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First application of ozone postharvest fumigation to remove smoke taint from grapes

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ABSTRACT

Smoke taint is becoming a worldwide problem for wine production because the number of fires increases in wine regions. It is the result of volatile phenols released by fire smoke which bind to berry sugars, translocate mainly through bunch stem and/or through berry skin. The actual used techniques to remove it from wine can modify wine quality provided that smoke taint is completely removed from wine. We report the first data on the ozone fumigation of harvested grape which were affected by the smoke taint. The experiments were run on table grapes in laboratory. The grape bunches were smoked by straw fire for 2 hours and then ozone fumigated for 4 hours in a cold room at 10°C. At a nonformal sensorial evaluation, the smoke taint which was very strong on smoked grapes, was not perceived after the ozone treatment, and the main volatile phenols (guaiacol and 4-methylguayacol) of the smoke taint were reduced significantly; the skin color slightly darkened in the berries affected by smoke while skin firmness increased; ozone did not recover these effects. Application of NIR-AOTF and E-nose on smoked grapes emphasizes the role of temperature but overall discovers the usefulness of the E-nose in the early detection of smoke taint.

Keywords

ozone, fire smoke, grape, NIR, electronic nose

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34 Introduction

35 In 2017, Italy has experimented the record of the forest surface affected by fire:
36 134107 ha between May and July, 72039 ha of forest surface has been burnt, mainly
37 in South Italy (Sicily, Calabria, Campania, Sardegna, Lazio, Toscana) (Legambiente,
38 2017). Because of the extreme biodiversity of Italian countryside, most of these fires
39 has touched vineyards or olive orchards, in many cases destroying them and, only in
40 few cases, the fire affected the wine quality with smoke taint. The reason of this is the
41 fires did not occur in harvest period (Ansa, 2017). In Napa Valley, the disastrous fire
42 at the beginning of October 2017 which burnt about 55000 ha, affected only the 10%
43 of the production because 90% of grapes had already been picked but, in this case,
44 the 10% of grapes had been in contact with smoke and the wine showed the smoke
45 taint. Before this year which has been disastrous for Australia due to huge wild fire,
46 approximately 50 million ha of land burned annually and about 80% of fire-affected
47 areas are in northern Savannah regions (Krstic, Johnson, and Herderich, 2015) and,
48 indeed, the first identification of smoke taint recorded in the wine industry, occurred in
49 Australia in 2003, as a result of the Camberra bushfires (Høj, Pretorius, and Blair,
50 2003). A range of organic compounds are emitted as volatile organic compounds
51 (VOCs) in wood smoke which may cause contamination of vines and grapes, and
52 contribute to the formation of undesirable smoke taint compounds in wine. Guaiacol
53 (2-methoxyphenol), 4-methylguaiacol and syringol (2,6-dimethoxyphenol) are the
54 most abundant volatile phenols in smoke and have an odour quality described as
55 smoky with a low sensory threshold; other VOCs are reported to contribute to smoke
56 taint such as cresols (*m*, *p*, *o*) and 4-methylsyringol (Krstic, Johnson, and Herderich,
57 2015). In grapes, these compounds are mostly glycoconjugated, thus nonvolatile,
58 with monoglucoside or disaccharide (Kennison et al., 2008). The bond between
59 volatile phenol and sugar can be broken by yeast during fermentation, in mouth by
60 means of glycosidases, or during wine aging thanks to chemical hydrolysis. In all
61 these cases, the smoke taint is easily perceivable. Beyond the use of different
62 practices in winemaking to reduce the smoke taint (Ristic et al., 2011), actually
63 commercial fining agents such as activated carbon, or reverse osmosis and solid
64 phase absorption (Fudge et al., 2012) are used to alleviate the smoke taint but not to

eliminate, sometime with problem on wine quality (activated carbon) or running cost (reverse osmosis). Ozone (O_3) has been used in winery as a sanitizing agent, and its use has been proposed at different stages of wine production to reduce the spoilage microbiota in grapes and to sanitize barrels, tanks, hoses and bottles. Postharvest ozone fumigation of grapes has been suggested as a means for wine production without SO_2 addition but also to increase polyphenols and wine aroma (Bellincontro et al. 2017) or to be used during wine grape dehydration to control fungi (Botondi et al., 2015 ; Cravero et al., 2016). Its strong oxidizing potential, 2.07 V, is used to remove the smoke smell from carpet, furniture, ventilation duct by destroying the smoke volatile molecules in fire restoration. Thus, here the first successful application of ozone on grapes to remove smoke taint is reported. NIR-AOTF and electronic nose applications were also carried out to identify metabolic changes occurring in berries as potential non destructive, rapid, tools for smoke taint identification at an early stage.

