± 27.4	0.010	Lime or citrus fruits	–	Slaghenaufi *et al.* (2021)
Linalool oxide	5989-33-3	17.2 ± 1.53	14.2 ± 1.31***	0.000	Floral, fruity	15 – 25	Styger *et al.* (2011); Wang *et al.* (2016)
Camphor	76-22-2	101.9 ± 8.69	94.4 ± 17.8	0.271	Camphor, fresh, minty	–	Amaro *et al.* (2022)
Linalool	78-70-6	704.3 ± 77.4***	836.3 ± 62.4	0.001	Floral with spicy tones and lemon aroma	50	Baron *et al.* (2017)
4-Terpineol	562-74-3	12.7 ± 0.518**	14.1 ± 1.21	0.005	Sweet, herbaceous	250	Yue *et al.* (2015)
Hotrienol	53834-70-1	9.70 ± 0.706	9.55 ± 0.873	0.705	Sweet, floral	–	Hui (2010)
Citronellyl acetate	150-84-5	16.1 ± 1.57	16.4 ± 1.77	0.783	–	–	
α-Terpineol	98-55-5	348.7 ± 20.8***	410.3 ± 39.0	0.001	Herbaceous, mint, grass, mandarin, citrus, fennel	250 – 300	Cejudo-Bastante *et al.* (2016); Wang *et al.* (2016); Ríos-Reina *et al.* (2020); Liu *et al.* (2022)
Citral	141-27-5	3.39 ± 0.569	3.53 ± 0.571	0.604	Lemon	–	Burdock (2004)
Geranyl acetate	105-87-3	4.91 ± 0.197	5.28 ± 0.593	0.092	–	–	
Citronellol	106-22-9	132.7 ± 6.51***	174.1 ± 13.9	0.000	Rose-like	40	Pardo *et al.* (2015)
Geraniol	106-24-1	52.2 ± 3.66**	59.6 ± 4.68	0.002	Rose-like	40 – 75	Pardo *et al.* (2015)
Nerol	106-25-2	161.9 ± 5.23**	181.7 ± 17.2	0.005	Fresh, sweet, rose-like	300	Pardo *et al.* (2015)
Dihydro methyl jasmonate	24851-98-7	8.51 ± 0.825	2.52 ± 0.374***	0.000	Sweet, fruity, floral aroma	15	Martínez-Gil *et al.* (2022); https://academic-accelerator.com/encyclopedia/methyl-dihydrojasmonate
Nerolidol	7212-44-4	53.2 ± 6.85	45.2 ± 7.03*	0.027	Floral green citrus woody waxy	7.5 – 150	Zalacain *et al.* (2007)

TABLE 2. (part 2/4).

