

Article

Characterization of Volatile Organic Compounds in ‘Rossa di Tropea’ Onion by Means of Headspace Solid-Phase Microextraction Gas Chromatography–Mass Spectrometry (HS/SPME GC–MS) and Sensory Analysis

Anna Taglienti ^{1,*} , Fabrizio Araniti ², Amalia Piscopo ³  and Antonio Tiberini ¹

¹ Research Centre for Plant Protection and Certification, Council for Agricultural Research and Economics, Via C.G. Bertero 22, 00156 Rome, Italy; antonio.tiberini@crea.gov.it

² Department of Agricultural and Environmental Sciences (DISAA), University of Milan, Via Celoria, 2, 20133 Milan, Italy; fabrizio.araniti@unimi.it

³ Department of Agricultural Science, University ‘Mediterranea’ of Reggio Calabria, Loc. Feo di Vito, 89122 Reggio Calabria, Italy; amalia.piscopo@unirc.it

* Correspondence: anna.taglienti@crea.gov.it; Tel.: +39-068-207-0228



Citation: Taglienti, A.; Araniti, F.; Piscopo, A.; Tiberini, A. Characterization of Volatile Organic Compounds in ‘Rossa di Tropea’ Onion by Means of Headspace Solid-Phase Microextraction Gas Chromatography–Mass Spectrometry (HS/SPME GC–MS) and Sensory Analysis. *Agronomy* **2021**, *11*, 874. <https://doi.org/10.3390/agronomy11050874>

Academic Editor: Hans Rediers

Received: 29 March 2021

Accepted: 27 April 2021

Published: 29 April 2021

Publisher’s Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Background: Plant viral infections induce changes in the host plant, which can potentially impact composition, organoleptic properties, and storability characteristics of plant products. In particular, onion odor and flavor are determined mainly by volatile organic compounds, and changes upon infection with onion yellow dwarf virus may deeply influence these characters. Methods: A time-course study of volatile organic compounds in onion yellow dwarf virus-infected versus healthy ‘Rossa di Tropea’ onion bulbs was performed using headspace solid-phase microextraction gas chromatography–mass spectrometry; sensory analysis performed at marketability stage of onion production was used to correlate such changes to the taste characteristics perceived by consumers. Results: Volatile organic compounds regulated in infection conditions were identified, mainly belonging to mono- and poly-sulfides classes. The most abundant compounds in the analyzed samples were propyl disulfide, allyl-isopropyl disulfide, and propanethiol; significantly different concentrations were observed for 7 out of 11 VOCs in virus-infected compared to healthy bulbs. Statistical analysis based on a partial least squares discriminant analysis model and hierarchical cluster analysis allowed us to cluster samples based on phytosanitary status and storage time and to identify the most responsible compounds for such classification. Conclusions: Onion yellow dwarf virus infection induces changes in volatile organic compounds in onion during storage. The impact of such regulated compounds on ‘Rossa di Tropea’ onion odor and flavor and correlation with sensory analysis are discussed.

Keywords: onion; gas chromatography; virus; volatile organic compounds

1. Introduction

In recent years, globalization and international trade have led to market saturation, particularly for agricultural food products, with a flattening of varieties and diversity due to the demanding global scale intensive productions [1]. Nevertheless, the recently growing consumers’ awareness of typical and local products represents a promising opportunity for relaunching and developing niche agricultural sectors [2], particularly in countries such as Italy, characterized by wide biodiversity in terms of landscapes, production areas, and local varieties/landraces [3]. The differentiation of local products includes several variables, such as quality, taste extolling, freshness, “rural” practices, and area of production, leading to recovering and requalification of old cultivations that previously could not face the global market [4,5]. Among them, onion, *Allium cepa* L., member of genus *Allium*, one of the most important and representative genera of the *Amaryllidaceae* family and widely

distributed in the northern hemisphere, is one of the oldest cropping plants [6]. Since ancient times, onions have been used as common foods and for treating many diseases (first citation in an Egyptian medical papyrus, *Codex Ebers*, 1550 b.C.).

Onion, as the whole *Allium* genus, is characterized by the presence of a wide range of volatile organic compounds (VOCs), mainly sulfur compounds, which are recognized as the main responsible for its unique flavor features [7]. The wide differences in odor among species (e.g., onions vs. garlic) depend on the type and amount of these sulfur compounds characterizing their VOCs profile. These compounds are all originated from S-alk(en)yl-l-cysteine-S-oxides (ACSOs) [8,9] by the enzymatic activity of alliinase, a C-S lyase present in the vacuoles [10]. ACSOs are located in the cytoplasm and come into contact with alliinase when the bulb is mechanically disrupted [11]. The enzymatic reaction produces sulfenic acids, which condense into thiosulfinates; these are considered “primary” aroma compounds produced in the first 30–60 s after cell wall rupture. Within 30 min after tissue crushing, thiosulfinates undergo further rearrangements, mainly producing mono- and polysulfides, which are referred to as “secondary” aroma compounds [12,13]. This pattern of compounds and reactions is common to most *Allium* species; the differences in the sulfur compounds profile, also impacting flavor among the species, are due to the structure and relative amounts of residues in the ACSO precursors [14]. In onion, the compound with 1-propenyl residue (isoalliin) is a major component, while the allyl residue (alliin) is quite or totally absent [15,16]. The sulfur compounds profile may also change among different varieties within onion species [17,18], some of them being particularly appreciated for their peculiar taste characters. ‘Rossa di Tropea’ onion is one of these varieties of choice; it is a production of particular interest whose cropping area is circumscribed in a small area of Calabria (Southern Italy). It is characterized by pink/red colored bulbs, worldwide known for their organoleptic features as mild to sweet flavor and their high content in flavonols and anthocyanins. In view of the above, and because of its economic implications on the territory from where it originates, it has been granted the protected geographical indication (PGI) trademark by the European Union.

