Research Article

Received: 2 August 2022 Revised: 8 October 2022 Accepted article published: 13 October 2022 Published online in Wiley Online Library: 3 November 2022

(wileyonlinelibrary.com) DOI 10.1002/jsfa.12268

The beneficial effect of leaf removal during fruit set on physiological, biochemical, and qualitative indices and volatile organic compound profile of the Cypriot reference cultivar 'Xynisteri'

Egli C. Georgiadou,[a,b*] Minas Mina,[b] Varnavas Neoptolemou,[a] Stefanos Koundouras,[c] Claudio D'Onofrio,[d,e] Andrea Bellincontro,[f] Fabio Mencarelli,[d,e] Vasileios Fotopoulos[a] and George A. Manganaris[a*]

Abstract

Background: 'Xynisteri' is the reference Cypriot white cultivar that, despite its significant societal and economic impact, is poorly characterized regarding its qualitative properties, while scarce information exists regarding its aroma profile. In the current study, the effect of leaf removal during fruit set (BBCH 71) on 6-year cordon-trained, spur-pruned grapevines was assessed and an array of physiological, biochemical, and qualitative indices were monitored during successive developmental stages (BBCH 75, BBCH 85, BBCH 87, and BBCH 89). Grapes were additionally monitored for the volatile organic compounds (VOCs) profile during the advanced on-vine developmental stages (BBCH 85–BBCH 89) with the employment of gas chromatography–mass spectrometry (GC–MS), Fourier-transform near infrared (FT-NIR) spectra and electronic nose (E-nose) techniques.

Results: Grape berries from the vines subjected to leaf removal were characterized by higher solid soluble sugars (SSC), titratable acidity (TA), tartaric acid, and ammonium nitrogen contents, while this was not the case for assimilable amino nitrogen (primary amino nitrogen). A total of 75 compounds were identified and quantified, including aliphatic alcohols, benzenic compounds, phenols, vanillins, monoterpenes, and C_{13}-norisoprenoids. Leaf removal led to enhanced amounts of glycosylated aroma compounds, mainly monoterpenes, and C_{13}-norisoprenoids. Chemometric analysis, used through FT-NIR and E-nose, showed that the aromatic patterns detected were well associated to the grape ripening trend and differences between leaf removal-treated and control grapes were detectable during fully ripe stage.

Conclusion: Leaf removal at fruit set resulted in an overall induction of secondary metabolism, with special reference to glycosylated aroma compounds, namely monoterpenes and C_{13}-norisoprenoids.

Supporting information may be found in the online version of this article.

* *Correspondence to: EC Georgiadou or GA Manganaris, Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, 3603 Limassol, Cyprus. E-mail: egli.georgiadou@cut.ac.cy (Georgiadou); E-mail: george.manganaris@cut.ac.cy (Manganaris)*

a *Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, Limassol, Cyprus*

b *Kyperounda Winery, P. Photiades Group, Limassol, Cyprus*

c *Laboratory of Viticulture, School of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, Greece*

d *Department of Agriculture, Food and Environment Science, University of Pisa, Pisa, Italy*

e *Nutraceuticals and Food for Health – Nutrafood, University of Pisa, Pisa, Italy*

f *Department for Innovation in Biological, Agro-food and Forest Systems (DIBAF) – Postharvest Laboratory, University of Tuscia, Viterbo, Italy*

Keywords: C_{13}-nosisoprenoids; E-nose; FT-NIR; GC–MS; indigenous cultivar; monoterpenes; volatile organic compounds (VOCs); chemometrics

ABBREVIATIONS

DMSO	dimethyl sulfoxide
E-nose	electronic nose.
FF	flesh firmness
FT-NIR	Fourier-transform near infrared
GC–MS	gas chromatography–mass spectrometry
RI	ripening index
SPAD	soil plant analysis development
SSC	solid soluble sugars
SWP	stem water potential
TA	titratable acidity
TSS	total soluble sugars
VOCs	volatile organic compounds

INTRODUCTION

The cultivation of grapes has significant societal and economic impact worldwide.[1] The potential of a grape cultivar is highly dependent on its content in secondary metabolism compounds, such as aromas or polyphenols. These compounds regulate both the nutraceutical and organoleptic properties of grape berries, including grape flavor and odor.[2]

Aromas in grapes are volatile organic compounds (VOCs) or glycosylated VOCs precursors belonging in terpenoids (monoterpenes, C_{13}-norisoprenoids, and sesquiterpenes), aliphatic C6 volatile compounds (alcohols and aldehydes), shikimate pathway derivatives (volatile phenols or benzene derivatives), methoxypyrazines, and volatile thiols (or mercaptans).[3] Different grape cultivars exhibit distinct aromas and flavors due to the presence or absence of specific compounds as well as to the variations in the ratios of the compounds that constitute the grape's aroma profile.[4] Terpenoids, in the form of glycoside conjugates are some of the most significant aroma compounds of grape berries.[5] For this reason, terpenoids, and primarily monoterpenes, have been extensively studied.[6] To enhance grape aroma, several approaches have been proposed, including agronomic practices such as leaf removal, irrigation, foliar fertilization, bunch thinning, canopy training systems, and exogenous compound application.[7-9,2]

Leaf removal is a preharvest technique applied to control the cluster zone microclimate, to improve the ripening of grapes and to reduce the incidence and severity of pests and diseases.[10,11] Leaf removal can be applied from pre-blooming to veraison, as well as before grape ripening.[12,13] Leaf removal allows grapes' exposure to the sun, increase berry temperature and as a result the metabolism of the grapes is affected both in terms of composition and quality.[14-16] However, the alteration of grape composition by leaf removal might also rely on factors other than microclimate, such as, changes in leaf-to-yield ratio, assimilation rates of the remaining leaves, changes in source-sink balance, berry size, and consequently there are different responses of grape composition to leaf removal concerning the timing and the level of intervention, as well as to factors such as grapevine genotype, training system, vine age, irrigation practice, and regional macroclimate.[17,18]

Regarding the timing, late leaf removal has been reported to be less effective than early leaf removal in modifying the chemical composition of grapes and subsequently the quality of the produced wine.[14] As far as the intensity of leaf removal is concerned, studies have shown that excessive light and thermal exposure of bunches should be avoided, as they can negatively affect the quality of the end product.[19,20] Moreover, prolonged exposure of grapes to sunlight can cause degradation of both aroma precursors and malic acid. Leaf removal can also change the physical characteristics of the grapes such as reduction of bunch density and berry size with early leaf removal, while other studies have shown that sun-exposed berries have thicker skin.[21,22]

Cyprus is one of the very few phylloxera-free areas in the world that allows the plantation of vine in their own roots and is dominated by indigenous cultivars, accounting for three-quarters of the total cultivated area. 'Mavro' and 'Xynisteri' cultivars dominate the cultivated area, mainly due to their adaptation to adverse climatic conditions with no irrigation. These cultivars upon sun-drying are destined additionally for the production of premium white wines and 'Commandaria', a Protected Designation of Origin (PDO) dessert wine.[23-25] Despite its significant societal and economic impact, 'Xynisteri' is poorly characterized.[26,27] Thus, the aim of this study was to assess the impact of leaf removal at fruit set in cv. 'Xynisteri' with special reference to its aromatic profile through the employments of an array of high-throughput analytical approaches.

MATERIALS AND METHODS

Plant material and experimental setup

Field experiments were carried out during 2020 in a 6-year-old commercial vineyard of own-rooted 'Xynisteri' grapevines (*Vitis vinifera* L.) in Agios Ioannis (34° 53′ 56.5″ N, 33° 00′ 48.4″ E) (Limassol district, Cyprus). Air temperature (°C), rainfall (in millimeters), and relative humidity (%, RH) were recorded by meteorological station installed in proximity to the study site (Supporting Information Fig. S2). Huglin index (HI) and growing degree-days (GDD) equation were calculated for climate characteristics.[28] HI was 2581 that corresponds to warm class limit and GDD was 2418 that corresponds to region V according to the Winkler index, thus typically appropriate for high production volumes, reasonable quality table wine or table grape cultivars destined for early season consumption.

