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Overview of Kaolin Outcomes from Vine to Wine: Cerceal White Variety Case Study

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Abstract: Kaolin protective effect was assessed in a white grapevine cultivar 'Cerceal' in 'Alentejo' Region (southeast Portugal) where plants face extreme conditions during the summer season. We addressed the hypothesis that kaolin effects lead to several changes in leaves, fruits, and wine characteristics on the primary and secondary metabolism. Results showed that kaolin reduces leaf temperature which provokes an improvement in physiological parameters such as net photosynthesis and water use efficiency. This protection interferes with berry color, leaving them more yellowish, and an increase in phenolic compounds were observed in all fruit tissues (skin, seed, and pulp). Additionally, both berry and wine characteristics were strongly affected, with an increase of tartaric and malic acid and consequently high total acidity, while the sugar concentration decreased 8.9% in berries provoking a low wine alcohol level. Results also showed that kaolin induces high potassium, magnesium, and iron, and low copper and aluminum concentrations. Moreover, the control wine showed higher content of esters related with hostile notes whereas wine from kaolin treated vines presented higher content of esters associated with fruity notes. Overall, the results strengthen the promising nature of kaolin application as a summer stress mitigation strategy protecting grapevine plants and improving fruit quality and creating more balanced wines.

Keywords: acidity attributes; fruit minerals; grapevine physiology; phenolic compounds; volatile compounds

1. Introduction

Viticulture and winemaking promote economic, social, and environmental benefits, through trademark, rural income, employment, and tourism [1]. Nonetheless, currently the greatest challenge for the wine industry is climate change, essentially under Mediterranean conditions [2], due to projected shifts in precipitation and temperature. Among the Portugal's wine regions, the Alentejo Demarcated Region (southeast) stands out due to the more pronounced harsh climate conditions and where water scarcity is a major problem. The reoccurrence of combined environmental stresses poses a risk to the crop yield and quality. Extreme temperature (>35 °C) through the growing season, as occurs in grape Portuguese areas, can harshly damage leaf photosynthetic efficiency and berry metabolism [3]. Water deficit affects berry quality in a developmental manner [4]. Grape leaf and

berry metabolite composition are affected by this climatic extreme and consequently affect the wine quality increasing sugar levels and thus the wine alcohol percentage. Changes also occurs at cellular level, where plants react to stress related stimuli by mediating the biosynthesis of an extensive range of chemical species with different properties, from compatible solutes [5] to complex phytochemicals. In spite of the confirmed primary metabolite contribution to an elevated plant resistance to stress [6–8], their secondary metabolites are mostly involved in defense and other facultative processes, such as biotic and abiotic stress responses [9,10]. Among the metabolic pathways involved in stress responses in grapevine berries, polyphenol metabolism is extremely important to fruit quality, due its composition of flavonoid classes, such as anthocyanins, flavonols, flavanones, and flavanols, which act as potent antioxidants, helping plants cope with abiotic stress. Polyphenols are defined as natural products [11] with different functions, such as defense against herbivores and pathogens, mechanical support (lignin), pollinator attractants, UV-B damage amelioration, and allelopathic effects [12].

Promoting the adoption of adaptation measures in winemaking regions is a pressing policy concern. Moreover, wine and its consumption are generally a cultural heritage in Europe [1]. Efforts in assessing both long- and short-term mitigation techniques have been promoted over the last years to adapt to the above described challenges [13]. There are long-term mitigation solutions such the use of new varieties and/or rootstocks. However, in the first line, the short-term strategies need to be in line and among several options, the use of reflective particle materials, such as kaolin (Kl), is a potentially viable practice in commercial vineyards [10,14–16]. Kaolin, $Al_2Si_2O_5(OH)_4$, is a white inert clay mineral that reflects in much higher amount the potentially damaging ultraviolet and infrared radiation than the photosynthetically active radiation, resulting in the decrease of leaf temperature and simultaneous increase in photosynthetic efficiency by decreasing photoinhibition [17]. It is well known that modifications in the leaf and fruit texture after pulverization as well as changes in the reflected light signature of the plant makes Kl a good repellent against a number of damaging insects [18,19]. At the same time, Kl is also effective in reducing leaf and fruit sunburn damage in several fruit crop species [6,10,20]. Under the quite stressful environmental conditions of the Douro Demarcated Region (NE Portugal), Kl treated leaves displayed decreased susceptibility to photoinhibition due to higher efficiency of photosystem II (PSII) and a more efficient photochemical quenching [14]. Under similar stress conditions, enzymatic activity assays [6] and transcriptional analyses described that Kl was able to increase sucrose concentration in leaves, sucrose transport, and phloem loading capacity [17,21]. In olive trees, leaves treated with Kl presented less oxidative damage, demanding a reduced antioxidant adaptation [22] and showed some changes in minerals content [23].

In spite of the fact that white grapevine varieties will be extremely affected by climate changes due their lower heat demands [24], there are no studies to our knowledge about the Kl effects on these varieties, neither in the respective wine biochemical characteristics. Therefore, the aim of the present study is to understand the effects of Kl pulverization in white grapevine variety 'Cerceal' in Alentejo Region, from the plant physiology to wine quality. For this purpose, several variables were studied, namely the repellent capacity, the leaf photosynthetic performance, as well as fruit and wine quality characteristics as alcohol degree, acids, and volatile compounds.

2. Materials and Methods

2.1. Weather Conditions and Kaolin Application

Meteorological conditions prevailing during the experimental periods are presented in Figure 1. The period of the study was very dry in both 2016 and 2017, except for some significant rainfall events at spring and in early autumn in 2016 (always below 35 mm d^{-1}), and light rain in the same period in 2017. Daily maximum air temperature (Tmax) was usually over 30 °C from May onwards, and over 40 °C in some days from July to September. The 5% (*w*/*v*) kaolin (Kl, Surround WP®; Engelhard Corp., Iselin, NJ, USA) application was done by spraying whole plants with a tractor, in June 6 (DOY, Day of the Year 129; Tmax = 30.0 °C) in 2016 and July 3 (DOY 155; Tmax, = 38.5 °C) in 2017. No significant

precipitation was registered till the end of the experiments. The field measurements and material collection prior to analysis were done in DOY 181 and DOY 222 in 2016 and in DOY 180 and DOY 209 in 2017, respectively.

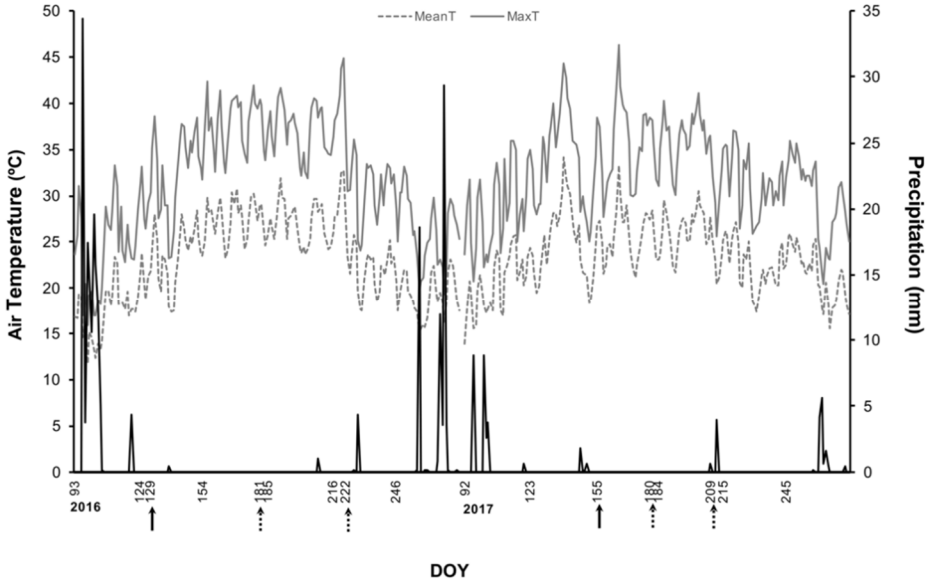

Figure 1. Daily mean temperature (Tmean, dashed grey line), maximum temperature (Tmax, grey line), and precipitation (black line) in 2016 and 2017. The filled arrows show the days of the kaolin application in 2016 (DOY 129) and 2017 (DOY155), and dashed arrows indicate the days of experimental measurements and material collection for prior analysis. DOY: day of the year.

2.2. Plant Material and Experimental Design

Samples were obtained from Cerceal, a white *Vitis vinifera* L., variety grafted on 1103P rootstock, located in a commercial vineyard "Herdade do Esporão" (38°22'48.1" N, 07°33'38.4" W), in southeast Portugal. The climate is of the Mediterranean-like type with dry and hot summers, moderate precipitation during the winter months, and dryness during the summer [25]. Records from a meteorological station, located 10 km away from the experimental vineyard, were collected. Vines were managed using the organic production mode. The white Cerceal cv. has a row with 200 m long (200 plants) in which two different conditions were set up: an experimental control (C; 100 plants) and another pulverized with kaolin (100 plants). The vines were 7 years old and were unilaterally cordon trained and pruned.