Many diseases and disorders affecting onion crops are mainly caused by fungi and systemic pathogens, such as bacteria and viruses [19]. In particular, onion yellow dwarf virus–OYDV (genus *Potyvirus*) has been reported to be the most widespread virus in onion and *Allium* spp., causing detrimental effects on crop yield and bulb quality [20]. The agronomic effects of viral infection in onion, and crops in general, are well documented, and several studies addressed to establish the effect of virus interference on host cellular metabolism and compound synthesis have been reporting the first results [21–23].

Volatile compounds contribute to the overall sensory characteristics of food products, often determining their acceptability and eating quality [24]. Many factors influence the VOCs emission rate and the type of compounds present in the VOCs fraction in vegetables: storage temperature, storage time, living plant tissue, and also the pathogen species possibly present in the samples [25–27]. In onion, numerous studies have been carried out on the correlation between volatile compounds and sensory attributes of bulbs, proving the importance of volatile composition for sensory qualities [28,29]. Several publications [30–32] report VOCs emission from experimentally inoculated onion bulbs, produced both from the infected plants or the pathogens growing in the commodity. However, these studies were limited to fungal pathogens, and very few studies about the modulation of VOCs profile in a plant–virus interaction patho-system can be retrieved in literature. In ‘Rossa di Tropea’ onion, little information is available regarding the effect of viral pathogens (particularly OYDV) on its organoleptic properties.

Moreover, it has to be underlined that, in case of infection by viruses, those pathogens being obligate parasites lacking an own biosynthetic system, all VOCs variations associated with infection are to be exclusively attributed to a change in the expression of the compounds profile by the plant itself.

This study aims to investigate the effect of a viral pathogen such as OYDV on the overall organoleptic properties in 'Rossa di Tropea' onion, mainly focusing on the determination of VOCs profile and their variations briefly after the inoculation and in postharvest storage.

2. Materials and Methods

2.1. Plant Materials and Experimental Setup

Plant material (i.e., onion bulbs) was obtained from an experimental trial established as follows. Seeds of 'Rossa di Tropea' onion (biotype locally named *Mezza campana*), collected from healthy plants as assessed by specific OYDV Real-Time RT-PCR test [33], were used as starting material. All the procedures performed during the different trial phases were carried out in an insect-proof screenhouse at "Mediterranea" University of Reggio Calabria, Italy. The experimental design was based on randomized blocks including two treatments (healthy-h and infected-i), each applied in three parcels. Overall, the trial was composed of 180 plants (each plant growing in one pot), 30 pots for each parcel, and six parcels, labeled from A to F. The cultivation practices mimicked those commonly performed in the open fields by farmers in 'Rossa di Tropea' onion production area. In particular, water and nutrient supplies were assured by daily irrigation, one round every 12 h (20 L/m² per h), 100 g/pot of diammonium phosphate in granules, respectively. Additionally, fungicide treatments were performed by Rizolex[®] and Signum[®] application, one pre-transplant (3 g/m²) and two post-transplant, in a range of 10 days (15 g/L), respectively. Three months after the transplant, an artificial inoculation was carried out in parcels A, D, and E, starting from fresh tissue material infected by an OYDV isolate, following previously described procedures [21]. For this study, three analytical time points were sampled, mirroring the 'Rossa di Tropea' onion bulb production steps: at harvesting time (t_0), i.e., at the complete development of plants and bulbs; an experimental check timepoint (t_1) during postharvest storage, i.e., 1 month after harvesting; and after storage (t_2), i.e., 3 months after harvesting, when the bulbs reach the marketable stage, corresponding to 2, 3, and 5 months post-inoculation, respectively. It has to be underlined that the middle sampling time t_1 was then observed to have no biological nor statistical significance for the scope of this work; hence, only results of the samples at harvesting and at the end of postharvest storage (i.e., t_0 and t_2) are reported. Each time point was represented by collecting three biological replicates (Table 1) sharing the same morphological features as bulb dimension (i.e., diameter) and weight (data collected only at t_0 for bulbs sampled at t_0 , both at t_0 and t_2 for bulbs sampled at t_2).

Table 1. Morphological features and weight of sample onion bulbs used for analyses. Sample IDs are coded with a progressive serial number identifying the individual and a letter corresponding to the belonging randomized block (B, C, F healthy; A, D, E infected); n.d. = not determined (sample collected at t_0).

Sample ID	OYDV	Diameter t_0 (mm)	Diameter t_2 (mm)	Weight t_0 (g)	Weight t_2 (g)
B2	absent	56.8	n.d.	239.4	n.d.
C7	absent	55.1	n.d.	232.2	n.d.
F5	absent	55.9	n.d.	236.6	n.d.
B21	absent	56.2	53.9	234.6	185.1
C29	absent	57.8	54.2	242.1	190.2
F22	absent	56.4	53.2	235.8	185.3
A3	present	58.8	n.d.	250.8	n.d.
D6	present	57.7	n.d.	244.6	n.d.
E4	present	58.5	n.d.	249.1	n.d.
A23	present	57.9	52.5	244.9	156.2
D25	present	58.2	53.1	248.7	158.7
E24	present	58.0	52.9	248.9	158.8

2.2. Phytosanitary Status Assessment

To ascertain sanitary status of infected (i) and healthy (h) plants after inoculation, leaf samples collected from all the 180 plants in the experimental trial were assayed by DAS-ELISA (BIOREBA, Reinach, Switzerland) and RealTime RT-PCR [33]. The sampling and analyses were performed 21 days post inoculum (d.p.i.). The infection rate was about 80% of inoculated plants; only bulbs from confirmed healthy and OYDV-infected plants in the respective parcels were harvested and sampled for downstream analyses.