The grapevines were spaced 2.1 m between rows and 1.5 m within the row, using the cordon-trained, spur-pruned system. Winter pruning took place during February, while organic manure was applied as basic fertilization. Soluble and dustable sulfur and *Bacillus thuringiensis* were applied during April–May and in June–August, they were reapplied to treat powdery mildew and *Lobesia botrana*. Moreover, the soil was ploughed three times at 25 cm, using a rotary hoe during March, May, and July. Furthermore, plants were tipped at the training wire (2 m) during June.

Experiment consisted of three plots (four grapevines per plot) per treatment [control (C) and leaf removal (LR)]; each experimental plot is considered as one biological replication. Experimental setup and the handling of sampling procedure is schematically

Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

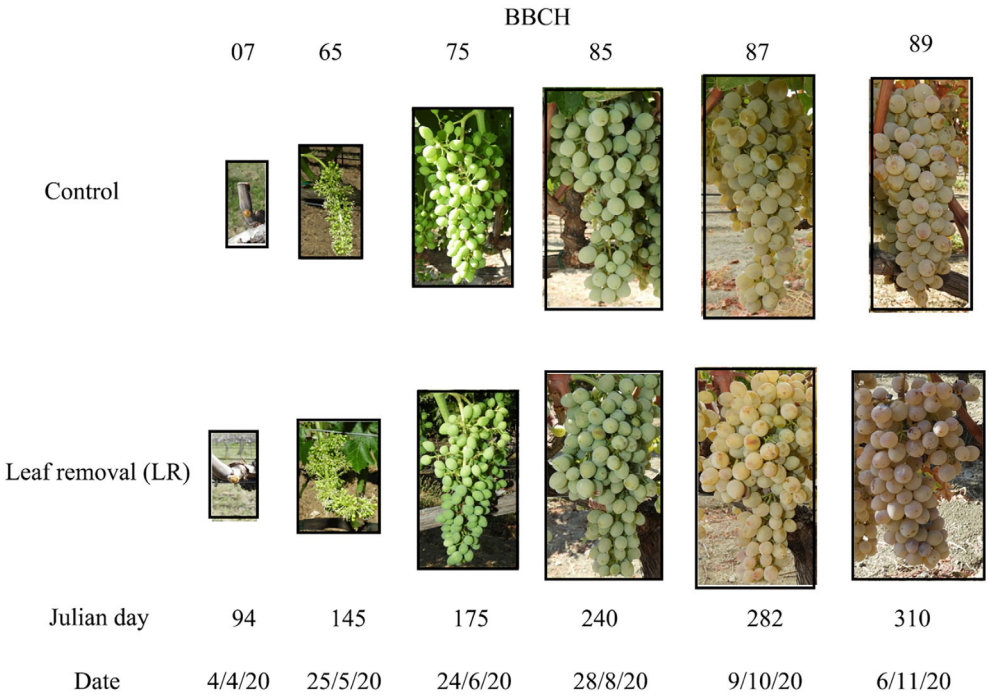

Figure 1. Phenological stages of control and leaf removal brunches according to the BBCH scale.[29,30]

described in Fig. S1. Grapevine developmental stages were determined based on the Biologische Bundesanstalt, Bundessortenamt, Chemische Industrie (BBCH) scale (Fig. 1).[29,30] Leaf removal treatment took place manually at fruit set (BBCH 71). All leaves around each grape bunch were removed while leaving enough leaves on the higher part of the vines to provide some shade (as can be seen in Fig. S1). The stem height was approximately 1 m. Leaf and berry samples were collected at BBCH 75, BBCH 85, BBCH 87, and BBCH 89. Leaves were additionally harvested prior to leaf removal.

Analysis took place in leaf, berry, and juice through the employment of an array of physiological, biochemical, qualitative indices, and analytical approaches as described later. For biochemical analysis, leaves (ten per plot) and berries (50 per plot) were flash frozen in liquid nitrogen in the vineyard, ground into powder in the laboratory, and stored at −80 °C until needed.

Physiological measurements, photosynthetic pigment analysis, and cellular damage indicators

Before leaf removal and at all four developmental stages (BBCH 75, BBCH 85, BBCH 87, and BBCH 89) stomatal conductance, stem water potential (SWP), and soil plant analysis development (SPAD) measurements were conducted in four leaves per plot between 12 p.m. and 2 p.m. ΔT-Porometer AP4 (Delta-T Devices, Cambridge, UK) was used to measure stomatal conductance according to the manufacturer's instructions. SWP values were determined on the leaves; the measurement included leaf enclosure in dark plastic bags for 60 min to allow water potential equilibration. SPAD measurements were conducted in four leaves per plot with a hand-held chlorophyll meter SPAD-502Plus (Konica Minolta Inc., Tokyo, Japan).

Photosynthetic pigments were extracted with dimethyl sulfoxide (DMSO) from four leaves per plot before leaf removal and at all developmental stages (BBCH 75, BBCH 85, BBCH 87, and BBCH 89) and were measured spectrophotometrically (Infinite 200 PRO; TECAN, Mannedorf, Switzerland) at 661, 643, 470, and 534 nm.[31] The concentrations of chlorophylls (Chl a, Chl b, and total) and carotenoids were quantified using the equations proposed by Misra and Dey,[32] whereas anthocyanins concentrations were calculated using the equations reported by Nikiforou *et al.*[33]

Cellular damage indicators [lipid peroxidation, hydrogen peroxide (H_2O_2) and nitrite-derived nitric oxide (NO)] were measured spectrophotometrically from leaves prior to leaf removal and at stages BBCH 75, BBCH 85, BBCH 87, and BBCH 89. The lipid peroxidation was extracted from malondialdehyde (MDA) content resulting from the thiobarbituric acid (TBA) reaction using the Lambert–Beer law, with extinction coefficient of 155 mmol L^{-1} cm^{-1}.[34] The H_2O_2 was processed based on the oxidation of iodide ion (I^-) to iodine (I), after the reaction of H_2O_2 with potassium iodide (KI).[35] The NO content was calculated using the Griess reagent.[36]

Qualitative attributes

Every week from end of veraison until harvest, 200 berries per plot were sampled in order to isolate the must with a commercial juicer. The quality attributes of must assessed were solid soluble sugars (SSC), titratable acidity (TA), ripening index (RI), pH, ammonium nitrogen, assimilable amino nitrogen, malic, and tartaric acid. For SSC, a PAL refractometer (PR-32a; ATAGO, Tokyo, Japan) was used. TA was measured using an automatic multiple positions titrator (862 Compact Titrosampler; Metrohm AG, Herisau, Switzerland), for each measurement, 5 mL of juice in 45 mL water was used for titrating 0.1 N sodium hydroxide (NaOH) to a pH end point of 8.1. Results were expressed as grams tar of taric acid per liter. For the RI the SSC/TA ratio was calculated and for the pH values the pH-meter (HI 2222, Hanna instruments, Inc., Woonsocket, RI, USA) was used. Ammonium nitrogen [a measure for the amount of ammonia (NH_4^+)] was determined using the indophenol method according to Scheiner[37] and assimilable amino nitrogen (primary amino nitrogen) using an *o*-

Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

phthalaldehyde/*N*-acetyl-L-cysteine (OPA/NAC) spectrophotometric assay.[38] L-Malic acid (L-malate) and tartaric acid (tartrate) were measured with assay kits (Megazyme, Bray, Ireland).