Materials and Methods

Laboratory tests

In the 1st test, white table grapes (*Vitis vinifera* var. Italia) were purchased at local market and two plastic crates, approximately 15 kg each (20-25 bunches), were hung from a tripod over a steel cylindrical tank (50 cm diameter and 100 cm height); a fire was lit inside the tank by using straw and, the smoke coming out from the tank opening, invested the bunches that were at a distance of about 50 cm from the tank opening. After two preliminary tests, the time of exposure was set in 2 hours. The grape temperature was raised from about 18°C up to 27 (± 2)°C (IR thermometer, Turoni srl, Forli, Italy) during the smoke treatment. After the smoke treatment, the grape bunches were divided into two lots: one lot of grapes were fumigated for 4 h with ozone gas (max 40 g h⁻¹ with 6% w/w of ozone) with a flow rate at maximum 150 NL / h (NL= normal litre) rate in a 9 m³ cold room (Ozone generator A series, PC Engineering, Uggiate Trevano, Italy); the second lot consisted in the smoked grapes which were untreated with ozone. The treatment temperature was 10 (± 1)°C and relative humidity (RH) 80 (± 5)%.

In the 2nd test, two sets of grape bunches were separated and treated with smoke as described above but for 1 hour: one set was smoked close to fire (HOT SMOKED) as in the 1st test while, in the 2nd set, smoke was collected in a stainless steel tank kept previously at 10°C, kept tightly close for 1 hour, then it was opened and grape bunches were placed inside and kept at 10°C, tightly closed, for 2 hours (COLD SMOKED); after the treatment respecting the time of the 1st test, the bunches were ozonated as aforementioned. The temperature of the berries in the hot smoke treatment was approximately 28°C while in the second one (COLD SMOKED) was 17°C. In this test we applied non destructive analysis with NIR and electronic nose (E-nose).

All the tests were repeated three times, one week after the other to have the same grape quality, always with purchased table grapes. Before smoke treatment and after ozone treatment, the berries were immediately analyzed or frozen for further analyses.

Chemical and physical analyses

Before and after smoke treatment or after ozone treatment, berries were detached by the stem and immediately analyzed for berry skin's color and resistance. 60 berries from different bunches of the two plastic crates, were pen-marked before smoke treatment and analyzed for color skin, using a CM-2600d colorimeter (Konica Minolta Inc., Ramsey, NY) set at SCE (specular component excluded), measuring CIELAB coordinates "L", "a", and "b". To measure berry skin resistance (puncture test), other 60 berries were analyzed by an Instron Universal Testing Machine mod. 3343 (Instron Ltd, High Wycombe, UK), adapted with a 1 mm diameter flat probe and bar speed was set at 10 mm / min. The value was expressed in terms of applied force to skin deformation at break point (N / mm). Spectrophotometric absorbance (Abs) was performed on juice coming from berries used in the skin resistance test. The total polyphenol content was measured by using the Folin-Ciocalteu method (Singleton, Orthofer, and Lamuela-Raventó, 1999). The volatile phenols, free and bound, in grape were measured by using GC-MS and the procedure by De Sanctis et al. (2015).

Non destructive analyses

Whole grape berries coming from smoke fumigation and subsequent ozone treatment were non-destructively analyzed by detecting NIR (Near Infrared) spectra by means of a Luminar 5030 Miniature Hand-held NIR Analyzer (Brimrose,