2.3. VOCs Analysis by HS/SPME GC-MS

A relatively recent technique called headspace solid-phase microextraction (HS-SPME) has been used to evaluate VOCs changes induced by OYDV infection. The HS-SPME technique is solvent-free and time-efficient, and it consists of immersing a thin fused-silica fiber in the headspace of a liquid or solid sample.

This fiber, coated with an extracting phase, can absorb several analytes (the class of metabolites absorbed depends on the fiber used), which are successively desorbed into the gas chromatography–mass spectrometry (GC–MS) apparatus for the analysis [34].

The HS/SPME GC–MS analysis was carried out as previously reported by Araniti et al. [35] with some modifications.

Briefly, 1 g of plant material, per sample and replicate ($n = 3$), was sealed in a 20 mL vial and allowed to equilibrate for 20 min at room temperature. Successively, the SPME grey fiber (StableFlex, divinylbenzene/Carboxen on polydimethylsiloxane coating; 50/30 μm coating, Supelco-Merck, Darmstadt, Germany) was exposed to plant VOCs for 20 min at room temperature.

The VOCs were identified using a gas chromatograph apparatus (G-Trace 1310 Thermo Fisher, Milan, Italy) coupled with a single quadrupole mass spectrometer (ISQ LT Thermo Fisher, Milan, Italy). The capillary column was a TG-5MS 30 m \times 0.25 mm \times 0.25 μm , and helium (purity 6.0) was used as carrier gas with a flow of 1 mL/min. Samples were injected in a split mode with a split ratio of 60. Injector and source were set at the temperature of 200 $^{\circ}\text{C}$ and 260 $^{\circ}\text{C}$, respectively. The temperature ramp used was settled as follows: 7 min 45 $^{\circ}\text{C}$, 45–80 $^{\circ}\text{C}$ rate 10 $^{\circ}\text{C}$ per min, 80–200 $^{\circ}\text{C}$ rate 20 $^{\circ}\text{C}$ per min, 3 min 200 $^{\circ}\text{C}$. Mass spectra were recorded in electronic impact (EI) mode at 70 eV, scanning the 45–500 m/z range. Compound identification was carried out, comparing the relative retention time and mass spectra of the molecules with commercial libraries (NIST 2017). Moreover, relative quantitation of these compounds were also accomplished by evaluating the relative percentage for each peak [peak area/total ion chromatogram (TIC) area] (Table 2).

Table 2. VOCs identified and quantified in HS/SPME GC–MS analysis of onion bulbs.

Entry	Compound	CAS n.
1	allyl-isopropyl disulfide	67421-85-6
2	3,5-diethyl-1,2,4-trithiolane	54644-28-9
3	1-nitro-2-prop-2-enylsulfanylpropane	127865-37-6
4	1-propyl-2-(4-thiohept-2-en-5-yl) disulfide	143193-11-7
5	2,4-dimethylthiophene	638-00-6
6	2-mercapto-3,4-dimethyl-2,3-dihydrothiophene	n.d.
7	acetic acid	64-19-7
8	methyl-propyl trisulfide	17619-36-2
9	methyl-propyl disulfide	2179-60-4
10	Propanethiol	107-03-9
11	propyl disulfide	629-19-6

2.4. Statistical Analysis

HS/SPME GC–MS quantitation data for each identified compound were subjected to Student *t*-test to establish the statistical significance of differences between healthy and infected groups at each sampling time.

For multivariate modeling, the collected HS/SPME GC–MS quantitation data were assembled in a data matrix containing samples in rows and features (amounts of each VOC) in columns. It was then normalized by constant sum, mean-centered, and divided by the standard deviation of each variable (auto-scaling) to be used as input for statistical analysis. No data filtering was applied, and no missing values were present in the matrix. The reduced and normalized data were then analyzed through the Biostatflow web application (biostatflow.org, v.2.9.2, access date 14 February 2020). Partial least squares discriminant analysis (PLS-DA) [36] was used for classification and relating the variation of VOCs profile to phytopathological status and sampling time. The optimal number of components was determined by evaluating the variance captured and statistics, considering R², Q², and accuracy parameters. The obtained model was based on two components and was validated by the leave-one-out cross-validation method (LOOCV) [37]. Furthermore, a permutation test was applied calculating the ratio of the between sum of squares to the within sum of squares (B/W-ratio) for the class assignment prediction ability of the model with a permutation number of 1000 [38,39]; the obtained model was correctly observed to be an outlier of the distribution of the 1000 randomly permuted models.

For the PLS-DA model obtained, variable important for projection (VIP) classifier was applied; the limit of significance value was placed equal to 1.5.

Hierarchical clustering analysis (HCA) was also applied on the same dataset in order to better elucidate the clustering of samples based on similarity and observe its consistency with phytopathological status/sampling time [40,41]. HCA was performed using Metaboanalyst software version 5.0 [42] considering Euclidean distance measure as a definition of similarity and Ward clustering algorithm.