2.3. Foliar Leaf Temperature

Leaf temperature was measured with an infrared thermometer (Infratrace, model KM800S, Comark Ltd., Hertfordhire, UK) with a 15° field view, at veraison and maturation in the midday period. Measurements were performed under clear sunny days and on sun-exposed and fully expanded leaves at the middle of the shoots (usually between 8th and 11th nodes on the shoot axes). The average temperature of three randomly selected leaves (in eight plants) per treatment ($3 \times 8 = 24$) was obtained by holding the thermometer at about 1 m above the foliar surface.

2.4. Physiological Parameters

Leaf gas exchange, chlorophyll a fluorescence, and OJIP test (open-source chlorophyll fluorometer based on the Kautsky induction curve) were obtained in both 2016 and 2017 years during the summer season (at veraison and maturation). In these times several field measurements were done.

2.4.1. Leaf Gas Exchange

Leaf gas exchange was measured with an infrared gas analyzer (LCPro+, ADC Bioscientific Ltd., Hoddesdon, UK), operating in the open mode. Measurements were carried out DOY 181 (veraison) and DOY 222 (maturation) in 2016 and DOY 180 (veraison) and 209 DOY (maturation) in 2017, in two time periods: morning (09:00–10:30) and midday (14:00–15:30). Net photosynthesis (P_N), stomatal conductance (g_s), transpiration rate (E), and intercellular CO_2 concentration/ambient CO_2 ratio (C_i/C_a) were estimated according to von Caemmerer and Farquhar (1981). To eliminate the possible effects of air humidity and temperature on transpiration, the P_N/g_s ratio, rather than the P_N/E ratio, was calculated to evaluate the intrinsic water-use efficiency (iWUE) [26].

2.4.2. Chlorophyll a Fluorescence Analysis and OJIP Test

Chlorophyll a fluorescence emission was measured at morning (09:00–10:30) and midday (14:00–15:30) on fully expanded leaves in both developmental stages with a Pulse Amplitude Modulation Fluorometer (mini-PAM, Photosynthesis Yield Analyzer; Walz, Effeltrich, Germany), using two scripts: (i) in the first script, the measurements were done on well sun exposed leaves. In this procedure, after a 35 s exposure to actinic light (1450 µmol m^{-2} s^{-1}), light-adapted steady-state fluorescence yield (F_s) was averaged, followed by exposure to saturating pulse light (6000 µmol m^{-2} s^{-1}) for 0.6 s to establish F_m'. The sample was then shaded for 5 s with a far-red light source to determine F_0'. (ii) In a second script, using the dark leaf clip (DLC-8), the same leaf portion used in first script was immediately dark acclimated for 30–45 min. After this, the maximum photochemical efficiency of PSII was given by $F_v/F_m = (F_m - F_0)/F_m$, where F_0 corresponds to the minimum fluorescence level excited by very low intensity of measuring light to keep PSII reaction centers open, and F_m corresponds to the maximum fluorescence level elicited by a pulse of saturating light (6000 µmol m^{-2} s^{-1}) which closes all PSII reaction centers. From these measurements, several fluorescence attributes were calculated [27,28]: photochemical quenching ($qP = (F_m' - F_s)/(F_m' - F_0')$), non-photochemical quenching ($NPQ = (F_m - F_m')/F_m'$), and efficiency of electron transport as a measure of the quantum effective efficiency of PSII ($\Phi_{PSII} = \Delta F/F_m' = (F_m' - F_s)/F_m'$). The photosynthetic electron transport rate was estimated as ETR (µmol m^{-2} s^{-1}) = $(\Delta F/F_m') \times PPFD \times 0.5 \times 0.84$, where PPFD is the photosynthetic photon flux density incident on the leaf, 0.5 is the factor that assumes equal distribution of energy between the two photosystems, and the leaf absorbance used was 0.84 because it is the most common value for C_3 plants [27]. The analysis of the fluorescence transients using the JIP test was performed according to our previous study [20].

2.5. Pests Control

To study the kaolin pesticide effect against *Panonychus ulmi* (Koch, 1836) and *Scaphoideus titanus* (Ball, 1932), 10 leaves of the upper third of canopy in 10 contiguous plants were observed and the percentage of leaves with symptoms were registered at veraison stage in 2017. The incidence was expressed as the percentage (%) of affected leaves.

2.6. Fruit Surface Color Index and Biometric Parameters

Berry color was determined by a spectrophotometer Konica Minolta Sensing's CM-2500c portable (Minolta Corp., Osaka, Japan) in 30 berries per treatment (three measurements were made around the equatorial belt of each berry) in the maturation stage, in 2017. This color system, evaluated lightness, L^* (0, black—100, white), chroma, C^*ab (0, achromatic), and hue angle, h_{ab} (0, red—90, yellow—180, green—270, blue) as previously described [29]. Fruit biometric parameters were measured (mm) and weighed (mg) individually in situ in triplicate (n = 30 per treatment and in each stage). The width and height were measured to calculate the average of grape berry radius in order to estimate the absolute volume (mm^3) of each one. Both color and fruit biometric parameters were obtained in the maturation stage in 2017.

2.7. Phenolic Compounds and Antioxidant Activity

The total phenolic contents from whole fruit in both veraison and maturation stages and from skin, pulp, and seed of the fruit in maturation stage were determined by the Folin–Ciocalteu method [30] during 2017. All samples were lyophilized and macerated with liquid nitrogen and MeOH 70% was used for the phenolic compound extraction. Briefly, the extract was added, 20 µL sample (4 mg/mL) or gallic acid standards in MeOH, 90 µL distilled H_2O, and 10 µL of Folin–Ciocalteau reagent solution. After 6 min, 80 µL of 7% Na_2CO_3 was added and mix gently. The reaction mixture was kept in dark for 2 h and its absorbance was measured at 750 nm in microplate. Total phenolics was expressed as mg gallic acid equivalents per gram of extract (mg g^{-1} DW) ($y = 1.076x + 0.059$, $R^2 = 0.99987$).

The aluminum chloride ($AlCl_3$) complex method at 510 nm was used for the quantification of the total flavonoids content of extracts [31] and was expressed as mg of catechin equivalents per gram of extract (mg CAE g^{-1} DW) ($y = 2.7814x + 0.0477$, $R^2 = 0.9993$). The *ortho*-diphenols content was estimated according to the colorimetric method based on a complex reaction with sodium molybdate dehydrate at 370 nm [32]. The results were expressed as mg of gallic acid equivalents per gram of extract (mg GAE g^{-1} DW) ($y = 3.173x + 0.166$, $R^2 = 0.9983$). The condensed tannins contents were determined according to the vanillin-HCl assay [33] at 500 nm. The results were expressed as mg of catechin equivalents per gram of extract (mg CAE g^{-1} DW) ($y = 1.874x + 0.0624$, $R^2 = 0.99708$).

The radical scavenging activity on ABTS radical was evaluated by the method of Trolox equivalent antioxidant capacity assay at 734 nm [31], and was done in the same samples used for total phenols. ABTS were expressed as mg of trolox equivalents per gram of extract (mg TE g^{-1} DW) ($y = -0.3004x + 0.1968$, $R^2 = 0.99969$). The radical scavenging activity on the DPPH radical was evaluated [31] being previously adapted to microplates [34]. DPPH were expressed as mg of trolox equivalents per gram of extract (mg TE g^{-1} DW) ($y = -0.1442x + 0.1039$, $R^2 = 0.99904$).

2.8. Total Soluble Proteins

The total soluble proteins were obtained from whole fruit in both veraison and maturation stages during 2017 and were extracted using an extraction buffer containing phosphate (Fisher Scientific, Loughborough, UK) of pH 7.5 mixed with EDTA (ethylenediaminetetraacetic acid) (Panreac, Barcelona, Spain). The work solution included the extraction buffer described above, PMSF (Phenylmethanesulfonyl fluoride) (Panreac, Barcelona, Spain), PVP (Polyvinylpyrrolidone) (Sigma, St. Louis, MO, USA) according to the method of Bradford (1976) [35] at 595 nm. All the absorbances of this work were determined using PowerWave XS2 microplate scanning spectrophotometer (Bio-Tek Instruments, Winooski, VT, USA). Total soluble proteins were expressed as mg bovine serum albumin equivalents per gram of extract (mg BSAE g^{-1} DW) ($y = 14.029x + 0.6234$, $R^2 = 0.981$).

2.9. Brix and Acidity Parameters

°Brix were measured ($n = 30$ per treatment), at maturation in 2017, using an ATAGO digital refractometer (CO., LTD., Tokyo, Japan). The Brix scale or degrees °Brix is numerically equal to the percent of sugar and other dissolved solids in the solution [36].

The physico-chemical parameters of grapes, such as pH and total acidity, and wine, such as alcohol degree and total acidity, were analyzed according to the OIV [37] methodologies. The tartaric and malic acid were measured enzymatically (Miura One, TDI S.A.).