Baltimore, MD), based on the AOTF-NIR principle. Two different measurements were performed on each intact berry, using the diffuse reflectance method of detection. Detection was conducted in the 1100-2300 nm range, with 2 nm wavelength increments and 10 spectra per average, which represented the spectral measurement of a single grape berry. Raw spectra were manipulated for absorbance ($\log 1/R$) transformation using SNAP! 2.03 software (Brimrose). The absorbance spectra were averaged to 1 spectrum for each sample; obtained measurements were addressed to a Principal Component Analysis (PCA) which was performed by Matlab R2013a (MathWorks®, Natick, MA, USA) and PLS Toolbox (Eigenvector Research, Inc., Manson, WA, USA). The same grape berries used in NIR detections were destined to the E-nose analysis. Whole berries were peeled, and skins and pulps were separated. 10.0 ± 0.1 g of peels, and 40.0 ± 0.5 g of pulps were collocated within glass vials having a volume of 15 and 50 mL, respectively. The vials were capped with a perforable septum which the head space into the E-nose device was aspired through. The used electronic nose was developed, designed and assembled at the University of Rome Tor Vergata. It is based on an array of eight quartz microbalances (QMB). QMBs 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 (López de Lerma et al, 2012). The flasks were placed in a thermostated bath under continuous stirring with a small magnetic stirring bar, for 30 min at $25 \pm 2^\circ\text{C}$. After that the equilibrated headspace was extracted by a constant flow of filtered air and delivered into the electronic nose sensor cell. A pure nitrogen flow was also used to clean the sensors, establishing the reference signal. Sensor signals were calculated as the resonant frequency shift between the two steady conditions corresponding to sensors exposed to pure nitrogen and to the sample diluted in nitrogen. The ensemble of sensor signals is composed in patterns (fingerprints) encoding the global composition of the headspace.

Statistical analysis

All chemical and physical data were subjected to one-way analysis of variance (ANOVA). Statistical significance of the differences was calculated using the Tukey B

test for $p < 0.01$ or 0.05 . Calculations were performed by Minitab 15 (Minitab, Inc., USA). Graphs were created with SigmaPlot 11.0 (Systat Software Inc., CA, USA). For NIR and E-nose, original data were treated for a normalization (Santonico et al., 2010) than employed for a Principal Component Analysis (PCA), and a Cluster Analysis performed by a Ward's method considering an Euclidean similarity index. Both PCA analyses calculated on the data derived from NIRs and E-nose measurements and the Cluster Analysis were performed by Matlab R2013a (MathWorks®, Natick, MA, USA) and PLS Toolbox (Eigenvector Research, Inc., Manson, WA, USA).

Results and Discussion

1st test

The grapes kept under smoke were easily detectable and the berries were inedible thus no official sensory evaluation was carried out. After the ozone treatment, the smoke odor disappeared almost completely. The color analysis revealed a significant decrease in "L" in the smoked grapes which was not recovered by the ozone treatment; "a" was slightly negative, the smoke treatment reduced the value and the smoke-ozonated grapes had the same values of the smoked one (not ozonated); finally, "b" values, after smoke treatment, did not change while, after ozone treatment of the smoked grapes, the values increased significantly (Table 1). The absorbance values at 420 and 520 nm showed a significant increase in the smoke-treated grapes while, after ozone treatment, the values decreased without, however, recovering to the initial levels (Table 1). Visually, the juice of smoked berry and, also, the one of the ozone-treated berries after smoke treatment, were slightly darker (the color of guaiacol oil is yellow) and this confirm the variation in "L" and "a" with a tendency from green to red, likely, as a consequence of phenol oxidation or phenol new synthesis. The shift of "b" (yellowness) in the ozone-treated berries might be an indication of the smoke VOC oxidation since these compounds are potent scavenger of reactive oxygen radicals. This seems confirmed by the absorbance values; the significant decrease in the absorbance after ozone treatment on smoked berries, might indicate the oxidation of volatile phenols whereas, the not complete recover to the initial value, it might be the well known phenol synthesis induced by ozone (Artes-