2.5. Sensory Analysis

The sensory analysis was performed by 10 panelists (seven women and three men, aged between 28 and 50 years old) trained according to ISO 8586:2012 guidelines [43] and selected on the basis of their familiarity with onion. Onion samples (infected and healthy) were cut into slices and coded with random three digit numbers. Onion samples were placed in plastic plates and given to the panelists in individual booths in an air-conditioned sensory evaluation room at 20 °C under incandescent white lighting. Water and unsalted bread were used to clean out tastes among samples. The onion sensory profile was expressed into four general attributes of acceptability (odor rating, taste rating, texture rating, and general acceptability) and in 12 specific attributes: color, pungency, peculiar odor, typical odor, sweetness, spiciness, savoriness, bitterness, typical aroma, peculiar aroma, pulp texture, and crunchiness. During the tasting evaluation, the consumers were asked to rate the samples for overall liking on a 9-point hedonic scale, where 0 = extremely low score and 9 = extremely high score [44]. The values of attributes are presented as means for infected and healthy samples in the form of a spider web graph.

3. Results and Discussion

The aroma of onion is mainly characterized by volatile sulfur compounds produced by the rearrangement of thiosulphinates; VOCs profile of fresh onion has been extensively studied, and its changes due to onion variety, soil characteristics (e.g., sulfur and nitrogen fertility), biotic stressors, and postharvest storage have been reported in the literature [17,45–49].

In this work, 11 VOCs were identified and quantified using HS/SPME GC–MS analysis in healthy and OYDV-infected onion bulbs at two sampling times; moreover, using a panel test, the sensory attributes of onion bulbs at the marketable stage (t₂) were investigated, aiming at highlighting likely differences between healthy and infected samples.

3.1. VOCs Analysis by HS/SPME GC-MS

VOCs profile was obtained by HS/SPME GC–MS analysis to investigate any difference in VOCs in response to virus infection and storage time. The HS/SPME GC–MS

analysis allowed us to identify 11 VOCs (Table 2) belonging to different chemical classes: monosulfides (compounds 3, 5, 10), polysulfides (1, 2, 4, 6, 8, 9, 11), and organic acids (7). The relative quantitation of these compounds was evaluated as the relative percentage for each peak at two sampling times t_0 and t_2 for healthy and OYDV-infected samples; the relative percentages of peak area average for each sample group are reported in Table 3. VOC ratio was also calculated for each compound as the ratio of average amounts in OYDV-infected onions relative to healthy onions, irrespective of the sampling time.

Table 3. Quantitation data (adimensional measure) of compounds in healthy and OYDV-infected bulbs at two sampling times.

Entry	Sampling Time Compound	t_0		t_2		VOC Ratio
		Control	OYDV	Control	OYDV	
1	allyl-isopropyl disulfide	5.67 ± 0.52	6.83 ± 1.09	33.15 ± 7.05 **	7.93 ± 0.32 **	0.38
2	3,5-diethyl 1,2,4-trithiolane	0.71 ± 0.30	1.12 ± 0.77	0.27 ± 0.03 **	1.07 ± 0.08 **	2.24
3	1-nitro-2-prop-2-enylsulfanylpropane	0.31 ± 0.17	0.13 ± 0.06	0.33 ± 0.23	0.28 ± 0.05	0.63
4	1-propyl 2,4-thiohept-2-en-5-yl disulfide	1.12 ± 0.63	0.57 ± 0.30	0.37 ± 0.03 **	0.12 ± 0.02 **	0.46
5	2,4-dimethyl thiophene	0.05 ± 0.02	0.07 ± 0.03	0.25 ± 0.01 **	0.37 ± 0.03 **	1.48
6	2-mercapto-3,4-dimethyl 2,3-dihydrothiophene	0.27 ± 0.19	0.27 ± 0.03	1.10 ± 0.04 **	0.44 ± 0.05 **	0.52
8	methyl-propyl trisulfide	0.29 ± 0.22	0.11 ± 0.05	2.34 ± 1.94	0.24 ± 0.06	0.13
9	methyl-propyl disulfide	1.57 ± 0.11 **	0.78 ± 0.11 **	3.59 ± 1.54	0.58 ± 0.09	0.26
10	propanethiol	10.97 ± 1.67 *	16.15 ± 1.63 *	4.76 ± 2.00	15.44 ± 5.49	2.01
11	propyl-disulfide	67.39 ± 3.48	60.13 ± 6.57	51.59 ± 9.94	61.11 ± 3.49	1.02

Values are expressed as mean ± standard error for three samples each group; Student's t test performed between healthy and infected at each sampling time: * significant difference at $p < 0.05$; ** significant difference at $p < 0.01$.

The most abundant compound in the analyzed samples (ca. 60% of the TIC), as reported in Table 3, was generally propyl disulfide (11), in accordance with previous literature [50–52]. In the genus *Allium*, polysulfides are produced by the rearrangement of thiosulfonates, which are, in turn, generated through alk(en)yl sulfenic acids (lachrymatory factor). Differences among ACSOs specific moieties (methyl, allyl, prop(en)yl) are found in the *Allium* genus, strongly depending on the species. Onion shows high levels of S-prop(en)yl l-cysteine-S-oxide (isoalliin); hence, thiosulfonates and polysulfides found in onion also have the same moieties of their precursor as major residues; conversely, high levels of allyl polysulfides are found in garlic. The kind and amount of such moieties have also been correlated with the peculiar kind of aroma typical of each of the species in the *Allium* genus: propyl sulfides have also been correlated with “fresh onion” taste, while allyl radical containing compounds are reported to cause pungent, radish- or garlic-like aroma [50].