Compound	CAS	Concentration (μg/L)		*p*-values	Odor descriptor	Odor threshold (μg/L)	References
		Control wine	**Kaolin treated grapevines wine**				
Norisoprenoids							
Vitispirane	65416-59-3	79.5 ± 13.6	21.3 ± 4.82***	0.000	Eucalyptus	800	Cheynier *et al.* (2010); Alexandre (2011); Wang *et al.* (2016)
β-Damascenone	23726-93-4	61.6 ± 4.40	63.6 ± 6.24	0.444	Cooked apple, floral, quince	4.5	Mendes-Pinto (2009)
Alcohols							
2-Butanol	78-92-2	195.7 ± 34.7***	374.9 ± 58.0	0.000	Fruity and wine	–	Wang *et al.* (2021)
1-Propanol	71-23-8	185.1 ± 21.1	20.3 ± 2.75***	0.000	Fresh, alcohol	306	Yue *et al.* (2015)
2-Methyl-1-propanol	78-83-1	195.4 ± 28.4	213.7 ± 51.4	0.366	Alcohol, wine like, nail polish	40000	Zea *et al.* (2007)
3-Pentanol	584-02-1	8.84 ± 0.496***	9.81 ± 0.497	0.001	Sweet herbal oily nutty	–	TGSC Information System (2023)
1-Butanol	71-36-3	196.3 ± 6.73***	246.0 ± 10.8	0.000	Medicinal, alcohol	150	Yue *et al.* (2015)
1-Penten-3-ol	616-25-1	1.87 ± 0.128***	20.5 ± 1.24	0.000	Ethereal horseradish green radish chrysanthemum vegetable tropical fruity	–	TGSC Information System (2023); http://www.ymdb.ca/compounds/YMDB15918
1-Pentanol	71-41-0	45.5 ± 6.28	50.8 ± 6.66	0.097	Bitter almond, synthetic, balsamic	676000	Zea *et al.* (2007)
1-Hexanol	111-27-3	1217.4 ± 163.0	1239.6 ± 113.8	0.742	Green, grass	8000	Li *et al.* (2008)
3-Hexen-1-ol	544-12-7	40.1 ± 2.07	39.0 ± 2.83	0.367	Green, bitter, fatty	1000	Peinado *et al.* (2004)
3-Octanol	589-98-0	20.2 ± 1.22	19.3 ± 2.13	0.248	–	–	
2-Octanol	123-96-6	11.2 ± 1.04	9.87 ± 1.39*	0.039	Mushroom oily fatty creamy grape	–	TGSC Information System (2023)
1-Octen-3-ol	3391-86-4	66.6 ± 3.29	64.4 ± 7.38	0.422	Mushroom	–	Gonçalves *et al.* (2014)
1-Heptanol	111-70-6	114.2 ± 9.76	106.1 ± 9.85	0.100	Wood, oil	–	Burdock (2004)
2-Ethyl-1-hexanol	104-76-7	23.1 ± 2.54***	31.8 ± 5.49	0.001	Waxy green orange aldehydic rose mushroom	–	TGSC Information System (2023)
3-Hepten-1-ol	10606-47-0	5.90 ± 0.449**	6.90 ± 0.794	0.005	Oily green metallic acrylate tomato spicy	–	http://www.perflavory.com/docs/doc1044351.html
4-Hepten-1-ol	20851-55-2	8.90 ± 1.05	9.88 ± 1.17	0.082	–	–	
(E)-2-Hepten-1-ol	33467-76-4	5.56 ± 0.408**	6.35 ± 0.689	0.009	Green, fatty	–	TGSC Information SystemÒ (2023)
2-Nonanol	628-99-9	80.2 ± 3.71*	88.5 ± 9.40	0.025	Fatty, mild, green, melon	–	Rocha *et al.* (2014)
1-Octanol	111-87-5	412.3 ± 20.5***	511.2 ± 46.3	0.000	Fresh, orange-rose	110 – 130	Pardo *et al.* (2015)
1-Nonanol	143-08-8	207.4 ± 26.3	163.7 ± 19.5***	0.001	Floral, green, fatty	58	Yue *et al.* (2015)
(6Z)-Nonen-1-ol	35854-86-5	10.5 ± 1.10	9.58 ± 1.93	0.230	–	–	
2-Undecanol	1653-30-1	16.8 ± 1.57	16.4 ± 2.23	0.672	–	–	
Benzenemethanol	100-51-6	587.5 ± 36.5	619.1 ± 83.1	0.312	Floral, sweet	900000	Zea *et al.* (2007); Xiao *et al.* (2017)
Phenylethyl Alcohol	60-12-8	2205.3 ± 420.5	2548.8 ± 421.5	0.103	Rose, honey	10000	Zea *et al.* (2007)
1-Dodecanol	112-53-8	22.0 ± 3.28	20.2 ± 4.26	0.332	Fat, wax	–	Burdock (2004)

TABLE 2. (part 3/4).