Hernandez et al., 2007; Carbone and Mencarelli, 2015; Bellincontro et al., 2017). Indeed, if we observe the polyphenol content (Table 1), the concentration of phenols raised significantly after smoke treatment confirming what observed by Noestheden et al. (2018) while the ozone treatment reduced partially the phenols, confirming the absorbance data. Cell response to ozone is very rapid; within 2 h after the beginning of O₃ exposure, O₃ induces H₂O₂ accumulation at the cell wall and plasma membrane levels from cell wall peroxidase activity, and changes induced by O₃ in cell wall components or by cell wall-produced H₂O₂ are possible initial steps of stress signal initiation (Vaultier and Jolivet, 2015). Volatile phenols such as guaiacol are very lipophilic but not hydrophilic {guaiacol solubility in water 1 to 10 mg / mL at 73° F (NTP, 1992)}. For this reason, their dissolution in a lipidic environment such as the berry skin is very rapid while, to be solubilized in water when they enter through stomata and lenticels of green tissue (stem) into the cells or when they pass through the berry pruinose layer to arrive to the epidermal cells, they need to glycosylate, requiring longer time and energy. Free volatile phenols (only the most important volatile phenols in smoke are reported) in berries after smoke treatment were significantly higher in concentration than the initial value while the ozone treatment of the smoked bunches, reduced the volatile phenol values of about 50% but without recovering to the initial level (Table 2). Ozone is known for its strong oxidant power and its effect on organic matter is concentration and time-dependent thus, the contact between ozone and easily oxidizable compounds such as volatile phenols, can provoke not synthesis but loss. In a preliminary winery trial, with higher concentration of ozone than the one used here, to remove smoke taint more rapidly, a must discoloration was observed (data not shown). Furthermore, a potential effect of heat coming from fire smoke on berry cells, should be studied as an effect on berries.

2nd test

At the informal sensorial analysis (odor), the 'HOT SMOKED' grapes, even if treated for a shorter time than in the 1st test, were easily detectable while the 'COLD SMOKED' not. The PCA performed on NIR data of the test with 'HOT SMOKED' or 'COLD SMOKED' grapes showed that three PCs explained more than the 95% of the residual variance: 77.76% (PC1), 13.22% (PC2), and 6.67% (PC3), respectively

(Figure 1). Untreated samples (not smoke treated) are segregated in opposite direction from 'HOT SMOKED' ones, and clusterization between them is mainly allowed for PC1. Taking into account vibrational spectroscopy is able to detect internal modification due to molecular changes, this result emphasizes the strong effect of heat whatever the smoked grapes were ozonated or not. In fact, 'COLD SMOKED' grapes, even after O₃ treatment ('COLD SMOKED_O3'), are close to CK (Figure 1 top, and bottom), while unsmoked grape berries-O₃ treated (CK_O3) are quite segregated from the CK, under the effect of PC1 and PC3. This last observation confirms that ozone treatment affects the grape quality but, in the same time, the proximity between CK and cold smoked grapes (ozonated or not) reveals that the changes occurring in grape due to 1 hour of smoke treatment are not so consistent and dramatic as the one caused by heat and smoke. The NIR result, showing that untreated and cold smoke-treated grape, ozonated or not, are very close in the PCA, would indicate that only smoke, without heating component, is a superficial effect not affecting greatly the main metabolism. In contrast, the ozone treatment on not smoked grape moves the sample far away from untreated sample (CK) in the PCA (Figure 1), highlighting the strong effect of ozone on grape metabolism, indeed while nothing is known on polyphenol or carotenoid pathway changes in smoked treated berries, it is known the effect of ozone on polyphenol synthesis (Mencarelli and Bellincontro, 2018). It might be that ozone, removing volatile phenols, at the same time, stimulates the synthesis of other phenols and this would explain the reason of high polyphenol content in smoked-ozonated-treated berries (1st test) and because it is not discriminated by NIR. In 2012, Fudge et al. obtained a good smoke taint classification in smoked grapes by using MIR spectroscopy while just recently, a field applied portable NIR spectrophotometer has been able to detect smoke taint in smoked grape and in different part of berries, skin and pulp, in a range of application of 700-1100 nm (Fuentes et al., 2017). In our experiment, the NIR applied scans from 1100 through 2300 nm allowing for a greater number of information, thus the obtained discrimination obtained only for hot smoked grape, opens the perspective that the temperature reached by the smoked berries is important for the NIR discrimination.

PCA performed on normalized volatile pattern E-nose detected, is reported in Figure 2. The scatter plot of PC1 vs PC2 (66.80%, and 22.72% of residual variance explained, respectively) well describes the clusterization among samples (Figure 2, Top). Untreated grape samples (CK) are located in the quadrant just opposite to the COLD SMOKED grapes and their separation is marked by PC1 and PC2; COLD SMOKED are also far away from COLD SMOKED-ozonated grapes. Ozonated control grapes (CK_O3) are separated from the CK by the influence of PC1 (Figure 2, Top) and, with minor evidence, by PC3 (Figure 2, Bottom). HOT SMOKED grapes are just in the middle together with HOT SMOKED-ozonated grapes. Thus, E-nose is able to discriminate better than NIR probably because the significant change is at aromatic level, based on the release of volatile phenols. The dendrogram related to the cluster analysis (Figure 3) confirms what aforementioned.