2.10. Trace Elements Quantification

Some element quantifications were obtained in 2017 in the grape berries at maturation and in the wine must, during fermentation process according to [38]. In the wine samples obtained from vines under different treatments (C and Kl) only the Al (aluminum) was quantified. Prior to the analyses each sample was vigorously shaken. An aliquot (0.5 g) of sample was weighed directly into the digestion vessels. The digestion was performed by adding HNO_3 (1.0 mL) and H_2O_2 (5.0 mL) to

each sample. The mixture was left at room temperature with a marble (preventing evaporation) for 24 h, and afterwards the marbles were removed, and the samples left overnight at room temperature. After this period, the sample was heated using a block heater at 50 °C during 1 h followed by 100 °C during 1 h (temperature at which the release of Nitric Oxide brown fumes starts), 120 °C during 1 h, and finally left overnight at 155 °C (usual time needed to obtain a clear digestion mixture), or until the solution was clear, with a glass marble on the top of the culture tube (to avoid drying before digestion and sample charring). After this period the glass marbles were removed, and the contents were dried at 155 °C. After cooling to room temperature, 10.0 mL of HNO3 matrix solution (1.5 mL of acid to 1000 mL of water) was added to the digested samples and stirred. Some of the solutions were diluted in order to allow the determination of the respective metals. All samples were analyzed in triplicate. The copper (Cu) and potassium (K) were determined by flame atomic emission spectrometry and calcium (Ca), magnesium (Mg), iron (Fe), and zinc (Zn) metals were analyzed by flame atomic absorption spectrometry (Thermo Scientific ICE 3000). The Al were analyzed by atomic absorption spectrometry in graphite furnace (Unicam 939 AA spectrometer, GF90 furnace). 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 standards were used, besides the blank, with the range of concentrations being selected according to the expected concentrations of the elements of interest.

2.11. Analysis of Wine Volatile Compounds by HS-SPME-GC–MS

Solid phase microextraction (SPME) was used for the extraction of volatile compounds from grapevine berries at maturation and wine from 2017 samples. One milliliter of sample was measured to a 20 mL headspace vial (La-Pha-Pack®, Langerwehe, Germany) and was capped with a white PTFE silicone septum (Specanalitica). The SPME operating conditions were extraction temperature 40 °C for 40 min, rotating speed 100 rpm, and desorption time 10 min at 250 °C. Analysis were carried out in a GCMS-QP2010 Plus (Shimadzu®, Kyoto, Japan) equipped with an AOC-5000 autosampler (Shimadzu®). A divinylbenzene/carboxen/polydimethylsiloxane (DVB/Car/PDMS) fiber (SUPELCO Analytical, Bellefonte, PA, USA) was used for headspace SPME sampling. For the analysis a capillary column Sapiens-5-MS (Teknokroma), 30 m, 0.25 mm (IS), 0.25 μm (film thickness) was used. The working conditions were as follows: injector temperature: 250 °C, injection mode: splitless during 1.5 min, detector temperature: 250 °C. High-purity helium (≥99.999%) was used as the carrier gas, column oven temperature was kept at 40 °C for 5 min, increased to 170 °C at a rate of 5 °C min^{-1}, 230 °C at 30 °C min^{-1}, and maintained for 4 min, then was raised to 300 °C at 30 °C min^{-1} and maintained for 2 min; carrier gas (He) with a flow of 2.00 mL min^{-1}. In MS interface temperature was 250 °C and ion source temperature was 250 °C. Mass spectra were acquired in electron ionization (EI) mode at 70 eV. in a m/z range between 29 and 300 with a scan speed of 555 scans s^{-1}. The compounds were identified using the mass spectra libraries, NIST 21, 27, 107, 147, and Wiley 229.

Firstly, the peak areas data of all compounds were extracted from the chromatograms and used to build the full data matrix from 'Cerceal' wines consisting of six observations (two treatments of wine samples, each one by three replicates) and 51 variables (volatile components).

2.12. Statistical Analysis

Statistical analyses were performed with SPSS 20.0 software. After testing for ANOVA assumptions (homogeneity of variances with the Levene's mean test, and normality with the Kolmogorov–Smirnov test), statistical differences among stages and treatments were evaluated by two-way factorial ANOVA, followed by the post hoc Tukey's test and in some cases only within the stage one-way ANOVA was done. Significant differences were considered for $p < 0.05$ and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ represent significant differences. 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 visualization was applied for 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 compounds detected in samples were compared between control and kaolin treated samples, through a two-sided Mann–Whitney test (using the SPSS software 20.0 (IBM, New York, NY, USA).

3. Results

3.1. Physiological and Pests Control Changes under Kaolin Application

Kaolin particle film showed several physiological effects. Regarding Figure 2, leaf temperature, which can be considered the first bound between weather conditions and plant health, was positively affected by Kl application in veraison and maturation in both years.

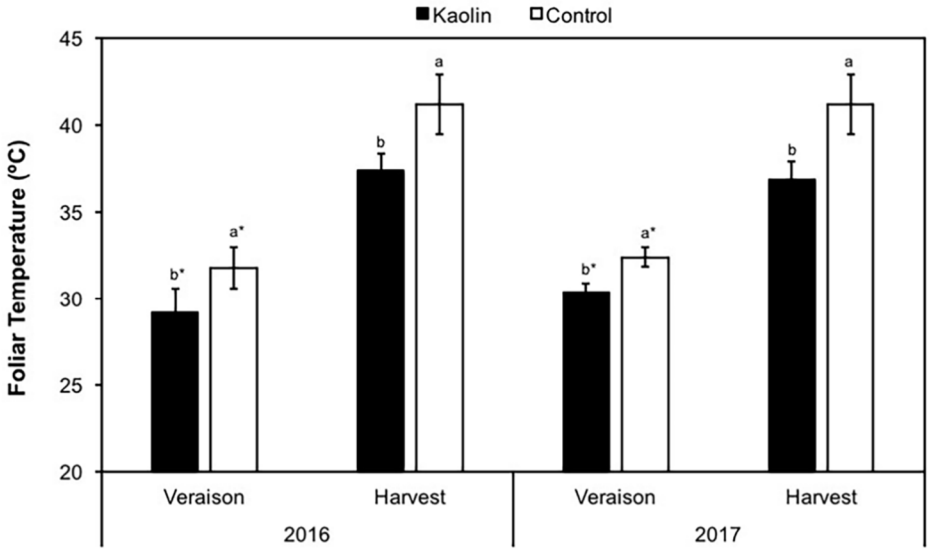

Figure 2. Leaf temperature of control and kaolin treated leaves in veraison and maturation in the midday period in 2016 and 2017. Values are presented as mean ± SD (n = 24 per treatment). Different lowercase letters represent significant differences between treatments (control vs. kaolin), in the same stage of the season, and * represents significant differences between stages within the same treatment ($p < 0.05$). Absence of superscript indicates no significant differences.

The kaolin treated leaves showed decreases of 8.2% and 9.3% and 6.4% and 10.4% in leaf temperature in veraison and maturation stages of 2016 and 2017, respectively. Regarding leaf gas exchange parameters (Table 1) the results showed low values of stomatal conductance (g_s), net photosynthesis (P_N), and intrinsic water use efficiency (iWUE) in the maturation stage in both years and periods of the day (morning and midday), comparing with the veraison stage. The Kl treatment leads to a significantly high P_N and iWUE and low C_i/C_a ratio in both stages and periods in 2016 and 2017.

Relating to g_s, the results only showed differences in the morning period in 2017 with high values in the Kl-treated plants. The transpiration rate (E) only presents a significant high value in the Kl-treated plants in the maturation stage in the morning period in 2016.

Table 2 presents parameters related to chlorophyll a fluorescence data. Regarding 2016, only differences were observed in the midday period, except for the ETR whose values were higher in the kaolin treated plants. Overall, kaolin application boosts F_v/F_m, Φ_{PSII}, qP, and ETR values, while leads to a decrease in the F_0 and NPQ values.

Table 1. Gas exchange parameters, namely transpiration rate (E, mmol m^{-2} s^{-1}), stomatal conductance (g_s, mmol m^{-2} s^{-1}), photosynthesis net (P_N, µmol m^{-2} s^{-1}), intrinsic water use efficiency (iWUE, µmol mol^{-1}), and ratio of intercellular to atmospheric CO_2 concentration (C_i/C_a), at morning and midday periods in veraison and maturation stages in kaolin and control leaves (n = 10). Different lowercase letters represent significant differences between treatments (control vs. kaolin), in the same period of the day (morning/midday) and stage of the season (veraison/maturation). * represents significant differences between stages of the season within the same period of the day ($p < 0.05$). Absence of superscript indicates no significant differences.