At harvest (BBCH 89), weight and number of all bunches of production grapes were measured from four grapevines per plot. The four grapevines per plot used to compute yield indicators, color parameters, and flesh firmness (FF), were different from the other four grapevines per plot used for all other measurements. Five bunches of grapes per plot were taken in the laboratory and weight, length, and width were measured. Berries from each grape bunch were removed and subsequently number/weight of berries from each bunch (weight of single berries), and weight of grape stalk from each bunch were calculated. Then, ten berries per replication were taken and length/width were measured with electronic caliber (IS11112; Insize, UK). Color parameters were measured with reflection colorimeter (CR-400; Konica Minolta, Osaka, Japan) to indicate the coordinates L^* (brightness or lightness; 0 = black, 100 = white), a^* ($-a^*$ = greenness, $+a^*$ = redness) and b^* ($-b^*$ = blueness, $+b^*$ = yellowness). Hue angle [($H°$) (0° = red-purple, 90° = yellow, 180° = bluish-green, 270° = blue)], chroma (degree of departure from gray to pure chromatic color) and the ratio a^*/b^* was also calculated. FF was measured with a texture analyzer (TA.XT plus; Stable Micro Systems, Godalming, UK), using a 3 mm diameter probe at a speed of 1 mm s^{-1} with a penetration depth of 5 mm and results were expressed in newtons. For color and FF, two measurements were made diametrically from equatorial sites of berry as technical replicates for the same berry. Following, skin, flesh, and seeds of the berries were removed carefully. The number of seeds and the fresh weight of skin, flesh, and seeds were measured and the percentage of fresh weight per berry (%) was calculated.

Total soluble sugars (TSS), sucrose, glucose, and fructose, were extracted from berry tissue (BBCH 75, BBCH 85, BBCH 87, and BBCH 89)[39] and determined spectrophotometrically as described elsewhere.[40,41]

Analysis of berry aroma compounds

Solid-phase extraction (SPE) was used for the analysis of berry glycosylated aroma compounds (aliphatic alcohols, benzenic compounds, phenols, vanillins, monoterpenes, C_{13}-norisoprenoids) as analytically described by D'Onofrio *et al.*[2] Samples were extracted from 100 fresh berries (for each plot) harvested during BBCH 85, BBCH 87, and BBCH 89.

For the preparation of grape samples, the skins of 100 berries were extracted and used for each replicate with 20 mL of methanol, flesh and juice were placed in a glass containing 100 mg of sodium metabisulfite, while seeds were disposed. After 1 h, the skins were placed together with the flesh and juice, to which 150 mL of the pH 3.2 tartaric buffer solution (2 g L^{-1} sodium metabisulfite, 5 g L^{-1} tartaric acid, and 22 mL L^{-1} NaOH 1 N) had been added and homogenized with the use of an immersion blender (Ultra-Turrax; IKA, Staufen, Germany). After centrifugation at 5000 × *g* for 5 min, the supernatant was collected in a flask, and the pellet was washed with an additional 100 mL of pH 3.2 tartaric buffer solution. After a further centrifugation, the supernatant was added to the first one, the volume adjusted to 400 mL by adding further tartaric buffer solution, and stored at −20 °C. A pectolytic enzyme (Vinozym FCEG) was added to the extract and incubated over night at room temperature to make it limpid. The next day, it was centrifuged, just before the SPE procedure.

For the extraction of aromatic compounds from these extracts, a total of 200 µL of 1-heptanol (40 µg mL^{-1}) was added as an internal standard and eluted through a 5 g C18 Cartridge (Mega Bond Elut; Agilent, Santa Clara, CA, USA), which had been activated with 20 mL of methanol and 50 mL of water. The cartridge was washed with 100 mL of water and then with 30 mL of dichloromethane so as to recover the fraction containing free compounds dehydrated with sodium sulfate anhydrous, and concentrated to 200 µL before the analysis. The glycosylated compounds were eluted with 30 mL of methanol. Methanol was evaporated under vacuum and the residue solubilized in 5 mL of a phosphate–citrate buffer (0.1 mol L^{-1} Na_2HPO_4 and 50 mmol L^{-1} citric acid; pH 5). The glycosidically-bound fraction was hydrolyzed with 600 µL of a glycosidic enzyme with strong glycosidase activity (CYTOLASE M102; Ferrari) and kept at 40 °C overnight (16 h). Next, were added 200 µL of 1-heptanol (40 µg mL^{-1}) as an internal standard. The mixture, containing the aglycones released by enzymatic hydrolysis, was then centrifuged and eluted through a 1 ×*g* C18 (Mega Bond Elut; Agilent) which had been previously activated with 5 mL of methanol and 10 mL of water. The fraction containing the aglycones was eluted with 6 mL of dichloromethane, dehydrated with sodium sulfate anhydrous, and concentrated to 200 µL before analysis.

Chromatographic analysis was performed, using an Agilent 7890A gas-chromatograph coupled with an Agilent 5975C quadrupole mass spectrometer (Agilent, Waldbron, Germany).[4] Helium was the carrier gas at a constant flow rate of 1 mL min^{-1}. The capillary column was an HP-Innowax [30 m length, 0.25 mm inner diameter (i.d.), 0.25 mm film thickness] from Agilent. The temperature program of the column oven started at 30 °C, then was increased at 30 °C min^{-1} to 60 °C for 2 min, at 2 °C min^{-1} to 190 °C, and at 5 °C min^{-1} to 230 °C for 10 min. The mass spectrometer detector scanned within a mass range of *m/z* 30–450. There were tentatively identified volatile compounds by comparing the mass spectra with those available in the data system library (NIST 08, National Institute of Standards and Technology, Gaithersburg, MD, USA, 2008) and using published retention indices (a positive characterization was achieved when a volatile compound was identified with a probability of > 70%), and when possible, the identity of the compounds were further confirmed by comparison of the retention times with 30 authentic standards. Calibration curves of some of these authentic standards were chosen to quantify the compounds of the same class sub-group (arranged by functional moiety) whose standards were not available (Supporting Information Table S2).[4] For gas chromatography–mass spectrometry (GC–MS) analysis, matrices of the original component data (metabolite concentrations *versus* treatment and developmental stages) were standardized in order to show (via a hierarchical clustering analysis heatmap) differences in the relative metabolite content using the gplots version 3.0.1 (heatmap.2 command; R Foundation for Statistical Computing, Vienna, Austria).

Chemometric analysis

Must samples were extracted from 200 fresh berries (for each plot) from BBCH 85, BBCH 87, and BBCH 89 as previously described for chemometric analysis with the employment of an electronic nose (E-nose) and Fourier-transform near-infrared (FT-NIR) techniques. The samples were centrifugated at 5500 rpm for 5 min at 4 °C and the supernatant was used.[42] A total of 20 mL of must for each replicate (three biological replicates per treatment) were incubated in glass vials with a volume of 50 mL at 25 °C for 20 min. The vials were capped with a perforable septum which the headspace into the E-nose device was aspired through. After the equilibration, the headspace was extracted by a constant flow of

filtered air and delivered into the E-nose sensor cell. The signals of the sensor were calculated as the resonant frequency shift between the signal of the sensors exposed to pure nitrogen (used as reference signals) and that obtained from the sample. The E-nose used is based on an array of eight quartz microbalances (QMBs) which are electromechanical resonators whose resonant frequency changes proportionally according to the mass adsorbed onto the sensor surfaces. Sensors were made with AT-cut quartz plates oscillating in the thickness-shear mode at a resonance frequency of 20 MHz. QMBs were functionalized by solid-state layers of metalloporphyrins.[43] Samples were analyzed in triplicate. Detected data as the array of eight-sensor signal were assembled in aromatic patterns characteristic of each must sample and used in chemometric calculation. FT-NIR spectra were transformed from transmittance to absorbance (log 1/*T*), then autoscaled and used as X-block variables for principal component analysis (PCA), and principal component regression (PCR) computations. In PCR, selected quality attributes of wine grapes (i.e. SSC, TA, tartaric acid and malic acid content, and pH) were used as dependent variables (Y-block).

Must samples (BBCH 85, BBCH 87, and BBCH 89) were prepared as described earlier were placed in a 10 mm cuvette and analyzed by FT-NIR spectra using an FT-NIR Neospectra with external light source and detector. The measurements were performed using the transmittance method of detection. Acquisition was conducted in the 1300–2600 nm range, with 8 nm wavelength increments and five spectra per average, which represented the spectral measurement of a single sample. Collected spectra were used for chemometric calculation. Data belonging to the E-nose patterns were normalized (mean normalization) then used for PCA and cluster analysis (by Ward's method) iterations. For all chemometric calculations, in both cases of FT-NIR spectra and E-nose data, a cross-validation by 'leave-one-out' method was performed. Multivariate computations were performed by using Matlab R2013a (MathWorks, Natick, MA, USA), and PLS Toolbox (Eigenvector Research, Inc., Manson, WA, USA). The results were graphically reported and interpreted as scoreplots, scatterplots, and hierarchical dendrogram.