Compound	CAS	Concentration (µg/L)		*p*-values	Odor descriptor	Odor threshold (µg/L)	References
		Control wine	**Kaolin treated grapevines wine**				
			Esters				
Ethyl isobutanoate	97-62-1	174.0 ± 17.32	190.0 ± 31.0	0.195	Strawberry, melon	15	Zea *et al.* (2007)
Propyl acetate	109-60-4	104.2 ± 12.10	339.4 ± 535.0	0.206	Fruity (pear–raspberry)	–	Burdock and Fenaroli (2005)
2-Methylpropyl acetate	110-19-0	338.9 ± 51.6	245.8 ± 26.6***	0.000	Sweet, apple, tropical, banana aroma and a similar fruity taste	71.8	Lytra *et al.* (2013)
Ethyl 2-methylbutanoate	7452-79-1	74.4 ± 8.48	73.5 ± 18.0	0.892	Green-fruity, apple-like	–	Burdock and Fenaroli (2005)
Ethyl 3-methylbutanoate	108-64-5	210.2 ± 16.0	222.0 ± 26.4	0.268	Fruity	15	Xiao *et al.* (2017)
Butyl ethanoate	123-86-4	24.2 ± 3.73***	46.2 ± 4.14	0.000	Not found	–	–
2-Methylbutyl acetate	624-41-9	115.0 ± 7.52	77.2 ± 17.1***	0.000	Black-fruit (blackcurrant and blackberry) and banana notes	181 – 359	Cameleyre *et al.* (2017)
Ethyl pentanoate	539-82-2	38.2 ± 3.06***	49.5 ± 2.08	0.000	Sweet fruity apple pineapple green tropical	5.1	Wang *et al.* (2021)
Ethyl 2-butenoate	10544-63-5	58.4 ± 2.79	32.8 ± 1.89***	0.000	Not found	–	–
Methyl hexanoate	106-70-7	25.4 ± 1.22***	31.1 ± 2.85	0.000	Ethereal fruity pineapple apricot strawberry tropical fruit banana bacon	–	TGSC Information System (2023)
Ethyl isohexanoate	25415-67-2	4.55 ± 0.571***	9.23 ± 0.776	0.000	Sweet fruity pineapple waxy green banana	5	TGSC Information System (2023); Luo *et al.* (2022)
Ethyl hexanoate	123-66-0	4931.1 ± 678.3**	6091.5 ± 987.3	0.010	Green apple aroma	14	Castilhos *et al.* (2020)
Hexyl ethanoate	142-92-7	408.2 ± 28.6	343.4 ± 43.4**	0.002	Pleasant fruity, pear	1500	Peinado *et al.* (2004)
Ethyl (E)-3-hexenoate	26553-46-8	25.4 ± 1.56*	30.5 ± 5.02	0.010	Green fruity, rum and brandy aroma	–	Wang *et al.* (2021)
Ethyl trans-2-hexenoate	27829-72-7	73.0 ± 5.70	61.8 ± 4.69***	0.000	Sweet, green, fruity with a vegetative nuance	–	TGSC Information System (2023)
Isobutyl hexanoate	105-79-3	21.3 ± 3.87	19.5 ± 1.92	0.231	Apple, fruity, cocoa	–	Burdock and Fenaroli (2005)
Heptyl acetate	112-06-1	10.0 ± 0.402	8.43 ± 1.00***	0.000	Green, waxy, fatty, citrus, aldehydic, winey and woody	–	TGSC Information System (2023)
Ethyl 6-heptenoate	25118-23-4	206.7 ± 27.0	205.8 ± 26.2	0.943	–	–	
Methyl octanoate	111-11-5	225.0 ± 17.5	264.8 ± 58.2	0.067	Orange	203	Xiao *et al.* (2017); Niu *et al.* (2019)
Hexyl butyrate	2639-63-6	20.7 ± 1.35***	24.7 ± 1.94	0.000	Green, fruity, estry and vegetative with a waxy nuance	–	TGSC Information System (2023)
Ethyl octanoate	106-32-1	13839.5 ± 2592.3***	19996.4 ± 2737.6	0.000	Caramel and fruity odor	5	Sánchez-Palomo *et al.* (2019)
Isopentyl hexanoate	2198-61-0	198.6 ± 18.9	228.3 ± 37.8	0.051	Pineapple, cheese	1000	Li *et al.* (2008)
Butyl lactate	138-22-7	56.9 ± 5.71	52.1 ± 5.93	0.096	–	–	
Propyl octanoate	624-13-5	58.2 ± 5.67***	102.3 ± 14.3	0.000	Coconut	–	TGSC Information System (2023)