Other well-represented VOCs in samples (ca. 13% and 12% of the TIC, respectively) were allyl-isopropyl disulfide (1) and propanethiol (10); they are widely reported in the literature as main components of VOCs profile and important sources of flavor in fresh onion [15,25]. Allyl-isopropyl disulfide is generated as described above for propyl disulfide, according to the different radical present in the corresponding ACSO. Propanethiol is widely reported in the literature to be present in small amounts compared to propyl disulfide [48,51]; proton-transfer reaction-mass spectrometry (PTR-MS) and GC, coupled with sulfur chemiluminescence detection (GC-SCD) experiments, elucidated that propanethiol undergoes oxidation and dimerization to propyl disulfide during GC-MS sampling and analysis [52]. Hence, propanethiol is likely to be present at higher amounts in the original sample, a fraction of the recorded amount of propyl disulfide being an artefact. Additionally, another eight compounds were identified in all sample groups and quantified at different amounts; most of them (mono- and poly-sulfides) were previously reported in VOCs profile of fresh onion [9,53]. To our knowledge, acetic acid, which was detected in all samples, has never been reported in raw onions. On the contrary, its formation joined with other organic acids was reported in cooked onions as a consequence of thermal degradation [28]. In this view, acetic acid in samples is likely to represent an artefact resulting

from thermal degradation occurring during GC analysis. Moreover, its amounts did not change with storage time nor upon virus infection. Due to these reasons, quantitation data on acetic acid were not included in Table 3 and discussed.

The HS/SPME GC–MS data on the identified VOCs were compared in healthy and OYDV-infected samples at each sampling time to highlight possible differences in some specific VOCs in response to OYDV infection and/or storage time. Student's *t*-test showed significant differences in concentration of 7 out of 11 VOCs in OYDV-infected vs. healthy bulbs at different sampling times (Table 3). Five of them were regulated at t_2 , and the other two compounds were regulated at t_0 ; no compound was found regulated at both sampling times, indicating a huge significance of postharvest storage in the evolution of VOCs profile. In detail, methyl-propyl disulfide (9) and propanethiol (10) were found regulated by OYDV infection at t_0 , the first being depleted and the second enriched in infected bulbs compared to healthy control. At t_2 , two compounds were recorded at higher levels in infected samples, i.e., 3,5-diethyl 1,2,4-trithiolane (2) and 2,4-dimethyl thiophene (5), while three were depleted upon infection, i.e., allyl-isopropyl disulfide (1), 1-propyl 2,4-thiohept-2-en-5-yl disulfide (4), and 2-mercapto-3,4-dimethyl 2,3-dihydrothiophene (6). All of them are sulfur compounds identified in onion as VOCs formed by rearrangements of thiosulfates produced by the enzymatic action of alliinase and are reported to play a role in flavor development [15,28]. These five compounds are relevant to the actual difference in VOCs profile between healthy and OYDV-infected 'Rossa di Tropea' onions when they reach the market and the consumers.

Conversely, it has to be underlined that the two VOCs potentially regulated upon infection at t_0 , but not at t_2 (9 and 10), should not be considered crucial for the VOCs profile of onion at marketable stage, nor to be correlated to sensory analysis, which was performed only at t_2 . Nonetheless, their regulation can provide an insight into the early plant response to OYDV infection shortly after inoculation. In detail, propanethiol (10) was upregulated in infected samples at t_0 with an overall VOC ratio of ca. 2, indicating a twofold increased emission in OYDV-infected samples. As mentioned above, propanethiol is one of the most represented VOCs identified in analyzed samples; it was previously indicated as the main source of the characteristic onion odor [52], and its increase was previously correlated with fungi infection in onion [32]. Methyl-propyl disulfide (9) was found in lower amounts in the infected group at t_0 , recording an overall VOC ratio of 0.26, indicating a fourfold decreased emission in OYDV infected samples. Such "mixed" sulfides are produced when two or more alliin homologs are found in the same tissue, giving rise, upon rupture, to asymmetrical sulfenic acid and, hence, to disulfides with two different moieties.

VOCs profile changes at t_2 in function of OYDV infection was even more important, indicating a likely impact on the flavor of the edible product at the marketable stage and correlation to panel test results. Compounds 2 and 5 recorded a higher emission in infected samples, with an overall VOC ratio of 2.24 and 1.48, respectively. Compound 2 (3,5-diethyl 1,2,4-trithiolane) is reported to be synthesized from acetaldehyde and hydrogen sulfide [54] and known to represent an intermediate in the formation of dimethylthiophene (5) [55]. The coherent increase of the two compounds in infected samples at the same sampling time (t_2) might reflect a general upregulation of their biosynthetic pathway; this may be due to an increase of one or more of their precursors. In accordance, acetaldehyde was already found to be around six-fold upregulated in diseased onion infected by *Fusarium oxysporum* [32]. These results may have an impact on the consumption and production of 'Rossa di Tropea' onion: OYDV infection is here demonstrated to alter VOCs profile in 'Rossa di Tropea' onion bulbs, especially at the marketable stage; this may also influence the control measures that are meant to manage these crops and protect them from virus infection. Compounds 1, 4, and 6 were instead emitted in lower amounts by OYDV-infected onions at t_2 . Allyl-isopropyl disulfide (1) was recently found in onion for the first time [56] and represented another example of "mixed" disulfide typical of the *Allium* genus. Being one of the most abundant VOCs in the analyzed samples, and yet recognized as one of the main ones responsible for flavor in onion, it is supposed to play a key role also in

modulating samples' flavor as a consequence of the infection. Allyl moiety has been correlated with pungent, radish-like odor, while n-propyl residue is usually associated with typical onion odor [50]; to our knowledge, no correlation to a specific odor has been observed relative to isopropyl residues in *Allium* sulfides.