Conclusions

The postharvest application of ozone gas on grapes to remove smoke taint appears a good tool to adopt. More research is needed to clarify metabolic aspect of smoke and heat or only smoke on berries and on the effect of ozone on volatile phenols and berry metabolism. In addition, E-nose could be an important tool for an early detection of smoke taint when it is not perceivable by sensory evaluation.

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Tables and Figures

Table 1. Colorimetric, rheological, and spectrophotometric data of initial (before smoking), smoke- and smoke+ozone-treated berries. Data represent the mean ($\pm$ SD) values of 60 berries from different bunches for color and rheological parameters and of 5 juices from the same berries for polyphenols and absorbance (Abs). Values with different letters within the same column, are significantly different ($p < 0.01$ or 0.05).

	a°	b°	L°	skin firmness (N)	skin elasticity (mm)	Abs 420	Abs 520	total polyphenols (mg/L)
before smoking	- 1.3 $\pm$ 0.2a	4.6 $\pm$ 0.3b	37.4 $\pm$ 0.6a	1.61 $\pm$ 0.11b	0.039 $\pm$ 0.008a	0.420 $\pm$ 0.12C	0.229 $\pm$ 0.08C	1045 $\pm$ 35C
smoke	- 0.8 $\pm$ 0.1b	4.7 $\pm$ 0.2b	35.9 $\pm$ 1.1b	1.82 $\pm$ 0.09a	0.046 $\pm$ 0.008a	0.491 $\pm$ 0.09A	0.280 $\pm$ 0.010A	1300 $\pm$ 53A
smoke+ ozone	- 0.6 $\pm$ 0.2b	5.3 $\pm$ 0.3a	35.7 $\pm$ 0.6b	1.80 $\pm$ 0.10a	0.044 $\pm$ 0.005a	0.466 $\pm$ 0.013B	0.249 $\pm$ 0.010B	1170 $\pm$ 41B

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Table 2 Main free volatile phenols (µg/L) of initial (before smoking), smoke- and smoke+ozone-treated berries from smoke or smoke+ozone-treated berries. Data represent the mean (± SD) values. Data are the mean of three GC-MS analyses from berries of different bunches. Values with different letters within the same row, are significantly different (p < 0.05).

	table grape		
	before smoking	smoke	smoke + ozone
guaiacol	8.4±1.0c	33.9±0.9a	18.9±1.1b
4-methylguaiacol	6.5±1.3c	18.2±1.6a	13.0±0.7b