TABLE 2. (part 4/4).

Compound	CAS	Concentration (μg/L)		*p*-values	Odor descriptor	Odor threshold (μg/L)	References
		Control wine	**Kaolin treated grapevines wine**				
Ethyl nonanoate	123-29-5	239.5 ± 31.2	276.4 ± 55.3	0.100	Rose, fruity	1300	Li *et al.* (2008)
Ethyl 2-hydroxy-4-methylpentanoate	10348-47-7	172.1 ± 8.51***	249.3 ± 21.5	0.000	"Fresh blackberry'' aroma	900 and 300 lg/l, respectively, in dearomatized red wine and model wine solution	Falcao *et al.* (2012)
Butyl octanoate	589-75-3	39.9 ± 5.43	39.2 ± 9.12	0.859	–	–	
Diethyl malonate	105-53-3	4.11 ± 0.323***	5.34 ± 0.485	0.000	Sweet, green, apple and fruity	–	TGSC Information System (2023)
3-Nonenoic acid ethyl ester	91213-30-8	15.4 ± 1.83*	18.4 ± 3.38	0.036	Not found	–	–
Methyl decanoate	110-42-9	81.2 ± 7.43**	97.9 ± 14.8	0.008	Oily, wine, fruity, floral	–	Morais *et al.* (2022)
Methyl benzoate	93-58-3	0.914 ± 0.152***	1.21 ± 0.118	0.000	Chemical with a phenolic and cherry pit note	–	TGSC Information System (2023)
Ethyl methyl succinate	627-73-6	137.4 ± 9.43***	196.0 ± 25.0	0.000	Not found	–	–
Ethyl decanoate	110-38-3	8879.8 ± 767.1*	9731.5 ± 822.9	0.037	Burnt, floral	200	Luo *et al.* (2022)
Isoamyl octanoate	2035-99-6	280.0 ± 41.8	323.9 ± 48.4	0.056	Fruity	125	Ferreira *et al.* (2000); Burdock (2005)
Ethyl benzoate	93-89-0	93.0 ± 7.31***	110.3 ± 10.4	0.001	Sweet, somewhat heavy-fruity taste, remotely reminiscent of Black currant and Grape.	60	Wang *et al.* (2021)
Ethyl succinate	123-25-1	5359.3 ± 317.3	5466.9 ± 917.3	0.744	–	–	
3,7-Dimethyl-2,6-octadienoic acid methyl ester	2349-14-6	17.5 ± 2.10**	20.9 ± 2.81	0.009	Not found	–	–
Propyl decanoate	30673-60-0	10.1 ± 0.494***	22.2 ± 3.95	0.000	Waxy fruity fatty green vegetable woody oily fruity	–	TGSC Information System (2023)
Ethyl 4-hydroxybutanoate		251.4 ± 33.1***	327.8 ± 37.4	0.000	Camellic aroma	–	TGSC Information System (2023)
2-Phenylethyl acetate	103-45-7	1025.7 ± 104.7	885.6 ± 104.4*	0.012	Flowery, rose, honey	250	Cortés-Diéguez *et al.* (2015)
Ethyl dodecanoate	106-33-2	1003.7 ± 93.1***	1456.1 ± 138.7	0.000	Flowery, fruity	1500	Yue *et al.* (2015)
Ethyl 3-phenylpropanoate	2021-28-5	20.3 ± 2.13**	25.3 ± 3.40	0.002	Fruity, sweet	40	Liu *et al.* (2018)
Ethyl palmitate	628-97-7	75.0 ± 8.90***	215.2 ± 16.8	0.000	Apple, sweet	1500	Zhao *et al.* (2021)
			Phenols				
Guaiacol	90-05-1	3.81 ± 0.381*	4.29 ± 0.398	0.017	Smoked, phenolic and medicinal odours	20 – 23	Pollnitz *et al.* (2004); Parker *et al.* (2012)
Phenol	108-95-2	17.5 ± 1.88***	21.5 ± 1.41	0.000	Sickeningly sweet, irritating	7 – 100	Parker *et al.* (2012)
4-Ethylguaiacol	2785-89-9	9.32 ± 1.49	0.276 ± 0.040***	0.000	Spicy clove medicinal woody sweet vanilla	–	TGSC Information System (2023)