Compound 6 was reported to arise from pyrolysis of bis (1-propenyl) disulfide [57]. It is considered a non-enzymatically produced volatile sulfur compound, having been reported to be over-synthesized in onions where lachrymatory factor synthase was suppressed [58]. This precursor is reported as a major VOC in onion, being the derivative of S-propenyl-L-cysteine-S-oxide, which is the most abundant ACSO in onion [15]. Compound 6 has also been recorded in *Meliaceae* by Liu et al. [59] but is not considered important in the aroma formation in *Amaryllidaceae* due to the presence of more pungent molecules.

It has also been proposed that 6 can rearrange to 3,4 dimethylthiophene, an isomer of (5), allowing us to hypothesize that the decrease of the former may be correlated to the increase of the latter at t_2 .

3.2. Statistical Analysis

In order to look over the collected data, a PLS-DA model was built, allowing us to reduce the dimensionality of such complex datasets, thus, enabling an easier visualization of any clustering of samples in function of phytopathological status and/or sampling time and to classify samples according to the pattern of quantified compounds.

Figure 1a shows the score plot for the score matrix of the two-components model obtained by PLS-DA: PC1 accounts for 12.9% of the captured variance in the model, while PC2 accounts for 23.8%. The distribution obtained shows that VOCs profile variables partially discriminated groups of samples, according to phytopathological status and sampling time. In particular, for both sampling times, healthy samples are mainly clustered at positive values of PC2, while OYDV-infected samples are found at negative values of PC2. Further, both PC1 and PC2 partially discriminate samples according to sampling time: samples at t_0 were mostly located in the lower-right quadrant of the graph (positive values of PC1, negative values of PC2), while samples at t_2 were mainly found in the upper-left quadrant (negative values of PC1, positive values of PC2).

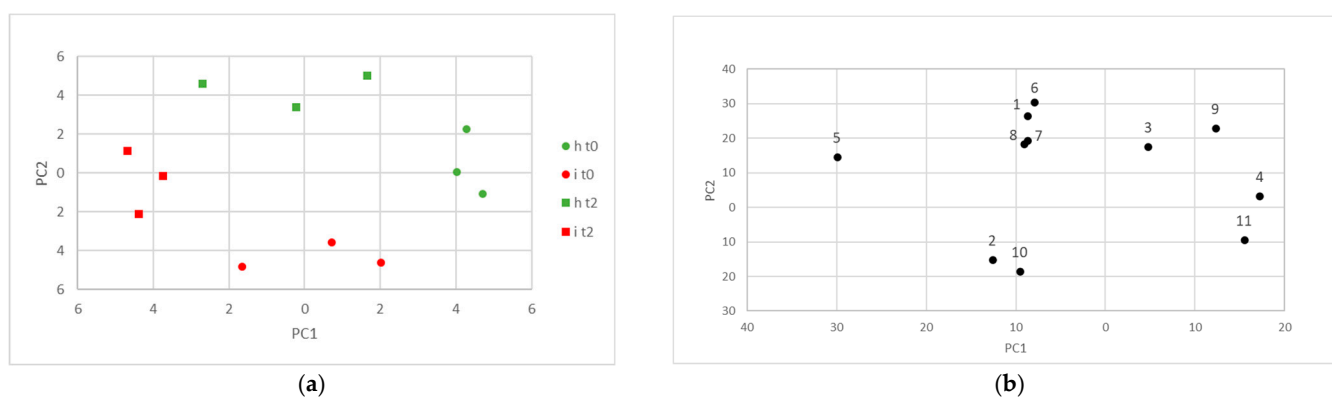


Figure 1. PLS-DA performed on the 11 VOCs identified in healthy (h) and OYDV-infected (i) samples for t_0 and t_2 sampling times. (a) Score plot for analyzed samples; (b) Loading plot for VOCs compounds (numbers correspond to those associated with compounds in Table 1).

Loading plot (Figure 1b) was useful for identifying which variables (i.e., quantified VOCs) have the most significant effect on each component in the model. High positive or negative loading values for a variable indicate that the variable strongly influences the component; loadings close to 0 indicate that the variable has a weak influence on the component. In particular, three compounds (1, 6, and 9) are found at high positive values of PC2 (i.e., >20); hence, their content is suggested to strongly influence PC2, which, as reported for the score plot, rules the discrimination between healthy and infected samples;

the other two VOCs (2 and 10) have high negative values of PC2 (i.e., <15), indicating that the sustained increase in their content in infected samples with respect to healthy is another factor influencing PC2, thus, driving group discrimination based on phytosanitary status. These results are in accordance with the direct comparison of amounts performed in the previous section for each quantified compound.

Figure 2 reports the results of the variable important for projection (VIP) classification for the PLS-DA model, identifying the variables (compounds) on which the built model mostly relies; overall, seven compounds were reported to have a high score (>1.5). Three of them were upregulated upon infection (5, 2, and 1), three were downregulated (6, 4, and 9), and one did not show any regulation correlated with virus infection with respect to healthy control (8). The most important variables were represented by compounds 6 and 5, in accordance with literature and previous discussion.