Statistical analysis

Statistical analyses were carried out using the SPSS v.17.0 (SPSS, Chicago, IL, USA) software package, based on the analysis of variance (one-way ANOVA) according to Duncan's multiple way test with a significance level of 5% ($P \leq 0.05$) and significant difference

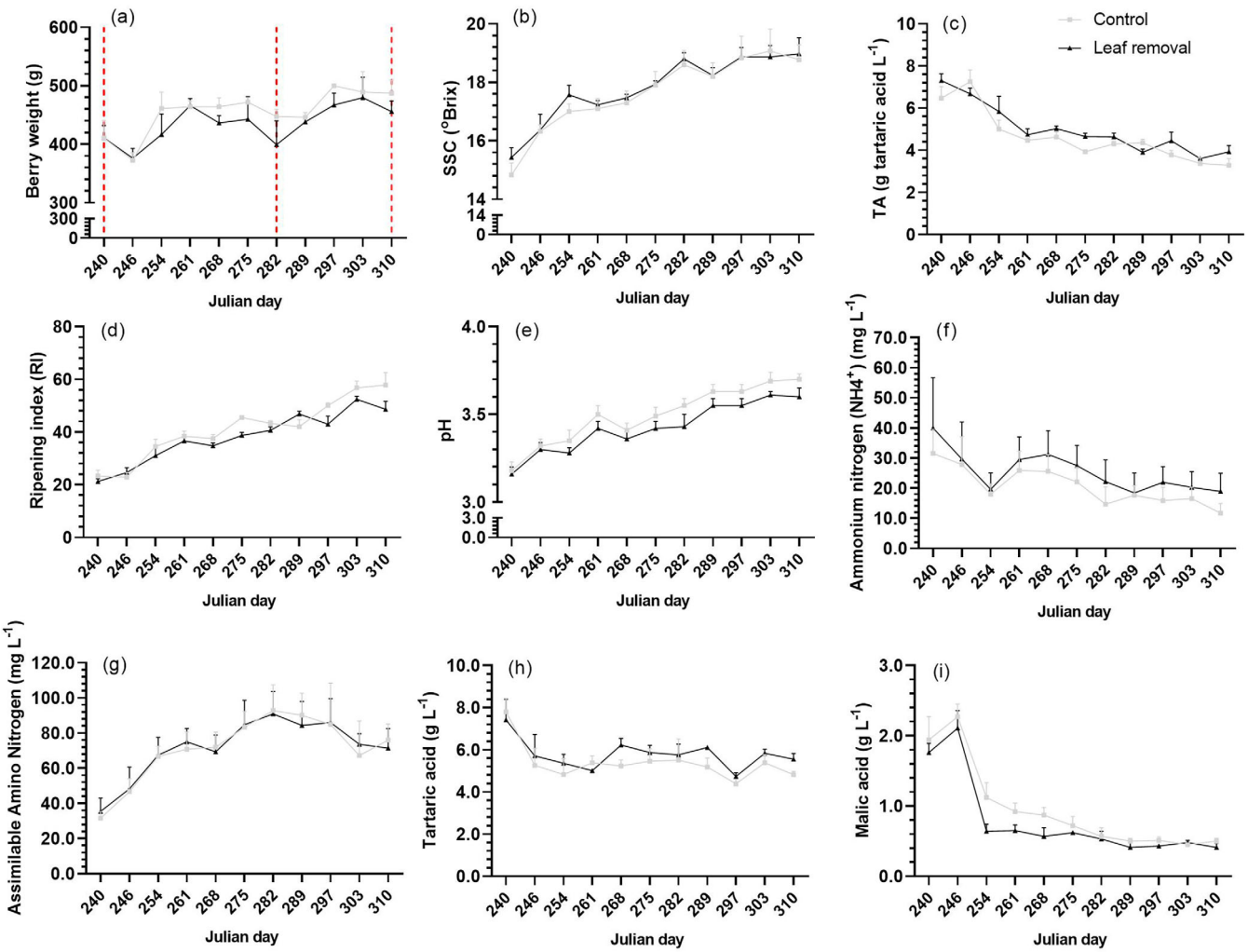

Figure 2. Berry weight (a), soluble solids content (SSC; b), titratable acidity (TA; c), ripening index (RI; d), pH (e), ammonium nitrogen (NH_4^+; f), assimilable amino nitrogen (primary amino nitrogen; g), tartaric acid (h), malic acid (i) of control and leaf removal experiment during berry development. The 240, 282, and 310 Julian day correspond to BBCH 85, BBCH 87, and BBCH 89 as shown with red dotted lines in (a). Results are the mean ± standard error (SE; $n = 3$).

Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

at 5% ($P \leq 0.05$) with *t*-test: paired two sample for means. Figures were generated using Prism 8.3.1 (GraphPad, La Jolla, CA, USA). Statistical analysis referring to GC–MS, E-nose, and FT-NIR analysis are additionally provided in the respective sections earlier.

RESULTS AND DISCUSSION

Effect of leaf removal on physiological measurements, photosynthetic pigment analysis, and cellular damage indicators

Physiological measurements, photosynthetic pigment analysis and cellular damage indicators were determined in leaves of 'Xynisteri' of control and leaf removal treatment (Table S1). SPAD units, stomatal conductance, chlorophylls (Chl a, Chl b, and total Chl content), carotenoids and anthocyanins dropped during ripening for both control and treated grapes, without any significant differences between treatments. Contrarily, SWP and cellular damage indicators (MDA, H_2O_2, NO) increased during the progress of on-vine developmental stages for both control and LR-treated vines. Leaf removal did not statistically affect SWP and cellular damage indicators.

Effect of leaf removal on qualitative attributes

Several qualitative attributes (SSC, TA, RI, pH, ammonium nitrogen, assimilable amino nitrogen, malic acid, and tartaric acid) were measured in grape must of cv. 'Xynisteri' of control and LR-treated (at fruit set). Although the skin was more reddish due to sun exposure compared with the control at leaf removal, the SSC was slightly higher on grapes from LR-treated vines (Fig. 2). Berry weight, pH, RI, and malic acid were lower on grapes from LR-treated vines. Similarly, another study on cv. 'Nebbiolo' grapes also showed that early leaf removal reduced berry number per bunch, the berry density and the size.[21] However, berries harvested following leaf removal treatment were characterized by higher TA, tartaric acid, and ammonium nitrogen related to control berries. This was not the case for while assimilable amino nitrogen (primary amino nitrogen) that presented similar values in control and LR-treated grapes (Fig. 2). Contradictory results have been presented in a study involving 'Xynisteri', where excessive sunlight and high daytime temperatures by late fruit-zone leaf removal (at veraison stage) showed a decrease in SSC and TA and an increase of pH, highlighting the significance on the timing of leaf removal application.[25]

Yield indicators, color parameters and flesh firmness were also measured in grape must of cv. 'Xynisteri' of control and LR-treated at production stage (BBCH 89). Phenotypic representation at BBCH 89 shows that control bunches were more green, while the leaf removal bunches were more red due to sun exposure. At BBCH 89, color parameters a^* and a^*/b^* ratio show higher values in the leaf removal berries manifesting higher coloration. However, hue angle was lower in leaf removal berries compared with control berries resulting from a more yellow coloration. In addition, FF was higher in LR-treated berries (Fig. S3).

TSS, sucrose, glucose, and fructose contents increased during ripening for both control and leaf removal treatments. Leaf removal (at fruit set) showed higher, yet not statistically significant, TSS, sucrose, glucose, and fructose contents (Fig. S4). Fructose was the most abundant type of sugar, followed by glucose and sucrose. Noteworthy, excessive sunlight and high daytime temperatures by fruit-zone leaf removal at veraison stage in 'Xynisteri' has resulted in a decrease in glucose and fructose.[25]

Effects of leaf removal on the glycosylated aroma compounds in berries

The concentrations of the berry glycosylated aroma compounds at BBCH 85, BBCH 87, and BBCH 89 are reported in Table S2. A total of 75 compounds were identified and quantified, including aliphatic alcohols, benzenic compounds, phenols, vanillins, monoterpenes, and C_{13}-norisoprenoids.