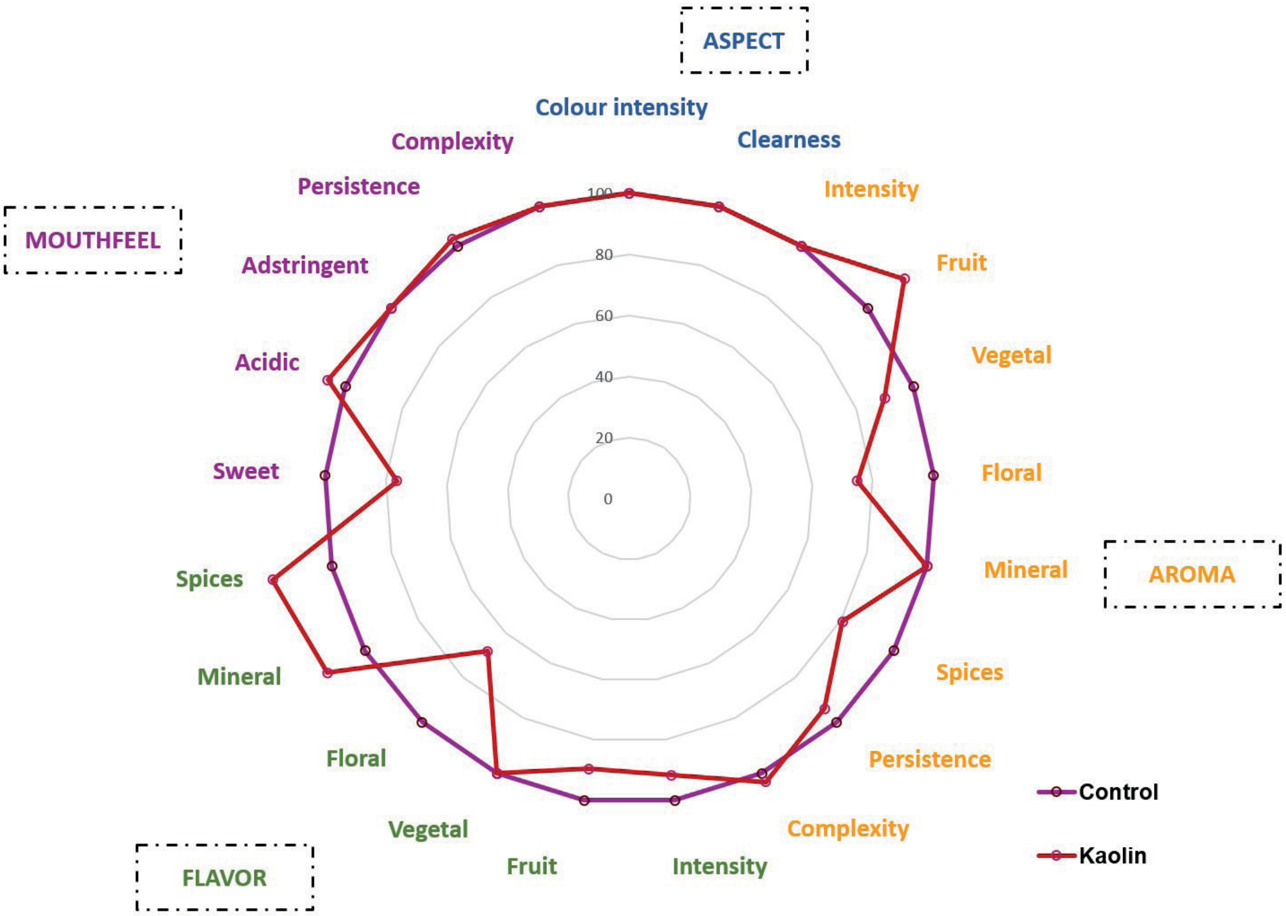

Figure 6. Sensory profile of the 'Touriga Nacional' cv. wines. The results are expressed as a percentage change (increases and decreases) in the attributes of wine from kaolin-treated plants compared to the wine from control vines. Significant differences are considered when $p < 0.05$, more specifically when *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$. The absence of superscript indicates no significant difference between treatments, according to the one-way factorial ANOVA.

to each other, as well as a profile that contrasted with the aforementioned 13 compounds, being positively correlated with pH (ranging from 0.673 to 0.868) and alcoholic degree (ranging from 0.716 to 0.996). Thus, it is possible to divide the volatile compounds in two different groups depending on their behaviour. The first group comprised linalool, α-terpineol, citronellol, geraniol, 1-butanol, 1-octanol, ethyl pentanoate, ethyl isohexanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl benzoate and phenol, most of which were associated with fruity notes. Meanwhile, the second group comprised limonene, trans-β-ocimene, linalool oxide, nerolidol, 1-nonanol, 2-methylpropyl acetate and 2-phenylethyl acetate, which were associated with floral notes.