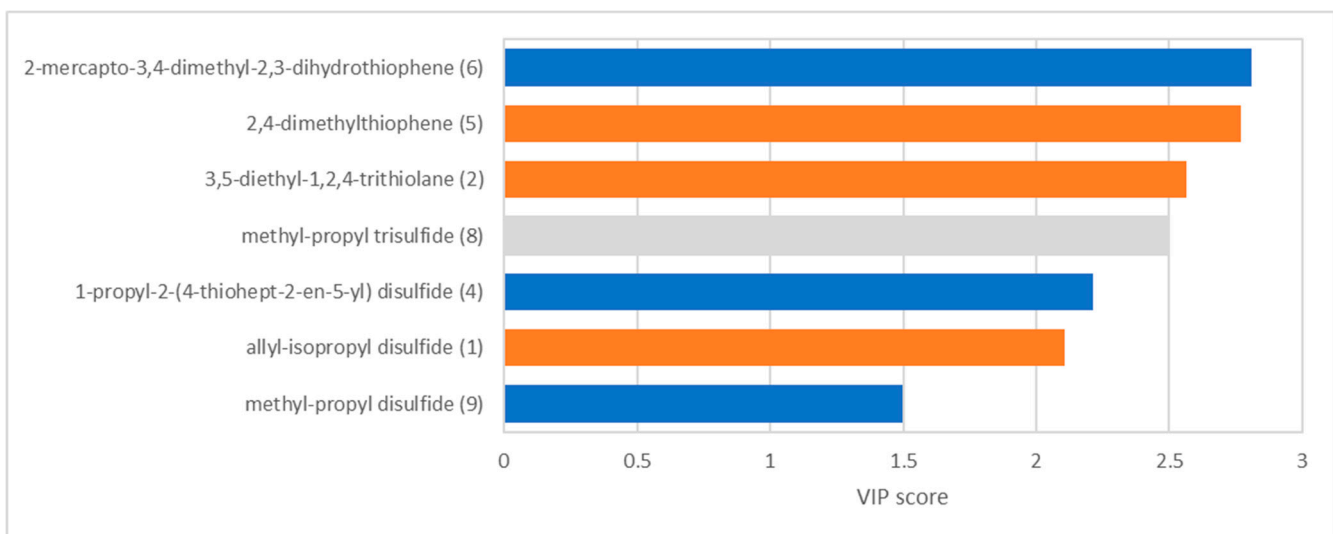


Figure 2. Variables important for the projection (VIP) values from Kruskal test on PLS-DA model. Upregulated compounds = orange bars; downregulated compounds = blue bars; unregulated compounds = grey bars.

Given the only partial group separation obtained by the PLS-DA model, HCA was also performed to fully understand hidden patterns and allow a better visualization of the actually similar samples. The dendrogram obtained by HCA is reported in Figure 3: it shows a clear separation of clusters referring to healthy and infected samples, respectively, at t_2 sampling time. Instead, healthy and infected samples collected at t_0 showed some similarity: bulbs with different sanitary status were observed to be quite mixed in the dendrogram and did not account for a distinct pattern according to phytosanitary status. This result indicates an effect of OYDV infection on the VOCs profile of onions at the marketable stage (t_2), and again, it underlines the huge importance of postharvest storage in the development of the taste and aroma perceived by consumers; this evolution has been observed previously in onion regarding other important compounds for taste and aroma, such as flavonoids, sugars, and aminoacids [60,61].

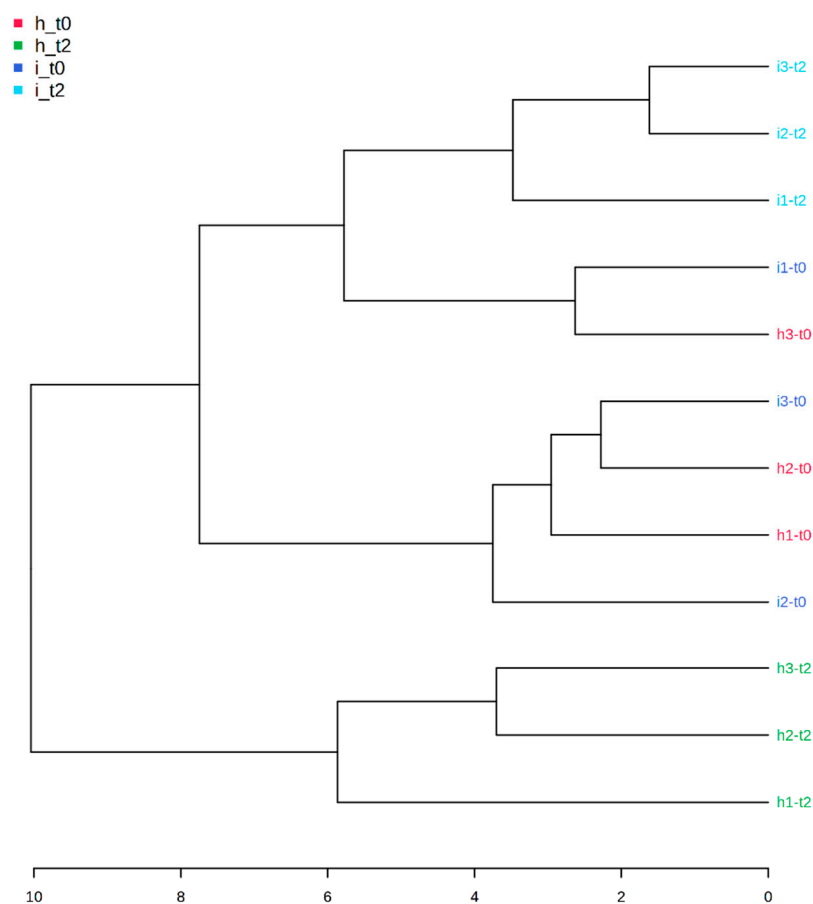


Figure 3. Dendrogram of samples based on HCA performed on the VOCs identified in healthy (h) and OYDV-infected (i) samples for t_0 and t_2 sampling times.