Stage	Treatment	Morning									
2016		E		gs		PN		iWUE		Ci/Ca	
Veraison	Kaolin	4.07 ±	0.632 *	251.9 ±	59.6 *	13.0 ±	3.04 *	50.3 ±	4.19 a *	0.706 ±	0.028 b *
	Control	4.10 ±	0.627 *	245.7 ±	47.9 *	10.3 ±	2.13 *	40.8 ±	6.29 b *	0.745 ±	0.037 a *
Harvest	Kaolin	2.27 ±	0.014 a	57.3 ±	11.3	2.23 ±	0.348 a	38.9 ±	5.32 a	0.772 ±	0.043 b
	Control	2.07 ±	0.109 b	51.7 ±	15.2	0.953 ±	0.144 b	18.4 ±	8.52 b	0.894 ±	0.025 a
2017											
Veraison	Kaolin	3.65 ±	0.958	266.6 ±	93.5 a *	10.7 ±	1.64 a *	48.2 ±	7.82 a *	0.755 ±	0.116
	Control	3.00 ±	0.641	149.1 ±	51.8 b *	7.2 ±	1.48 b *	40.4 ±	8.71 b *	0.767 ±	0.095
Harvest	Kaolin	3.10 ±	0.897	154.3 ±	66.9 a	8.66 ±	2.16 a	56.1 ±	2.34 a	0.744 ±	0.015
	Control	3.00 ±	0.634	138.0 ±	55.7 b	3.95 ±	1.28 b	28.6 ±	5.05 b	0.840 ±	0.023
		Midday									
2016											
Veraison	Kaolin	3.74 ±	0.742 *	173.5 ±	57.3 *	10.4 ±	2.57 *	59.9 ±	6.57 *	0.661 ±	0.025 b *
	Control	3.81 ±	0.663 *	171.5 ±	48.0 *	9.75 ±	3.03 *	56.8 ±	5.48 *	0.684 ±	0.034 a *
Harvest	Kaolin	2.15 ±	0.465	101.8 ±	21.1	4.07 ±	1.07 a	40.0 ±	5.39 a	0.766 ±	0.046 b
	Control	2.16 ±	0.428	92.3 ±	36.3	1.89 ±	0.382 b	20.4 ±	11.7 b	0.859 ±	0.059 a
2017											
Veraison	Kaolin	2.52 ±	0.218	105.0 ±	10.6	7.36 ±	1.50 a *	70.1 ±	13.1 a *	0.644 ±	0.081 b *
	Control	2.79 ±	0.317	102.8 ±	15.0	5.48 ±	0.702 b *	53.3 ±	8.75 b *	0.720 ±	0.045 a *
Harvest	Kaolin	2.67 ±	0.604	111.1 ±	37.4	6.93 ±	1.60 a	62.3 ±	7.8 a	0.675 ±	0.049 b
	Control	2.45 ±	0.661	91.0 ±	27.6	3.21 ±	0.879 b	35.3 ±	5.75 b	0.797 ±	0.035 a

Table 2. Chlorophyll a fluorescence parameters, namely basal fluorescence (F_0), maximum quantum efficiency of photosystem II (F_v/F_m), effective PSII efficiency (Φ_{PSII}), photochemical quenching (qP), electron transport rate (ETR, μmol $e^{-}m^{-2}$ s^{-1}), and non-photochemical quenching (NPQ) at morning and midday periods in veraison and maturation stages in kaolin and control leaves (n = 10). Different lowercase letters represent significant differences between treatments (control vs. kaolin), in the same period of the day (morning/midday) within the stage of the season (veraison/maturation), and * represents significant differences between stages of the season within the same period of the day ($p < 0.05$). Absence of superscript indicates no significant differences.

Treatment **Stage**	**Morning**											
2016 Veraison	**F_0**		**F_v/F_m**		**Φ_{PSII}**		***qP***		**ETR**		**NPQ**	
Kaolin	514.5 ±	99.9 *	0.743 ±	0.051	0.225 ±	0.044 *	0.803 ±	0.052	141.4 ±	17.4 a *	0.821 ±	0.436
Control	536.5 ±	100.9 *	0.700 ±	0.033	0.193 ±	0.041 *	0.896 ±	0.197	121.3 ±	15.6 b *	1.26 ±	0.349
Harvest												
Kaolin	405.0 ±	97.9	0.798 ±	0.057	0.314 ±	0.059	0.616 ±	0.131	252.0 ±	18.0 a	0.870 ±	0.200
Control	448.5 ±	121.4	0.688 ±	0.085	0.333 ±	0.064	0.639 ±	0.175	237.6 ±	14.3 b	0.707 ±	0.216
2017 Veraison												
Kaolin	485.0 ±	36.9 b *	0.759 ±	0.025 a	0.117 ±	0.027 a *	0.365 ±	0.079 a *	88.5 ±	10.1 a *	3.52 ±	0.281 *
Control	603.2 ±	38.7 a *	0.675 ±	0.026 b	0.084 ±	0.041 b *	0.289 ±	0.061 b *	63.8 ±	13.2b *	3.94 ±	0.997 *
Harvest												
Kaolin	1684.0 ±	56.7 b	0.773 ±	0.039 a	0.457 ±	0.009 a	1.00 ±	0.014 a	345.2 ±	17.3 a	2.43 ±	0.774 b
Control	1816.6 ±	50.9 a	0.622 ±	0.070 b	0.429 ±	0.018 b	0.97 ±	0.004 b	324.2 ±	9.85 b	4.22 ±	0.804 a
	Midday											
2016 Veraison	**F_0**		**F_v/F_m**		**Φ_{PSII}**		***qP***		**ETR**		**NPQ**	
Kaolin	372.0 ±	40.2 b *	0.753 ±	0.013 a *	0.207 ±	0.040	0.414 ±	0.012 *	130.1 ±	25.3 *	0.71 ±	0.154 b
Control	473.8 ±	52.0 a *	0.733 ±	0.003 b *	0.228 ±	0.022	0.388 ±	0.012	143.4 ±	13.8	1.79 ±	0.079 a
Harvest												
Kaolin	521.0 ±	24.0 b	0.634 ±	0.018 a	0.238±	0.032 a	0.563 ±	0.061 a	180.1 ±	13.9 a	1.14 ±	0.031
Control	597.3 ±	23.3 a	0.536 ±	0.046 b	0.204 ±	0.045 b	0.618 ±	0.032 b	154.4 ±	13.8 b	1.39 ±	0.195
2017 Veraison												
Kaolin	510.2 ±	58.9 b *	0.731 ±	0.017 *	0.059 ±	0.002 *	0.190 ±	0.061 *	45.1 ±	11.6 *	2.75 ±	0.263 b *
Control	599.0 ±	13.8 a *	0.702 ±	0.029	0.054 ±	0.001 *	0.198 ±	0.024 *	40.8 ±	9.26 *	4.09 ±	0.085 a *
Harvest												
Kaolin	359.8 ±	59.4 b	0.789 ±	0.014 a	0.183 ±	0.015 a	0.404 ±	0.019 a	138.3 ±	14.7 a	2.08 ±	0.132 b
Control	427.8 ±	68.7 a	0.718 ±	0.015 b	0.176 ±	0.012 b	0.391 ±	0.088 b	103.7 ±	6.89 b	3.15 ±	0.024 a

Figure 3 shows the higher values of Kl treated plants in parameters related to the *OJIP* test, specifically the efficiency of energy conservation in the electron transport (Ψ_0) and performance index (PI_{ABS}) in both years and stages in morning and midday periods.

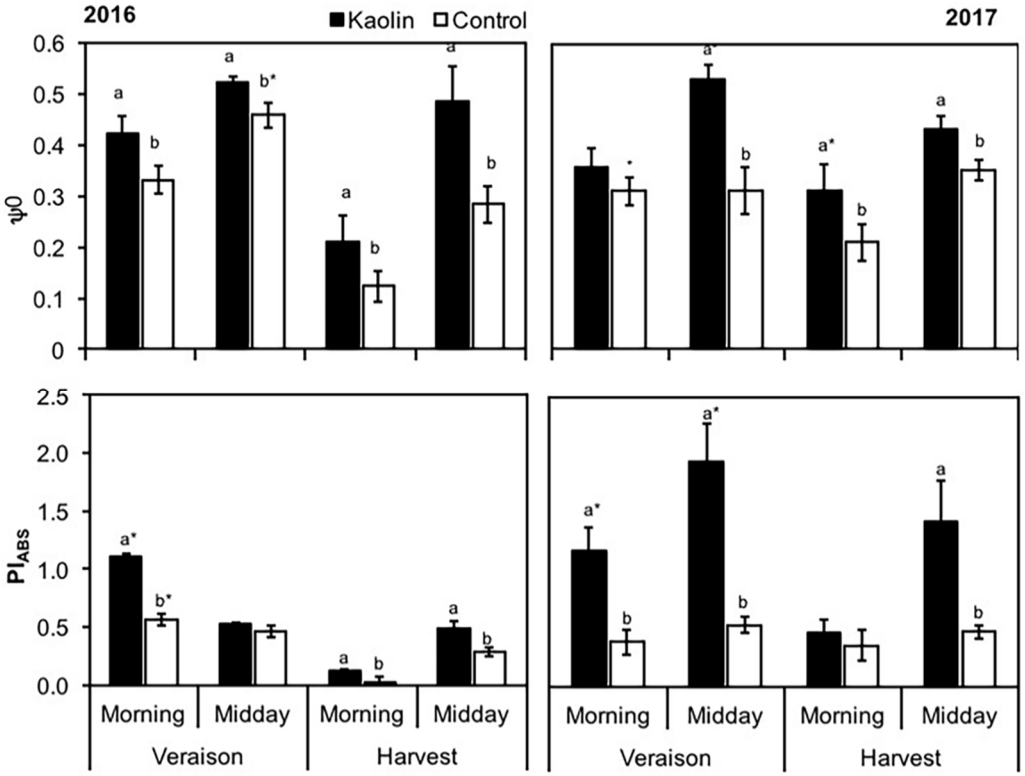

Figure 3. JIP parameters in control and kaolin treated grapevines from veraison and maturation stages in the midday period in 2016 and 2017. Values are presented as mean ± SD (n = 10). Different lowercase letters represent significant differences between treatments (control vs. kaolin), in the same period of the day (morning/midday) within the stage of the season (veraison/maturation), and * represents significant differences between stages of the season within the same period of the day ($p < 0.05$). Absence of superscript indicates no significant differences.