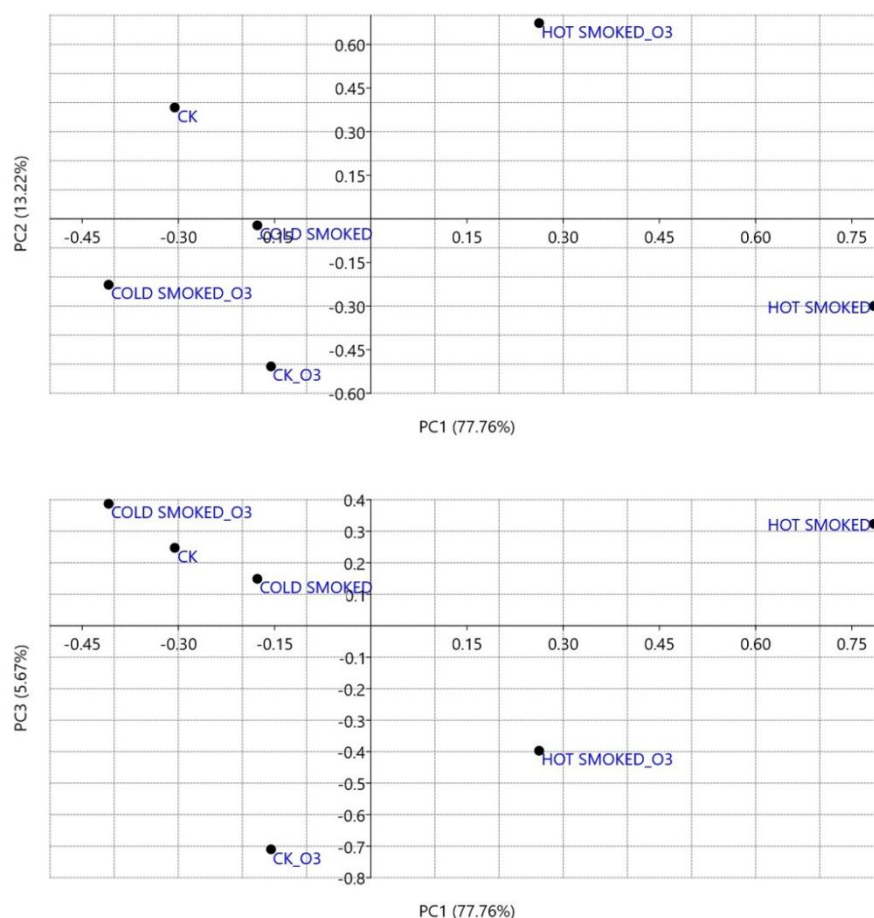


Figure 1 Score plots of PCA performed on NIR spectra detected on untreated grape berries (CK), and smoke treated berries (COLD and HOT SMOKED). Same grape samples are also reported after ozone exposure (_O3). The graph on the top represents the plotting of PC1 versus PC2, while PC1 versus PC3 is reported in the bottom.

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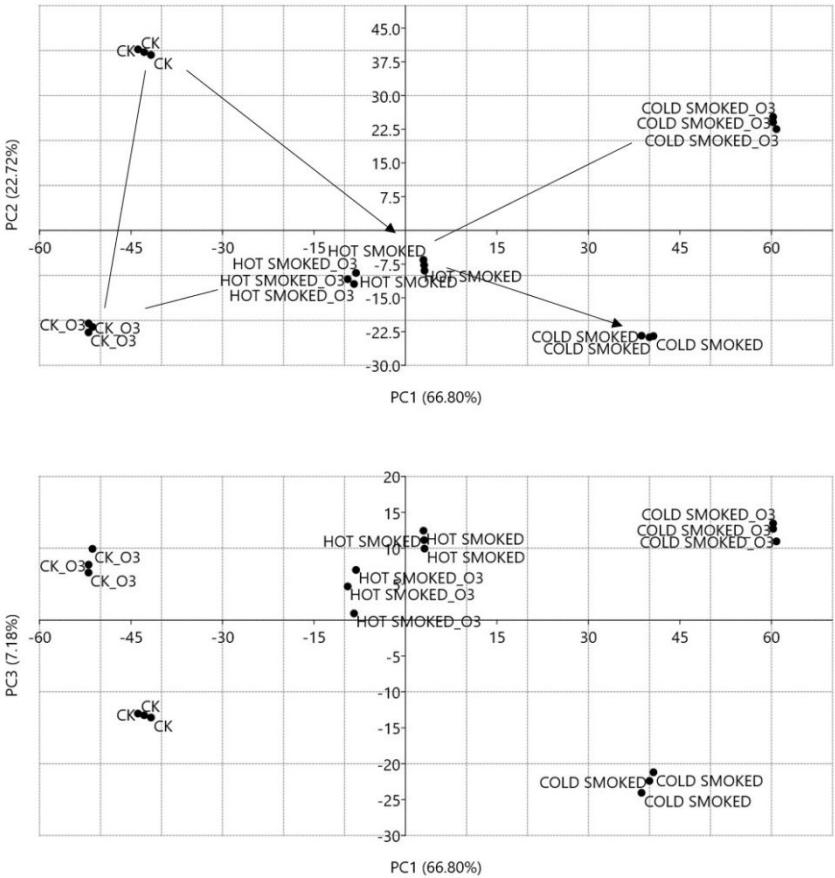
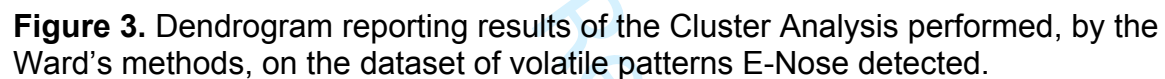


Figure 2. Score plots of PCA performed on the data referred to volatile patterns E-Nose detected on untreated grape berries (CK), and smoke treated berries (COLD and HOT SMOKED). Same grape samples are also reported after postharvest ozone fumigation (_O3). The graph on the top represents the plotting of PC1 versus PC2, while PC1 versus PC3 is reported in the bottom.



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