The total amount of glycosylated aroma compounds in the berries obtained following leaf removal treatment was higher by 3% in BBCH 85 (end of veraison), 15% in BBCH 87 (middle ripe), and 17% in BBCH 89 (fully ripe) compared with the control (Table S2). The two classes of aroma that contributed mostly to this increase were monoterpenes, and C_{13}-norisoprenoids. In particular, monoterpenes in LR-treated berries were 10–24% higher than control, while C_{13}-norisoprenoids were 11–34% higher during ripening (Fig. 3).

Leaf removal led to higher concentrations of aroma in BBCH 87 compared with all other developmental stages and control, across all six groups of glycosylated aroma compounds (aliphatic alcohols, benzenic compounds, phenols, vanillins, monoterpenes, and C_{13}-norisoprenoids), while it led to higher concentrations of monoterpenes and C_{13}-norisoprenoids in BBCH 89 (Fig. 3). Early leaf removal (EL29 and EL31) on 'Sauvignon blanc' grapes also resulted in increased monoterpenes and C_{13}-norisoprenoids contents.[44,45] Such results can be attributed to increased light exposure due to leaf removal on both the biosynthesis of monoterpenes as well as carotenoids, which are precursors of C_{13}-norisoprenoids.

A total of 25 monoterpene and 15 C_{13}-norisoprenoids compounds were detected. Notably LR-treated grapes at harvest (BBCH 89) were characterized by enhanced concentrations of α-terpineol and exo-2-hydroxycineole. An increase of monoterpenes such as α-terpineol and linalool in response to leaf removal, especially toward the advanced stages of berry development have been also reported in 'Sauvignon Blanc' grapes.[44] Among the detected C_{13}-norisoprenoids compounds, actinidol A and

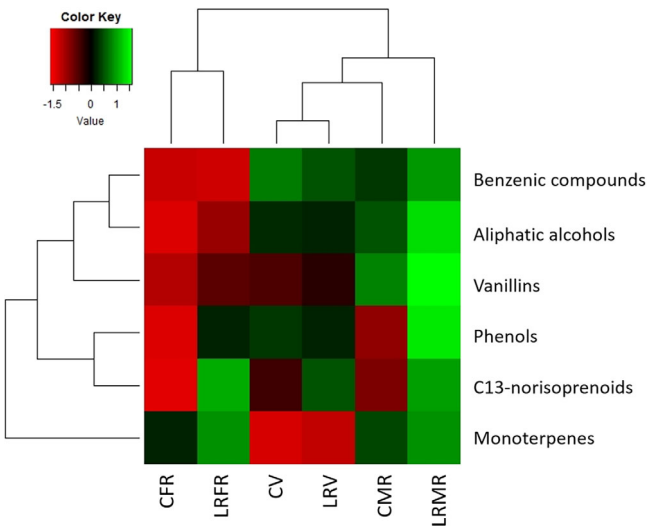

Figure 3. Heat map representing differences in the concentrations of six groups of glycosylated aroma compounds (aliphatic alcohols, benzenic compounds, phenols, vanillins, monoterpenes, C13-norisoprenoids) at BBCH 85, BBCH 87, and BBCH 89 for control and treated grapes (leaf removal). C, control; LR, leaf removal; V, BBCH 85 (end of veraison); MR, BBCH 87 (middle ripe); FR, BBCH 89 (fully ripe). This data were standardized as described in Material and Methods section. Up-regulation is indicated in green; down-regulation is indicated in red. A scale of color intensity is presented as a legend. Concentrations of the six groups of aroma compounds are shown in Table S2.

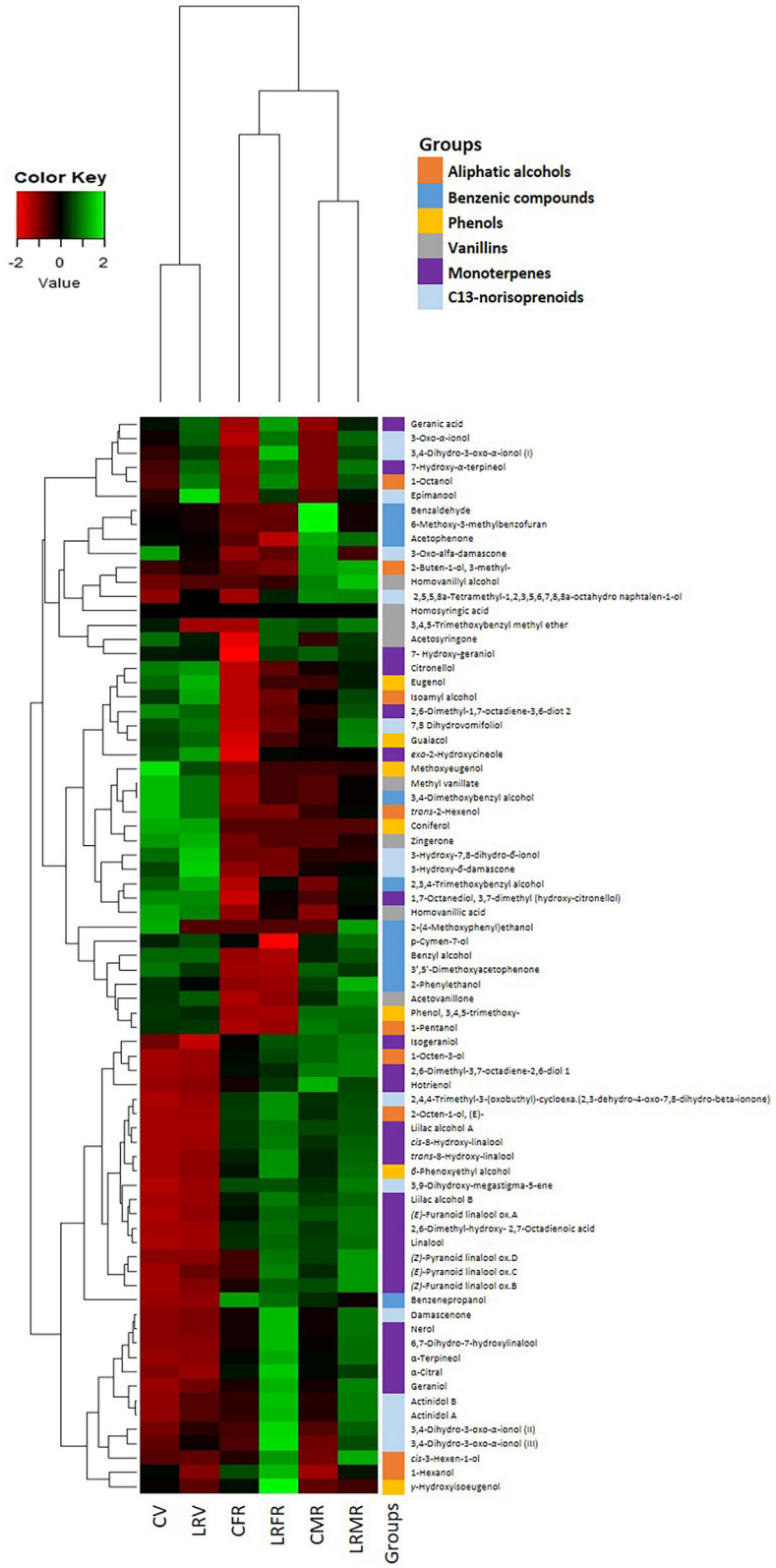

Figure 4. Heat map representing differences in the concentrations of 75 glycosylated aroma compounds from the six groups (aliphatic alcohols, benzenic compounds, phenols, vanillins, monoterpenes, C13-norisoprenoids) at BBCH 85, BBCH 87, and BBCH 89 for control and treated grapes (leaf removal). C, control; LR, leaf removal; V, BBCH 85 (end of veraison); MR, BBCH 87 (middle ripe); FR, BBCH 89 (fully ripe). This data were standardized as described in Material and Methods section. Up-regulation is indicated in green; down-regulation is indicated in red. A scale of color intensity is presented as a legend. The group of each aroma compound is indicated using a color box next to the compound name. Concentrations of the 75 aroma compounds from the six groups are shown in Table S2.

Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

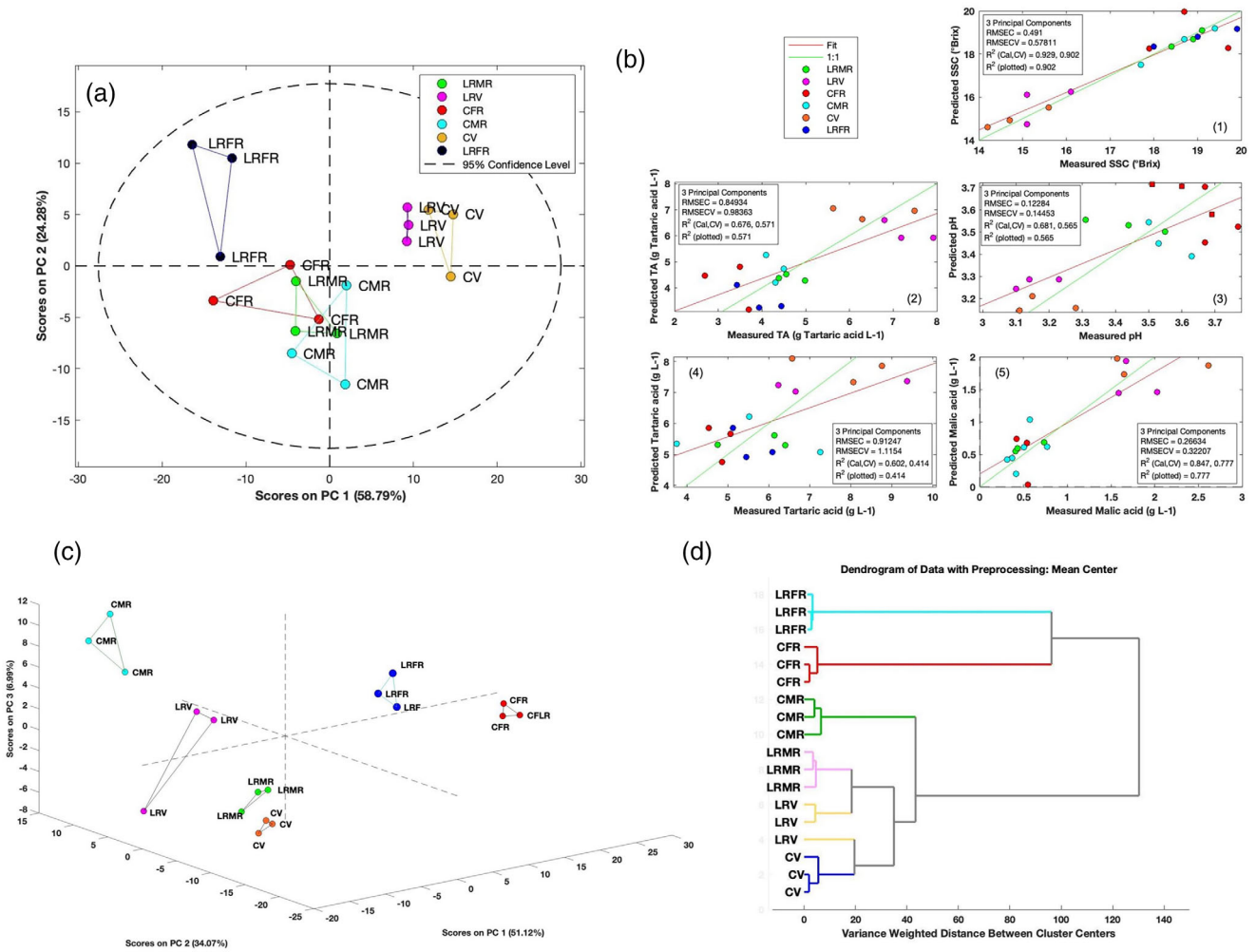

Figure 5. (a) Scoreplot (PC1 *versus* PC2) of PCA calculation performed on FT-NIR spectra detected; (b) scatterplots of computed PCR referred to regressive models developed for SSC, TA, pH, tartaric acid, and malic acid. Each graph includes indexes for statistical correlation (e.g. R^2 in calibration and prediction), and robustness (root mean standard error, in calibration and prediction; RMSEC and RMSECV, respectively); (c) Scoreplot (PC1 *versus* PC2 *versus* PC3) of PCA calculation performed on E-nose measurements; (d) dendrogram of cluster analysis (by Ward's method) performed on E-nose measurements of musts derived from control and leaf removal experiment at BBCH 85, BBCH 87, and BBCH 89. C, control; LR, leaf removal; V, BBCH 85 (end of veraison); MR, BBCH 87 (middle ripe); FR, BBCH 89 (fully ripe).

actinidol B both showed a steady concentration increase with the progress of developmental stage, ranging from ~35% difference in BBCH 85 to a two-fold increase in BBCH 89 (Fig. 4).

The compounds belonging to the phenols and vanillins groups also showed enhanced concentrations in LR-treated grapes during advanced on-vine developmental stages (BBCH 87 and BBCH 89 stages). In particular, phenols had a 32% and 23% increase for the BBCH 87 and BBCH 89 stages, respectively (Fig. 4; Table S2). Out of the seven phenolic compounds, *b*-phenoxyethyl alcohol and coniferol registered the highest increase. Vanillins showed *c.* 16.5% increase for both stages, with acetovanillone and zingerone compounds having 26% increase on average for both stages. Finally, aliphatic alcohols and benzenic compounds showed a 10% increase for BBCH 87 (middle ripe) in LR-treated grapes. A notable exception is the 1-octanol aliphatic alcohols, which was increased by 1.45 to 1.71-fold during grape berry ripening.

Effects of leaf removal on the FT-NIR and E-nose

Figure 5(a) reports the scoreplot representing PCA results of the FT-NIR detection. The first and second principal components (PC1 and PC2) described the 58.8% and the 24.3% of the explained variance, respectively, while four PCs are required by the computation for explaining, overall, more the 95% of variance, so reducing the error of the residual variance under 5%. Along PC1, the most significant discrimination among samples, based on FT-NIR spectra and their vibrational response in must samples, was observed. It starts from the upper right quadrant where 'control' and 'leaf removal' samples at the end of veraison (CV and LRV; BBCH 85) appear to be quite similar even though well segregated from the other samples. Moving to the left, it is possible to find 'control' and 'leaf removal' samples at middle ripe (CMR and LRMR; BCH 87) being collocated on the border of lower right and left quadrants and they are practically overlapped. Only spectra referred to the fully ripe stage (BBCH 89) of grapes seem to show significant differences between 'control' (CFR) and 'leaf removal' (LRFR) samples, as they are located in the lower left and in the upper left quadrants, respectively. These results suggest that the observed discrimination between musts belonging to the end of veraison (BBCH 85) and fully ripe stages (BBCH 89), respectively, with no significant differences between control and

leaf removal samples should be associated to the grape ripening evolution mean. The latter is NIR-monitorable because of the vibrational response of the principal grape organic molecules and their modification which are, in turn, strictly related to the ripening process.[46,47] Only concomitantly to the final stage of monitoring (fully ripe), the possible consequences of the leaf removal treatment appear to affect the internal characteristics of grape samples, influencing the spectral differences observed on the musts coming from treated and untreated samples, respectively. This last assumption is significantly corroborated by the results of the performed PCR (Fig. 5(b)) were FT-NIR spectra were associated, by a regressive approach, to the principal quality attributes of wine grapes describing the ripening behavior as well as defining the technological maturity. Particularly for SSC and malic acid content (Fig. 5 (b(1), b(3))), significant correlations (R^2 in calibration and in prediction) were obtained (0.929, 0.902 and 0.847, 0.777, respectively), as well as quite low errors performed by the models in calibration and prediction [root mean standard errors; root mean square error of calibration (RMSEC) and root mean square error of cross-validation (RMSECV), respectively] were observed. For the other tested attributes (in detail, titratable acidity, pH, tartaric acid and malic acid content; Fig. 5 (b(2)–b(5)), respectively) the results were not so performing but, in any case, they demonstrated the promising relationship between spectra and analytical data. Overall, all the performed regressive models suggested that the FT-NIR spectra were able to follow the grape ripening process, as aforementioned.