The results revealed that the incidence of *Panonychus ulmi* was reduced by 10% in leaves sprayed with Surround WP® (Kl) compared to control fruit (C—20%; Kl—10%) (Figure S1). Furthermore, *Scaphoideus titanus* incidence was also significantly reduced by 30%, after Kl sprays (C—30%; Kl—0%).

3.2. Kaolin Effects on Fruit Quality

In white grapes varieties it is predictable that skin berry color changes during grape maturation, from an initial green to a yellow/brownish at maturation. The Kl effects on the color skin parameters in the maturation stage were evaluated and results are shown in Table 3. Accordingly, Kl leads to a decrease in a^* and b^* parameters, i.e., a rise of green and a little decline of yellow color, giving thus more yellowish coloring to the skin compared to the brownish coloring to the skin in control fruits. Additionally, a decrease was observed in the chroma values (C*ab) and no differences were obtained in the lightness (L*). Relevant differences existed in hue (h_{ab}), being higher in fruits from Kl treated plants. Furthermore, C*ab is positively correlated with b^*, independently from treatment, showing correlation coefficients (r) of 1.00 and 0.988, for Kl and control treatments, respectively (all Pearson's correlations were significant at $p < 0.05$, data not shown). Usually, hue (h_{ab}) showed an inverse correlation with a^*. Our results are in agreement with this tendency with $r = -0.992$ for Kl and -0.991 for control treatments (all correlations were significant at $p < 0.05$).

Table 3. Kaolin effect on colorimetric parameters analyzed for all grape skin color variants and °Brix of fruits picked in the maturation period, and total acidity (g L^{-1} of tartaric acid), pH, tartaric and malic acid concentration (g L^{-1}) of fruits picked in the veraison and maturation stages, in 2017. Values are presented as mean ± SD. Significant differences were presented between treatments and considered for $p < 0.05$ and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ represent significant differences; ns indicates no significant differences.

	Kaolin		Control		*Significance*
Veraison					
Total acidity	10.5 ±	0.767	8.76 ±	0.386	
pH	2.92 ±	0.049	3.05 ±	0.009	***
Tartaric acid	7.04 ±	0.516	5.59 ±	0.308	***
Malic acid	1.82 ±	0.36	2.35 ±	0.417	*
Harvest					
Colour parameters					
*b**	13.9 ±	1.63	15.1 ±	1.87	*
*C*ab*	14.0 ±	1.62	15.4 ±	1.99	***
h_{ab}	91.3 ±	3.92	81.9 ±	6.11	***
°Brix	17.5 ±	1.03	19.2 ±	2.16	*
Total acidity	4.88 ±	0.176	4.48 ±	0.036	***
pH	3.44 ±	0.090	3.61 ±	0.070	**
Tartaric acid	3.88 ±	0.161	3.25 ±	0.251	***
Malic acid	0.910 ±	0.105	0.683 ±	0.049	***

Kaolin application shows influence in the °Brix and acidity parameters (Table 3). A decrease of 8.9% was observed in the °Brix of fruits from Kl treated plants. As expectable, a decrease in the total acidity as well as in the tartaric and malic acids concentration from veraison to maturation stage were observed. However, Kl berries presented 16.6% and 8.2% higher total acidity in veraison and maturation stage, respectively. Regarding pH in Kl fruits, the results showed an increase between veraison and maturation stage, the treated fruits being 4.3% and 4.7% more acidic than control ones, respectively. Both tartaric and malic acids decreased from veraison to maturation stage. In the veraison stage Kl fruits presented 20.6% more tartaric acid and 22.3% less malic acid concentration. However, in the maturation stage the tartaric acid concentrations remained 16.2% higher in Kl fruits. The malic acid concentration decreased 24.9% in Kl berries compared to the fruits from the control plants.

Results showed that although fruit height was not affected by foliar Kl application, neither differences were observed between stages, the diameter was modified, also leading a different fruit volume (Table 4) in the veraison stage. Therefore, the fruit diameter and volume in this stage were significantly higher in kaolin-treated plants, these fruits being approximately 8.6% wider and with 14.3% more volume. In the maturation stage no differences were observed.

In Table 4 some fruit quality parameters related with secondary metabolism and primary metabolism (protein content) are presented. Generally, the secondary metabolism decreased from veraison to maturation stage and the protein content increased. The Kl application does not have an effect on the total phenols and tannins concentration in the veraison stage (Table 4). However, the flavonoids, *ortho*-diphenols, and protein content as well as DPPH presented lower values than control fruits and ABTS showed a higher value in the same stage. From veraison to maturation the Kl effect changes the tendency. At maturation stage the fruits from Kl treated plants showed higher total phenols (+11.6%), *ortho*-diphenols (+1.83%), tannins (+6.45%), and protein content (+9.42%). Regarding antioxidant activity obtained by ABTS and DPPH methods the Kl fruits also presented higher values (+5.36% and 12.7%, respectively) in this stage (Table 4). Deepening the study, Table 5 shows the same secondary metabolism parameters and the antioxidant activity in different berry tissues, namely seed, skin, and pulp. Independent of the treatment, our results showed, as expected, higher content of phenols, flavonoids, and tannins and also higher ABTS values in the seeds. However, the skin presented the higher content of *ortho*-diphenols as well as higher DPPH value (antioxidant activity). Kl treated

plants presented berries with higher content of flavonoids (+10.3%), *ortho*-diphenols (+32.5%), tannins (+27.3%), and DPPH value (+25.8%) in the seeds; higher content of total phenols (+13.1%), flavonoids (+25.5%), *ortho*-diphenols (+29.4%), tannins (+7.46%), and ABTS value (+11.0%) in the skin; higher content of total phenols (+39.7%), flavonoids (+41.1%), *ortho*-diphenols (+13.8%), and tannins (+14.4%) in the pulp.

Table 4. Kaolin application effect on height (mm), diameter (mm), and volume (mm^3) of berries, and phenols (mg g^{-1} DW), flavonoids (mg g^{-1} DW), *ortho*-diphenols (mg g^{-1} DW), tannins (mg g^{-1} DW), protein (mg g^{-1} DW), and antioxidant activity (mg g^{-1} DW; obtained by ABTS and DPPH methods) of whole fruits at the veraison and at maturation stages in 2017. Values are presented as mean ± SD. Different lowercase letters represent significant differences between treatments (control vs. kaolin), in the same stage of the season (veraison/maturation) and * represents significant differences between stages of the season within the same treatment ($p < 0.05$). Absence of superscript indicates no significant differences.

Parameters		Kaolin		Control		Kaolin		Control	
		Veraison				Harvest			
Biometry	Height	12.1 ±	0.616	11.5 ±	0.814	13.2 ±	0.429	12.9 ±	0.705
	Diameter	11.3 ±	0.434 a *	10.4 ±	0.783 b *	12.1 ±	0.449	12.3 ±	0.515
	Volume	136.7 ±	11.4 a *	119.6 ±	17.1 b *	159.6 ±	10.6	159.4 ±	14.1
Biochemestry	Phenols	76.2 ±	3.09 *	76.5 ±	3.04 *	56.0 ±	1.32 a	50.2 ±	1.52 b
	Flavonoids	27.5 ±	1.54 b *	33.0 ±	1.02 a *	15.2 ±	0.776	15.0 ±	0.699
	Ortho-diphenols	196.1 ±	8.00 b *	216.4 ±	8.96 a *	150.1 ±	1.47 a	147.4 ±	1.22 b
	Tannins	42.8 ±	1.26 *	45.4 ±	1.73 *	19.8 ±	0.289 a	18.6 ±	0.289 b
	ABTS	208.5 ±	7.10 a *	124.3 ±	15.1 b *	176.9 ±	2.80 a	167.9 ±	3.78 b
	DPPH	207.3 ±	5.44 b	220.8 ±	0.832 a *	212.8 ±	7.94 a	188.8 ±	11.9 b
	Protein	9.18 ±	0.140 b *	9.75 ±	0.140 a *	15.1 ±	0.071 a	13.8 ±	0.405 b

Noticeably, the mineral analysis also showed that mature berries and must from vines treated with kaolin had a significantly lower quantity of aluminum (Al) and copper (Cu) and high quantity of potassium (K), iron (Fe), magnesium (Mg), and zinc (Zn) than berries from the control vines (Table 6).