In another study carried out by our team, we showed that kaolin application influenced grapefruit metabolism by increasing the phenolic content, tartaric acid content and total acidity (Dinis et a., 2020). In the present study the wines were deep reddish in colour and contained total and coloured anthocyanins and total and polymeric pigments. Meanwhile, a decrease in pH and alcoholic degree was observed due to kaolin application. No significant differences were observed in Al concentration, since the fruits did not absorb the powdered Al. No significant differences were found between the two types of wines in terms of sensorial attributes, indicating that the kaolin did not affect the wine's appearance, aroma, flavour or mouthfeel. However, the wines from the kaolin-treated vines were perceived as being fruitier, as observed by other authors (Ou *et al.*, 2010; Coniberti *et al.*, 2013; Brillante *et al.*, 2016). Although a number of studies have been carried out on the effect of kaolin on berries, little is known about its effect on wines, in particular on the volatile composition of wines. This knowledge gap is even more pronounced regarding red wines, since the few existing studies have been on white wines.

Based on the Pearson's correlation, it is possible to divide the volatile compounds into two groups: a group containing compounds that are associated with a fruitier flavour and are positively correlated with most of the biochemical and oenological parameters (except for pH and alcoholic degree); and a group comprising compounds with a more floral flavour and that are positively correlated with pH and alcoholic degree.