With respect to the E-nose measurements, Fig. 5 (c, d) present the resulting scoreplot of the PCA calculation, and the dendrogram of the performed cluster analysis, respectively. PC1 (51.1%), PC2 (34%), and PC3 (7%) described about the 92% of the explained variance, and four PCs are required by the PCA computation for minimizing the residual error under the 5% (Fig. 5(c)). Hierarchical clustering among treated and untreated samples, in turn associated to the three stages of monitoring, is well appreciable by the graphical dendrogram (Fig. 5(d)). The observed indications of the FT-NIR results are significantly confirmed by the two sample clusters generated by the multivariate computation performed on the volatile patterns E-nose detected. The greater formed cluster is associated with fully ripe stage of monitoring, and it is clearly segregated from the second generated cluster including grape samples belonging to the other two developmental stages. Within the first cluster also differences between leaf removal and control samples are distinguishable, as well as previously observed for spectra measurements. Collectively, the aromatic patterns detected with the employment of the E-nose are well associated to the grape ripening trend as also described by similar studies.[48,49] In addition, we hereby postulate that the beneficial effect of early leaf removal on the volatile profile seem to be also detectable by the sensor array of the E-nose device.

Finally, some significant relationships were observed between grouped VOCs and E-nose results. In detail, comparing the heatmap *versus* the cluster analysis and the PCA scoreplot in Figs 3, 5 (c, d) it is possible to observe that one cluster generated by CFR and LRFR samples is present in both GC–MS and E-nose computed data. The other samples are grouped into the same bigger cluster in the case of PCA and cluster analysis of E-nose results, as well as the heatmap computed on the basis of grouped VOCs. This second cluster is well distinguished from the first one, even though the relative connection between CV, LRV, CMR, and LRMR samples is exactly not the same in the two calculations for E-nose and GC–MS analysis. These results confirmed the general trends of the samples strictly associated to the respective ripening trends, while the little differences found between the two techniques are a confirmation about the role of E-nose measurements. The E-noses are non-selective and non-discriminative devices able to recognize an aromatic pattern, or a global aromatic profile of a matrix, and unable to detect single VOC or grouped aromatic molecules if not carefully trained to do it.[50]

CONCLUSIONS

This study showed that leaf removal during fruit set affected the composition of berries obtained from 'Xynisteri' cultivar during three developmental stages (BBCH 85, BBCH 87, and BBCH 89). In particular, leaf removal affected the VOC profiles at fully ripe stage with a distinct increase being observed in glycosylated aroma compounds and monoterpenes, and C_{13}-norisoprenoids in particular. Moreover, leaf removal showed an increase in the levels of must quality characteristics such as SSC, TA, tartaric acid and ammonium nitrogen, exception made for assimilable amino nitrogen (primary amino nitrogen) that remained unaffected. The present study showed that leaf removal during fruit set is an important cultivation practice that can be effectively used towards intensifying the glycosylated aroma compounds of 'Xynisteri' grapes. Results reported herein derived from one growing season and need to be repeated to be further validated. Furthermore, to what extent the increased aroma in grapes is transferable to the 'Xynisteri' wine need to be dissected.

ACKNOWLEDGEMENTS

This work was co-funded by the European Regional Development Fund and the Republic of Cyprus through the Research and Innovation Foundation (Project: POST-DOC/0718/0066).

AUTHOR CONTRIBUTIONS

Egli C. Georgiadou: conceptualization, methodology, formal analysis, investigation, data curation, visualization, project administration, writing – original draft. Minas Mina: conceptualization, supervision, writing – review and editing. Varnavas Neoptolemou: investigation. Stefanos Koundouras: methodology, writing – review and editing. Claudio D'Onofrio: methodology, formal analysis, data curation, writing – review and editing. Andrea Bellincontro: methodology, formal analysis, data curation, writing – review and editing. Fabio Mencarelli: methodology, formal analysis, data curation, writing – review and editing. Vasileios Fotopoulos: supervision, writing – review and editing. George A. Manganaris: conceptualization, supervision, writing – review and editing.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