3.3. Kaolin Application Consequences in White 'Cerceal' Wine

As Kl is an aluminum silicate, we quantified the aluminum concentration in the wine (Table 7). Accordingly, the Al content is significantly lower (−12.9%) in wine of Kl treated plants than in the control one. The alcohol degree was also lower in Kl wine (3.57%) and an increase in total acidity, malic and tartaric acid were obtained (+16.3%, 11.1%, and 7.08% in Kl samples, respectively).

We also evaluated the wine volatile compounds (Table 8). The heatmap (Figure 4) shows a graphical representation of the chromatographic data (presented in Table 8) achieved for the 51 volatile components, allowing a rapid visual evaluation of the wine's volatile profiles. The chromatic scale of the heatmap allows access the relative amount of each volatile component (from dark blue, minimum, to dark red, maximum). Whereas the dendrogram (Figure 4) built from the HCA is an exploratory tool that reveals two clusters corresponding to the two types of wines, i.e., control and kaolin. From the 51 volatile components detected, 53% (corresponding to 27 components) exhibited differences statistically significant between both types of wines (differences corresponding to $p < 0.05$).

Table 5. Phenols, flavonoids, *ortho*-diphenols and tannins, and antioxidant activity (ABTS and DPPH) (mg g^{-1} DW) in the seeds, skins, and pulps of fruits from Kl treated and untreated (control) plants. Values are presented as mean ± SD. Significant differences were presented between treatments and considered for $p < 0.05$ and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ represent significant differences; ns indicates no significant differences.

	Tissues	Kaolin		Control		*Significance*
Phenols	Seed	99.4 ±	4.40	95.7 ±	4.42	ns
	Skin	49.5 ±	1.28	43.0 ±	0.853	**
	Pulp	22.4 ±	0.942	13.5 ±	0.554	***
Flavonoids	Seed	46.7 ±	1.00	41.9 ±	1.90	*
	Skin	13.7 ±	0.476	10.2 ±	0.289	***
	Pulp	2.58 ±	0.127	1.52 ±	0.046	***
Ortho-diphenols	Seed	198.3 ±	16.9	133.9 ±	2.00	**
	Skin	219.0 ±	7.38	154.6 ±	1.27	***
	Pulp	164.1 ±	1.97	141.5 ±	11.2	*
Tannins	Seed	57.5 ±	1.42	41.8 ±	0.946	***
	Skin	20.1 ±	0.144	18.6 ±	0.144	***
	Pulp	9.26 ±	0.382	7.93 ±	0.250	***
ABTS	Seed	297.1 ±	7.56	296.6 ±	2.30	ns
	Skin	185.9 ±	7.56	165.4 ±	3.97	*
	Pulp	109.6 ±	29.6	88.9 ±	15.2	ns
DPPH	Seed	245.3 ±	6.25	182.1 ±	5.21	***
	Skin	291.0 ±	5.51	277.2 ±	11.9	ns
	Pulp	228.5 ±	0.425	229.8 ±	1.13	ns

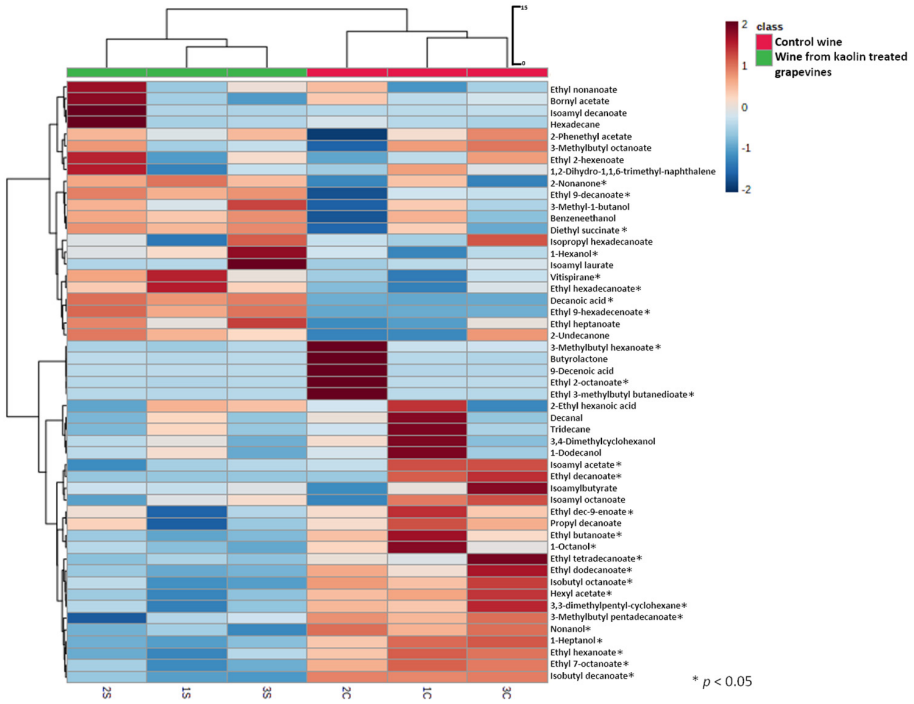

Figure 4. Heatmap and dendrogram representation of the 51 volatile components from 'Cerceal' cv. wines under study: control and kaolin treated grapevines, which reveals the distinction among wines. The content of each compound was illustrated through different colors (from dark blue, minimum, to dark red, maximum). Dendrogram for the HCA results using Ward's cluster algorithm to the data set was also included. Differences corresponding to $p < 0.05$ were considered significant and were marked with *.

Table 6. Mineral composition of white grape berries and must in different stages of 'Cerceal' cv. from kaolin treated and untreated (control) plants. Values are presented as mean ± SD. Different lowercase letters represent significant differences between treatments (control vs. kaolin), in the same stage of the season (veraison/maturation) and * represents significant differences between stages of the season within the same treatment ($p < 0.05$). Absence of superscript indicates no significant differences. N.D. means that concentration was so low that it was not detected.

Treatment		Stage	Al (μg g^{-1})		K (mg g^{-1})		Ca (mg g^{-1})		Fe (μg g^{-1})		Mg (mg g^{-1})		Cu (μg g^{-1})		Zn (μg g^{-1})	
Fruit	kaolin	Harvest	35.7 ±	2.62 b	15.7 ±	0.651 a	4.01 ±	0.240	20.5 ±	0.800 a	0.612 ±	0.049 a	1.46 ±	0.050 b	14.8 ±	0.500 a
	Control		38.9 ±	0.351 a	13.2 ±	1.40 b	4.02 ±	0.255	18.6 ±	2.410 b	0.452 ±	0.002 b	1.48 ±	0.015 a	6.99 ±	1.33 b
Must	kaolin	15 September	821.0 ±	1.21 a *	548.3 ±	2.52 c	27.2 ±	1.40. a *	0.994 ±	0.004 a *	65.2 ±	0.751 a	9.10 ±	0.001 a *	N.D.	
		23 September	561.4±	0.586 b	910.3 ±	1.53 a *	23.4 ±	0.889 b *	0.997 ±	0.002 a *	53.7 ±	0.300 b	8.00 ±	0.001 b *	N.D.	
		4 October	185.5 ±	0.854 c *	844.7 ±	1.15 b *	29.2 ±	0.709 a *	0.889 ±	0.009 b *	65.0 ±	0.153 a *	1.80 ±	0.001 c *	N.D.	
	Control	15 September	960.9 ±	0.001 a	511.7 ±	2.52 c	34.7 ±	0.577 a	0.858 ±	0.008 a	66.2 ±	0.361 b	20.6 ±	0.003 c	N.D.	
		23 September	564.0±	0.002 b	772.7 ±	3.06 a	29.6 ±	0.611 b	0.819 ±	0.019 b	55.2 ±	0.265 c	14.0 ±	0.001 a	N.D.	
		4 October	326.4 ±	1.22 c	706.3 ±	2.51 b	24.7 ±	1.25 c	0.767 ±	0.009 c	73.3 ±	0.379 a	14.1 ±	0.002 b	N.D.	

Table 7. Aluminum concentration (μg g^{-1}), total acidity (g L^{-1} of tartaric acid), pH, alcohol degree (%, *v*/*v*), and tartaric and malic acid concentration (g L^{-1}) of wine of white 'Cerceal' cv. from Kl treated and untreated (control) grapevines. Values are presented as mean ± SD. Significant differences are presented between treatments and considered for $p < 0.05$ and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ represent significant differences.

	Kaolin		**Control**		***Significance***
Aluminium	96.3 ±	2.82	110.6 ±	0.151	**
Alcohol degree	13.5 ±	0.191	14.0 ±	0.123	*
Total Acidity	6.34 ±	0.168	5.45 ±	0.081	***
Malic acid	1.30 ±	0.006	1.17 ±	0.026	***
Tartaric acid	2.57 ±	0.058	2.40 ±	0.001	***

Table 8. Kaolin effect on volatile composition (VOCs) of 'Cerceal' cv. wines determined by HS-SPME/GC-MS. Values are presented as mean area ± SD.