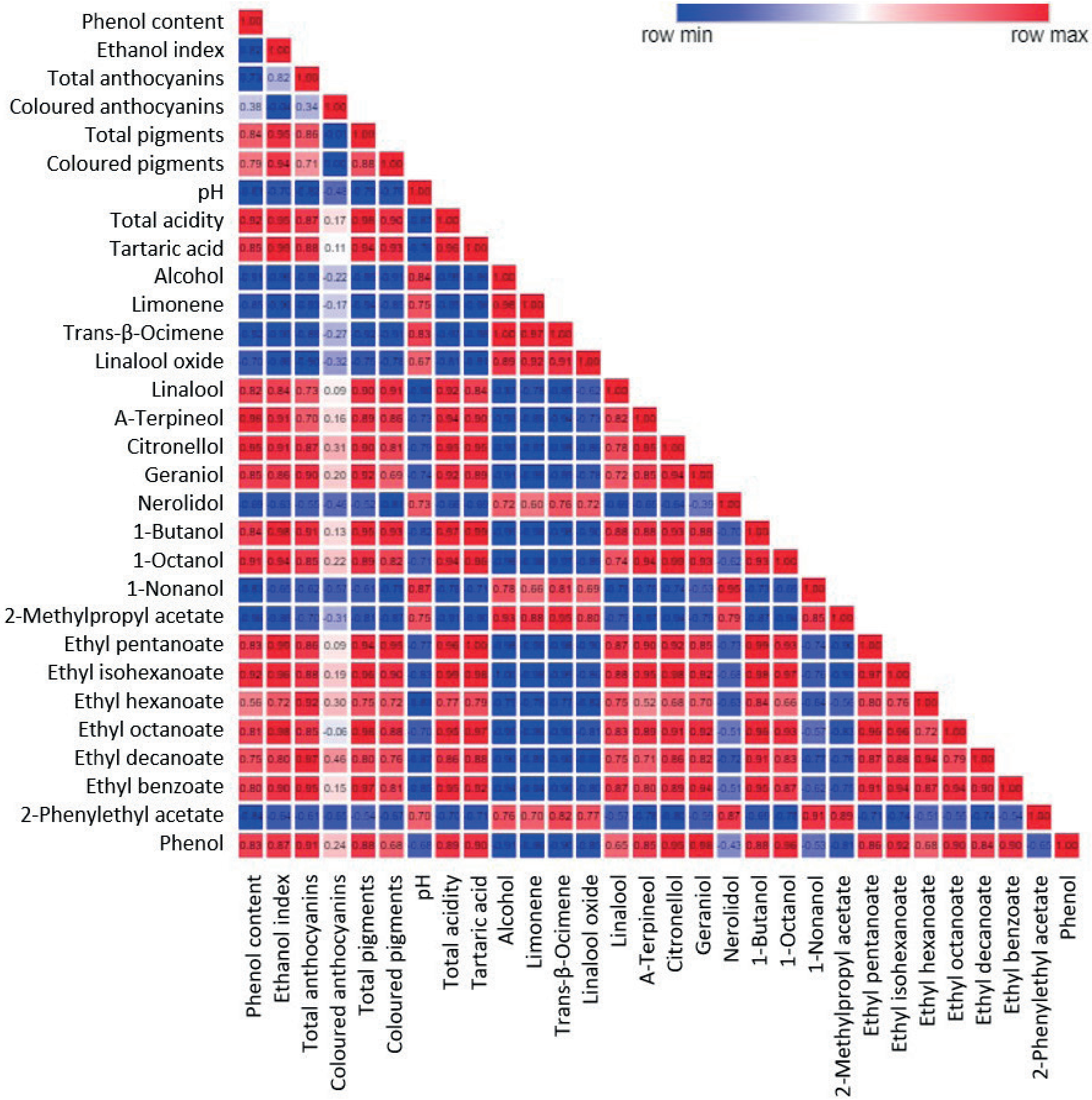

FIGURE 7. Pearson's correlation matrix of oenological and biochemical attributes and volatile compounds represented by a heat map with a colour scale from red ρ = +1 to blue ρ = −1. Confidence level: 5 %.

In sum, kaolin application is a promising low-cost strategy for mitigating climate change, increasing fruit and wine quality, protecting vines against abiotic stress and producing more balanced wines. Nevertheless, these applications should be contrasted in future studies, since the data here corresponds to a single experiment.

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