REFERENCES

1 Fajardo TV and Quecini V, Comparative transcriptome analyses between cultivated and wild grapes reveal conservation of expressed genes but extensive rewiring of co-expression networks. *Plant Mol Biol* **106**:1–20 (2021).
2 D'Onofrio C, Matarese F and Cuzzola A, Effect of methyl jasmonate on the aroma of Sangiovese grapes and wines. *Food Chem* **242**: 352–361 (2018).
3 D'Onofrio C, Changes in volatile compounds, in *Sweet, Reinforced and Fortified Wines: Grape Biochemistry, Technology and Vinification*, ed. by Mencarelli F and Tonutti P. Hoboken, NJ, John Wiley and Sons, pp. 91–104 (2013).
4 D'Onofrio C, Matarese F and Cuzzola A, Study of the terpene profile at harvest and during berry development of *Vitis vinifera* L. aromatic varieties Aleatico, Brachetto, Malvasia di Candia aromatica and Moscato bianco. *J Sci Food Agric* **97**:2898–2907 (2017).
5 Lund ST and Bohlmann J, The molecular basis for wine grape quality – a volatile subject. *Science* **311**:804–805 (2006).
6 Mateo JJ and Jiménez M, Monoterpenes in grape juice and wines. *J Chromatogr A* **881**:557–567 (2000).
7 Zoecklein BW, Wolf TK, Pelanne L, Miller MK and Birkenmaier SS, Effect of vertical shoot-positioned, smart-Dyson, and Geneva double-curtain training systems on viognier grape and wine composition. *Am J Enol Vitic* **59**:11–21 (2008).
8 Wen YQ, Zhong GY, Gao Y, Lan YB, Duan CQ and Pan QH, Using the combined analysis of transcripts and metabolites to propose key genes for differential terpene accumulation across two regions. *BMC Plant Biol* **15**:240 (2015).
9 Li XY, Wen YQ, Meng N, Qian X and Pan QH, Monoterpenyl glycosyltransferases differentially contribute to production of monoterpenyl glycosides in two aromatic *Vitis vinifera* varieties. *Front Plant Sci* **8**: 1226–1239 (2017).
10 Poni S, Casalini L, Bernizzoni F, Civardi S and Intrieri C, Effects of early defoliation on shoot photosynthesis, yield components, and grape composition. *Am J Enol Vitic* **57**:397–407 (2006).
11 Feng H, Yuan F, Skinkis PA and Qian MC, Influence of cluster zone leaf removal on pinot noir grape chemical and volatile composition. *Food Chem* **173**:414–423 (2015).
12 Silvestroni O, Lanari V, Lattanzi T, Palliotti A and Sabbatini P, Impact of crop control strategies on performance of high-yielding Sangiovese grapevines. *Am J Enol Vitic* **67**:407–418 (2016).
13 Alessandrini M, Battista F, Panighel A, Flamini R and Tomasi D, Effect of pre-bloom leaf removal on grape aroma composition and wine sensory profile of semillon cultivar. *J Sci Food Agric* **98**:1674–1684 (2017).
14 Sternad Lemut M, Sivilotti P, Franceschi P, Wehrens R and Vrhovsek U, Use of metabolic profiling to study grape skin polyphenol behavior as a result of canopy microclimate manipulation in a 'pinot noir' vineyard. *J Agric Food Chem* **61**:8976–8986 (2013).
15 Baiano A, De Gianni A, Previtali MA, Del Nobile MA, Novello V and Palma D, Effects of defoliation on quality attributes of Nero di Troia (*Vitis vinifera* L.) grape and wine. *Food Res Int* **75**:260–269 (2015).
16 Osrecak M, Karoglan M and Kozina B, Influence of leaf removal and reflective mulch on phenolic composition and antioxidant activity of merlot, Teran and Plavac Mali wines (*Vitis vinifera* L.). *Sci Hortic* **209**:261–269 (2016).
17 Main GL and Morris JR, Leaf-removal effects on Cynthiana yield, juice composition, and wine composition. *Am J Enol Vitic* **55**:147–152 (2004).
18 Chorti E, Kyraleou M, Kallithraka S, Pavlidis M, Koundouras S and Kotseridis Y, Irrigation and leaf removal effects on polyphenolic content of grapes and wines produced from cv. 'Agiorgitiko' (*Vitis vinifera* L.). *Not Bot Horti Agrobot Cluj-Napoca* **44**:133–139 (2016).
19 Downey MO, Dokoozlian NK and Krstic MP, Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. *Am J Enol Vitic* **57**:257–268 (2006).
20 Petropoulos S, Kallithraka S and Paraskevopoulos I, Influence of some viticultural practices on the polyphenolic content ofwines produced from cv. Agiorgitiko (*Vitis vinifera* L.). *J Int des Sci la Vigne du Vin* **45**: 235–243 (2011).
21 Nicoletti I, Bellincontro A, De Rossi A, De Sanctis F, Tiberi D, Pietromarchi P *et al*., Postharvest dehydration of Nebbiolo grapes grown at altitude is affected by time of defoliation. *Aust J Grape Wine Res* **19**:358–368 (2013).
22 Pastore C, Zenoni S, Fasoli M, Pezzotti M, Tornielli GB and Filippetti I, Selective defoliation affects plant growth, fruit transcriptional ripening program and flavonoid metabolism in grapevine. *BMC Plant Biol* **13**:30–45 (2013).
23 Constantinou S, Gomez-Caravaca AM, Goulas V, Segura-Carretero A and Manganaris GA, Metabolic fingerprinting of must obtained from sun-dried grapes of two indigenous Cypriot cultivars destined for the production of 'Commandaria'; a protected designation of origin product. *Food Res Int* **119**:189199 (2017).
24 Constantinou S, Gomez-Caravaca AM, Goulas V, Segura-Carretero A, Koundouras S and Manganaris GA, The impact of postharvest dehydration methods on qualitative attributes and chemical composition of 'Xynisteri' grape (*Vitis vinifera*) must. *Postharvest Biol Technol* **135**: 114–122 (2018).
25 Constantinou S, Gómez-Caravaca AM, Goulas V, Fernandez-Gutierrez A, Koundouras S and Manganaris GA, Leaf removal at veraison stage differentially affects qualitative attributes and bioactive composition of fresh and dehydrated grapes of two indigenous cultivars of Cyprus. *J Sci Food Agric* **99**:1342–1350 (2019).
26 Copper AW, Collins C, Bastian SEP, Johnson TE and Capone DL, Preliminary investigation of potent thiols in Cypriot wines made from indigenous grape varieties Xynisteri, Maratheftiko and Giannoudhi. *Oeno One* **55**:223–234 (2021).
27 Copper AW, Johnson TE, Danner L, Bastian SEP and Collins C, Preliminary sensory and chemical profiling of Cypriot wines made from indigenous grape varieties Xynisteri, Maratheftiko and Giannoudhi and acceptability to Australian consumers. *Oeno One* **53**:229–248 (2019).
28 Hall A and Jones GV, Spatial analysis of climate in winegrape-growing regions in Australia. *Aust J Grape Wine Res* **16**:389–404 (2010).
29 Meier U, *Growth Stages of Mono-and Dicotyledonous Plants*. Blackwell Wissenschafts-Verlag, Germany (1997).
30 Zhao G, Gao Y, Gao S, Xu Y, Liu J, Sun C *et al*., The phenological growth stages of *Sapindus mukorossi* according to BBCH scale. *Forests* **10**: 462 (2019).
31 Richardson AD, Duigan SP and Berlyn GP, An evaluation of noninvasive methods to estimate foliar chlorophyll content. *New Phytol* **153**: 185–194 (2002).
32 Misra BB and Dey S, Developmental variations in sesquiterpenoid biosynthesis in east Indian sandalwood tree (*Santalum album* L.). *Trees* **27**:1071–1086 (2013).
33 Nikiforou C, Filippou P, Manetas Y and Fotopoulos V, Winter leaf redness in mastic tree (*Pistacia lentiscus* L.) is associated with increased cellular damage levels and modified nitric oxide and hydrogen peroxide biosynthesis. *Adv Plants Agric Res* **1**:180–184 (2014).
34 Filippou P, Antoniou C and Fotopoulos V, Effect of drought and rewatering on the cellular status and antioxidant response of Medicago truncatula plants. *Plant Signal Behav* **6**:270–277 (2011).
35 Loreto F and Velikova V, Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiol* **127**:1781–1787 (2001).
36 Christou A, Manganaris GA, Papadopoulos I and Fotopoulos V, Hydrogen sulfide induces systemic tolerance to salinity and non-ionic osmotic stress in strawberry plants through modification of reactive species biosynthesis and transcriptional regulation of multiple defence pathways. *J Exp Bot* **64**:1953–1966 (2013).
37 Scheiner D, Determination of ammonia and Kjeldahl nitrogen by indophenol method. *Water Res* **10**:31–36 (1976).
38 Dukes BC and Butzke CE, Rapid determination of primary amino acids in grape juice using an o-phthaldialdehyde/*N*-acetyl-L-cysteine spectrophotometric assay. *Am J Enol Vitic* **49**:125–134 (1998).
39 Hadjipieri M, Georgiadou EC, Costa F, Fotopoulos V and Manganaris GA, Dissection of the incidence and severity of purple spot physiological disorder in loquat fruit through a physiological and molecular approach. *Plant Physiol Biochem* **155**:980–986 (2020).
40 Jin ZM, Wang CH, Liu ZP and Gong WJ, Physiological and ecological characters studies on Aloe vera under soil salinity and seawater irrigation. *Process Biochem* **42**:710–714 (2007).
41 Edewor-Kuponiyi TI, Determination of fructose content in *Anacardium occidentale*. *Int J Agri Food Res (IJAFR)* **2**:13–19 (2013).
42 Santonico M, Bellincontro A, De Santis D, Di Natale C and Mencarelli F, Electronic nose to study postharvest dehydration of wine grapes. *Food Chem* **121**:789–796 (2010).

43 Di Natale C, Paolesse R and D'Amico A, Metalloporphyrins based artificial olfactory receptors. *Sens Actuators B* **121**:238–246 (2007).
44 Young PR, Eyeghe-Bickong HA, du Plessis K, Alexandersson E, Jacobson DA, Coetzee Z *et al*., Grapevine plasticity in response to an altered microclimate: sauvignon blanc modulates specific metabolites in response to increased berry exposure. *Plant Physiol* **170**: 1235–1254 (2016).
45 Du Plessis K, Young PR, Eyéghé-Bickong HA and Vivier MA, The transcriptional responses and metabolic consequences of acclimation to elevated light exposure in grapevine berries. *Front Plant Sci* **8**: 1261 (2017).
46 Dambergs R, Gishen M and Cozzolino D, A review of the state of the art, limitations, and perspectives of infrared spectroscopy for the analysis of wine grapes, must, and grapevine tissue. *Appl Spectrosc Rev* **50**: 261–278 (2015).
47 Power A, Truong VK, Chapman J and Cozzolino D, From the laboratory to the vineyard—evolution of the measurement of grape composition using NIR spectroscopy towards high-throughput analysis. *High-Throughput* **8**:21 (2019).
48 Aleixandre M, Santos JP, Sayago I, Cabellos JM, Arroyo T and Horrillo MC, A wireless and portable electronic nose to differentiate musts of different ripeness degree and grape varieties. *Sensors* **15**: 8429–8443 (2015).
49 Aghilinategh N, Dalvand MJ and Anvar A, Detection of ripeness grades of berries using an electronic nose. *Food Sci Nutr* **8**:4919–4928 (2020).
50 Modesti M, Taglieri I, Bianchi A, Tonacci A, Sansone F, Bellincontro A *et al*., E-nose and olfactory assessment: teamwork or a challenge to the last data? The case of virgin olive oil stability and shelf life. *Appl Sci* **11**:8453 (2021).

Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.