Retention Time (min)	**Compound**	**Formula**	**'Cerceal' Wines VOCs Composition**	
			Control	**Kaolin**
3.423	3-Methyl-1-butanol	C5H12O	1.18E+09 ± 9.68E+07	1.29E+09 ± 7.14E+07
5.133	Ethyl butanoate	C6H12O2	6.90E+06 ± 7.44E+05	5.47E+06 ± 1.63E+05
7.740	1-Hexanol	C6H14O	1.61E+06 ± 1.96E+05	2.06E+06 ± 3.69E+05
7.911	Isoamyl acetate	C7H14O2	1.35E+08 ± 8.29E+06	1.22E+08 ± 3.82E+06
9.300	Butyrolactone	C4H6O2	1.56E+06 ± 2.42E+06	2.31E+05 ± 3.48E+04
11.448	1-Heptanol	C7H16O	4.35E+06 ± 6.98E+05	1.45E+06 ± 2.43E+05
12.399	Ethyl hexanoate	C8H16O2	3.47E+08 ± 1.66E+07	2.75E+08 ± 1.83E+07
12.877	Hexyl acetate	C8H16O2	1.18E+07 ± 9.53E+05	8.57E+06 ± 6.96E+05
13.921	Ethyl 2-hexenoate	C8H14O2	4.06E+05 ± 1.07E+05	4.56E+05 ± 1.60E+05
14.309	Isoamylbutyrate	C9H18O2	1.99E+05 ± 3.32E+04	1.90E+05 ± 2.83E+03
14.916	1-Octanol	C8H18O	1.34E+06 ± 4.59E+05	7.15E+05 ± 1.19E+05
15.488	2-Nonanone	C9H18O	1.54E+06 ± 4.72E+05	2.22E+06 ± 1.29E+05
15.694	Ethyl heptanoate	C9H18O2	5.62E+06 ± 9.98E+05	7.87E+06 ± 1.04E+06
16.149	Benzeneethanol	C8H10O	1.77E+08 ± 1.38E+07	1.92E+08 ± 2.43E+06
16.857	2-Ethyl hexanoic acid	C8H16O2	2.00E+05 ± 1.54E+05	2.03E+05 ± 9.78E+04
18.083	Nonanol	C9H20O	1.27E+06 ± 7.12E+04	8.15E+05 ± 8.99E+04
18.285	Diethyl succinate	C8H14O4	1.40E+07 ± 1.50E+06	1.62E+07 ± 2.74E+05
18.477	Ethyl 7-octenoate	C10H18O2	1.98E+09 ± 6.41E+07	1.54E+09 ± 8.84E+07
19.043	Decanal	C10H20O	9.95E+05 ± 5.35E+05	6.58E+05 ± 2.39E+05
19.666	3,4-Dimethylcyclohexanol	C8H16O	4.97E+05 ± 2.80E+05	3.17E+05 ± 8.95E+04
20.151	Ethyl 2-octenoate	C10H18O2	2.53E+06 ± 3.69E+06	2.64E+05 ± 7.79E+04
20.319	3-Methylbutyl hexanoate	C11H22O2	2.36E+06 ± 2.06E+06	8.03E+05 ± 1.27E+05
20.430	2-Phenethyl acetate	C10H12O2	3.67E+06 ± 3.04E+06	4.96E+06 ± 7.77E+05
20.937	Vitispirane	C13H20O	6.80E+05 ± 2.78E+05	1.42E+06 ± 3.72E+05
21.266	Bornyl acetate	C12H20O2	2.71E+05 ± 4.59E+04	2.87E+05 ± 1.79E+05
21.474	2-Undecanone	C11H22O	2.45E+05 ± 1.18E+05	3.64E+05 ± 3.76E+04
21.553	Ethyl nonanoate	C11H22O2	7.32E+06 ± 2.36E+06	9.56E+06 ± 3.52E+06
21.633	Tridecane	C13H28	1.00E+06 ± 1.01E+06	4.06E+05 ± 4.22E+05
22.939	Isobutyl octanoate (Caprylic acid isobutyl ester)	C12H24O2	2.07E+06 ± 2.49E+05	1.15E+06 ± 2.27E+05
23.067	1,2-Dihydro-1,1,6-trimethyl-naphthalene	C13H16	1.33E+06 ± 1.23E+05	1.32E+06 ± 2.75E+05
23.609	9-Decenoic acid	C10H18O2	5.56E+08 ± 7.86E+08	2.19E+07 ± 4.16E+06
23.814	Decanoic acid	C10H20O2	7.19E+06 ± 3.32E+06	3.73E+08 ± 2.62E+07
24.050	Ethyl 9-decenoate	C12H22O2	3.43E+08 ± 2.87E+08	8.49E+08 ± 5.84E+07
24.361	Ethyl decanoate (Capric acid ethyl ester)	C12H24O2	8.17E+08 ± 6.91E+08	9.13E+06 ± 2.12E+05
24.600	Ethyl dec-9-enoate	C12H22O2	1.22E+07 ± 3.60E+06	5.36E+06 ± 4.53E+06
25.073	Ethyl 3-methylbutyl butanedioate	C11H20O4	5.60E+06 ± 9.22E+06	1.80E+05 ± 4.80E+04
25.484	3-Methylbutyl octanoate	C13H26O2	1.25E+07 ± 9.03E+06	1.21E+07 ± 4.42E+06
25.562	Isoamyl octanoate (Isoamyl caprylate)	C13H26O2	1.77E+06 ± 1.47E+06	1.10E+06 ± 6.58E+05
26.247	1-Dodecanol	C12H26O	1.12E+06 ± 6.61E+05	7.38E+05 ± 2.61E+05
26.615	Propyl decanoate	C13H26O2	2.47E+05 ± 4.43E+04	1.39E+05 ± 8.04E+04
27.925	Isobutyl decanoate (Isobutyl caprate)	C14H28O2	1.64E+06 ± 1.03E+04	1.06E+06 ± 7.47E+04
28.947	Ethyl 9-hexadecenoate (Ethyl oleate)	C18H34O2	4.29E+05 ± 8.58E+04	4.83E+06 ± 5.82E+05
29.091	Ethyl dodecanoate (Ethyl laurate)	C14H28O2	2.77E+08 ± 3.66E+07	2.02E+08 ± 7.67E+06
30.043	3,3-dimethylpentyl-cyclohexane	C13H26	1.39E+06 ± 1.94E+05	8.55E+05 ± 1.52E+05
30.205	3-Methylbutyl pentadecanoate	C20H40O2	9.99E+06 ± 1.05E+06	3.65E+06 ± 3.12E+06
30.279	Isoamyl decanoate	C15H30O2	8.45E+05 ± 1.87E+05	3.96E+06 ± 6.07E+06
32.478	Ethyl tetradecanoate (Ethyl myristate)	C16H32O2	3.53E+06 ± 2.97E+06	2.43E+05 ± 3.27E+05
32.514	Hexadecane	C16H34	6.16E+05 ± 4.46E+05	2.50E+06 ± 3.82E+06
32.893	Isoamyl laurate	C17H34O2	7.83E+05 ± 6.20E+05	3.10E+06 ± 4.36E+06
33.970	Ethyl hexadecanoate (Ethyl palmitate)	C18H36O2	4.52E+06 ± 4.05E+05	5.54E+06 ± 4.88E+05
34.216	Isopropyl hexadecanoate (Isopropyl Palmitate)	C19H38O2	3.03E+05 ± 1.02E+05	2.76E+05 ± 1.39E+05

4. Discussion

4.1. Kaolin Modulates Grapevine Plant Physiology and Pest Control

The kaolin particle film was initially developed for suppression of arthropod pests and diseases by its repellent effect [39,40]. Our results confirm that Kl spraying reduces the incidence of pests in grapevines (Supplementary data), reinforcing their repellent benefit as reported in other crops e.g., apple, pear, and olive trees [40–42]. Increased plant productivity results from insect control with Kl has also been documented [42,43]. This work, as well as a previous one done by our group in red grape cultivars in the Douro valley (NE Portugal) [44] reinforce that the treatment of grapevine leaves with the inert clay mineral kaolin increases also physiological capacity of plants [44]. The clay particles protect leaves from excessive radiation leading to a lower leaf temperature (Figure 2) and thus better iWUE, extremely linked to the lower ABA accumulation [14], reducing potentially damage triggered by visible and ultraviolet radiation and therefore decreases heat stress and sunburn injury [45]. Grapevine Kl plants under better temperature and irradiation conditions showed higher P_N values (Table 1). In control leaves nonstomatal limitations to photosynthesis were evident, as revealed by the decrease of P_N and iWUE and the increase of C_i/C_a ratio (Table 1), either through CO_2 diffusion and carboxylation efficiency [46], and/or photochemical perturbations. At this level, the decrease in P_N under saturating light conditions was associated with a reduction of Φ_{PSII}, F_v/F_m, and ETR (Table 2). Additionally, linking the decrease of F_v/F_m with the increase of F_0, these results suggest that the photoprotective capacity of these leaves was surpassed and photoinhibitory damage in the PSII occurred [47], as seen previously in plant reaction to high temperature and water stress [48]. The Kl photoprotection induction is also supported by the higher qP and lower NPQ values, which suggest an effective radiative and nonradiative dissipation of the excess energy, avoiding the photosystem damage by oxidation [49].

4.2. Kaolin Boosts Grapevine Fruit Quality

It is known that during grape berry development a complex series of physicochemical modifications, such as changes in size, color, chemical composition, and flavor occurs [50]. Temperature influences both cell division and enlargement and in excess, such as in our study (>35 °C), reduce growth rate and size. However, solar radiation is also crucial for berry growth [51], which could explain that Kl treatment induces 11.2% lower increase in berry diameter than control ones, from veraison to maturation stages, supposedly due to more shaded fruits provoked by a high number of healthy leaves that exist because of the leaf Kl protection from sunburn. White berries from *V. vinifera* varieties are the consequence of the inactivation of anthocyanin biosynthesis, thus that grape green-yellowish color is mainly linked to catabolic pathways instead of specific pigment accumulation [52]. During the maturation process a degradation of carotenoids and chlorophyll pigments occurs [53] being, according C*ab coordinates, the yellowish color of the fruits related with the b* value. In spite of the high b* value of control fruits, both Kl and control berries have a positive b* (Table 3) and, consequently, yellowish color. However, the negative a* value of the Kl fruits (greener) compared with the positive one (more reddish) obtained for control berries, reveals that Kl fruits are more yellowish compared with the brownish-yellow of control ones. This difference could be related with the low chlorophylls/carotenoids ratio, and the ripening characteristic oxidative burst, promoting the appearance of the yellowish color of the fruits [53]. During the berry ripening stage, pH should increase, mainly related with the decline of tartaric and malic acid [54]. These acids decrease and were observed in berries of both treatments, resulting in a decrease of overall total acidity (Table 3). Tartaric acid concentration decreases from veraison to maturation stage, contrary to other studies reporting that their content remains relatively constant in the grape berry and is not related to climatic conditions [55]. Inversely, malic acid concentration in grapes depends of several factors, such as climate conditions, especially irradiance [55] and temperature [56], as the most important ones. The positive protection of Kl treatment leads to berries with higher tartaric and malic acid concentration (Table 3). Probably, this is due to the sun

protection of fruits by the healthy leaves and their shade effects on lower acid degradation. It is known that higher level of organic acids (particularly tartaric acid) is a positive characteristic of grapevine varieties in warm climates, such in this studied region and others most threatened by climate change [57]. For this reason, Kl treatment showed potential to produce well balanced wines avoiding the intensive need of acidification of must/wine. As referred before, organic acids (malic and tartaric acids) and sugars concentration showed opposite behavior [54] as we observed in our study. In fact, Kl fruits have more tartaric and malic acid and lower sugar concentration than control berries (Table 3). These results in white grapevine fruits, as previous ones in red berries [10,17,21], reinforce that Kl treatment boosts, mainly in the mature grape berry (maturation period), the quantities of phenolic compounds, including total phenolics and tannins, leading also to an augment of antioxidant activity (Table 4). This happened because in response to kaolin there is a global stimulation of phenylpropanoid and flavonoid-flavonol pathways at the gene expression and/or protein activity levels [21]. This fact should have major implications in fruit and wine quality, while protecting plant against abiotic stress. An analysis focused on secondary metabolism in the different fruit tissues showed as predictable that the major quantity of phenolics are found in the seed, after in the skin, and only a little percentage in the pulp [58]. However, we observed that Kl provokes an increase of total phenols, flavonoids, tannins, and *ortho*-diphenols compared with the control (Table 5). Amongst polyphenols, *ortho*-diphenols are known as the most significant in relation to their antioxidant activity which is related with hydrogen donation, i.e., their capacity to improve radical stability by forming an intra-molecular hydrogen bond between the hydrogen of their hydroxyl group and their phenoxyl radicals [59]. Grape berry mineral content is also important and involved in wine chemical composition. Among the several minerals present in grape berries, potassium (K^+) usually represents the most abundant cation because is accumulated during the entire period (pre- and post-veraison) with a huge rise at the beginning of ripening [60]. The Kl use leads to an enhance of K^+. Looking from a technological point of view, this effect is beneficial because K^+ influences the pH of musts and wines and thereby their chemical and microbiological stability, in addition to the perception of wine flavor [61]. Taking into account that the factors that affect K^+ accumulation include their soil availability and weather conditions effects, this study reinforces the Kl protective capacity against summer stress, since the pedoclimatic conditions were similar for all plants under study. This high accumulation is reflected in the must when the Kl application also boosts their content (Table 6). Magnesium is considered to be a phloem-mobile element and its amount increased during ripening but at different rates depending on the response of berry to vine water relations [62]. This finding allows us to highlight the Kl capacity on the WUE avoiding water stress, which enabled the increase the quantity of Mg^{++} in Kl fruits and must. With magnesium, other mineral elements such as calcium and copper also play a role in osmotic balance. In spite of copper being an essential micronutrient for all living organisms, including humans [63], elevated copper concentrations in grapevine can cause oxidative spoilage leading to browning of white wine as well as haze formation [64], which will hardly happen in the Kl must when the copper quantity is much lower than the control one. Of the several questions that winemakers have about Kl application, the most frequent one refers to aluminum. As Kl is an aluminum silicate ($Al_2Si_2O_5(OH)_4$) there was a fear that high quantities of this metal would be found in grapes and wines whose plants were sprayed with it. However, these results confirm that the powdered aluminum was not absorbed into the fruits, nor to the musts (Table 6), which is reflected in the wine (Tables 7 and 8), and even its concentrations are even lower than those of the control vines. One possible explanation is that the kaolin that falls to the soil may change the pH of the soil, making it less acidic, leading to less solubility of Al in the soil and consequently less absorption of Al by the vines.

4.3. Positive Effect of Kaolin Application in White Wine Balance

One of the main challenges for the wine industry is climate change, because it has an enormous effect on vine phenology and physiology and consequently on yield and grape composition. Among climate change-related consequences, the advanced maturation times and temperatures are the most

significant because these lead to an increase in grape sugar concentrations, which provokes high wine alcohol and lower acidities, especially in warm regions [65]. White varieties with highest level of tartaric and malic acid are suggested to be of great interest for breeding new cultivars. These are the principal organic acids in wine representing 70–90% of total grape acidity [66]. Their concentration is one of the most key quality characteristics of grapes for wine production, and has an essential impact on the color, flavor, and stability of wine [67]. Relating to white wines from warm regions, malic acid can even have a positive effect on wine balance. In such cases malolactic fermentation may result in significant changes of the wine aromatic profile and in increase of lactic and buttery characteristics and a decrease in fruity characteristics [68]. Looking to our results, Kl pulverization should be also used in white cultivars in areas with severe summer conditions due their capacity to trigger high total acidity (higher malic and tartaric acid) and lower alcohol degree (Table 7) which are characteristics well appreciated by the new wine consumers. After analysis of volatile compounds, it is possible to see that, in general, the control wine exhibited higher content of esters with C_{11} to C_{18} and decanoic acid, which if present in an amount higher than their aroma threshold, may contribute with unpleasant notes, such as wax, soap, or fatty [69,70]. On the other hand, in general, wine from kaolin treated grapevines presented higher content of esters with $<C_{12}$, which are associated with fruity notes [69]. Additionally, control wine presents higher content of vitispirane, a C_{13}-norisoprenoid. As observed for several fruits, including grape berries, during ripening diverse reactions modulated by enzymes may occur, namely degradation processes, such as carotenoids cleavage resulting in formation of norisoprenoids [71,72]. Hydroxylated C_{13}-norisoprenoids often occur in plants as glycosides and can be liberated from these by enzymatic or acid hydrolysis and then transformed into aroma compounds, such as vitispirane. The statistically significant differences observed between both types of wines in the content of vitispirane may infer the impact of kaolin treatment of grapevines on the carotenoids cleavage. Vitispirane is associated with camphor and eucalyptus notes [73], but as its odor threshold is relatively high (800 µg/kg, wine) [68]. Further research is needed, namely sensorial analysis assays, to properly evaluate the impact of the observed statistically significant differences related with wine volatile profiles on the aroma characteristics of control wine and wine from kaolin treated grapevines.

5. Conclusions

We showed that kaolin application triggered an improvement in plant physiology, especially under conditions of abiotic stress, and can also be considered as an alternative to synthetic pest control. Moreover, kaolin application significantly influences the grape fruit metabolome in a way that provides berries with high phenolic compounds, tartaric and malic acids, total acidity, and lower sugar content. Besides, it is essential to reinforce that a good influence was observed in wine having higher acidity and lower alcohol levels and seems to have improved the aroma. In sum, foliar kaolin application in grapevine leaves shows great potential as a summer stress mitigation strategy because it clearly impacts on berry and wine quality as a result of many molecular and biochemical changes in key primary/secondary metabolic pathways.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/9/1422/s1, Figure S1: Kaolin effect on *Panonychus ulmi* and *Scaphoideus titanus* pests.

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