3.3. Sensory Analysis

The sensory analysis of onion samples was reported in a spider graph (Figure 4) that allowed us to compare the perceived attributes for infected and healthy ‘Rossa di Tropea’ onions at t_2 sampling time, when onions reach the marketable stage after postharvest storage. The infected onions manifested higher scores for color, pungency, spiciness, and bitterness than healthy ones, which manifested instead higher scores in the other sensory descriptors, such as sweetness, pulp texture, and crunchiness. Very low peculiar odor (ranging from 0.67 to 2.65) and aroma (ranging from 0 to 1.89) were evidenced in both samples, whereas typical aroma and odor resulted in high scores (5.1 and 4.8, respectively). Among the four general attributes of acceptability, texture rating was higher in healthy onions (5.20) than infected ones (4.86), and it can be correlated with some effect of infection to the vegetable tissues since viral infections are known to induce structural alteration starting from the cell wall [62,63]. The evaluation of general acceptability obtained a good score on both infected and healthy onion samples with values of 5.30 and 5.23, respectively.

The statistical data elaboration evidenced significant differences only for few attributes between healthy and infected samples. The taste attributes accounting for the major differences were bitterness and sweetness, suggesting an influence of virus infection on these characteristics. In fact, infected onion samples showed a lower sweetness (5.42) compared to healthy onions (6.09); higher bitterness (2.89) and spiciness (4.34) for the first ones were also recorded. As panel test results show an enhanced pungency, spiciness, and bitterness in the infected group, it is likely that the allyl moiety in compound **1** is not crucial in defining these characters, as compound **1** is underrepresented in infected onions.

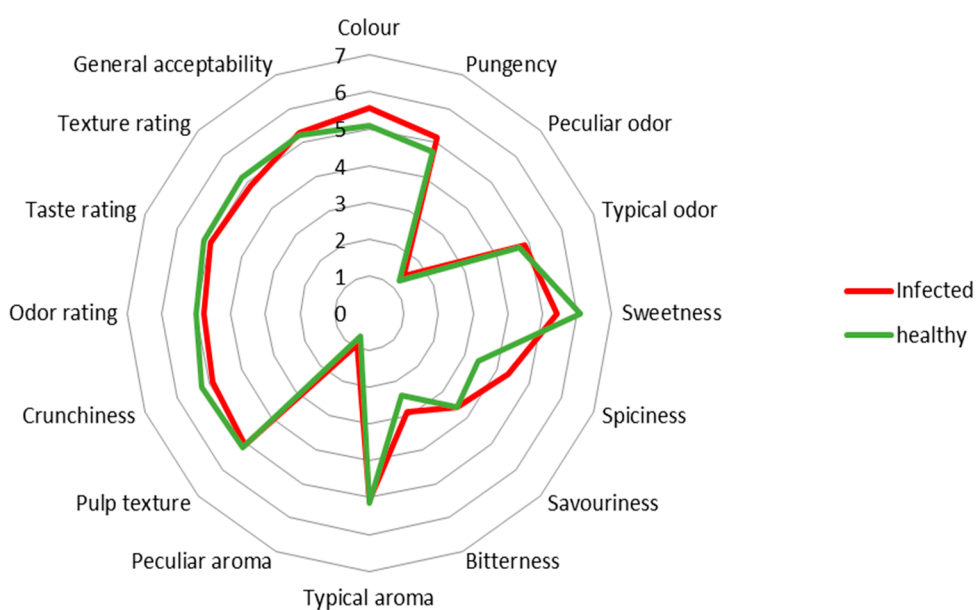


Figure 4. Spider web graph of the sensory attributes of infected (red line) and healthy (green line) onion bulbs.

4. Conclusions

In this study, the quantitation of VOCs in 'Rossa di Tropea' onion bulbs by means of HS-SPME GC-MS indicated significant differences between healthy and OYDV-infected samples in the content of seven VOCs at harvest and after postharvest storage. All of them belong to the sulfur compounds class, which is well known to be crucial in the development of odor and taste in *Allium* species.

Multivariate statistical analysis based on this quantitation discriminated between samples according to their sanitary status at t_2 sampling time, indicating the significance of virus infection during postharvest storage in the development of VOCs profile evolving to the taste and aroma perceived by consumers. The VOCs mostly accounting for such clustering were also identified as 2-mercapto-3,4-dimethyl-2,3 dihydrothiophene (6), 2,4-dimethylthiophene (5), and 3,5-diethyl-1,2,4-trithiolane (2). The sensory analysis also accounted for differences in some sensory attributes, and in one general attribute of acceptability (i.e., texture), between healthy and infected samples at the marketable stage. All these results suggest an impact of OYDV infection on the taste of this particular onion variety; this change involves several compounds, and it might indicate their importance in postharvest storage and the development of taste and aroma in 'Rossa di Tropea' onion.

Author Contributions: Conceptualization, A.T. (Antonio Tiberini); formal analysis, A.T. (Anna Taglienti); investigation, A.T. (Anna Taglienti), A.T. (Antonio Tiberini), F.A., and A.P.; resources, A.T. (Antonio Tiberini); writing—original draft preparation, A.T. (Anna Taglienti); writing—review and editing, A.T. (Anna Taglienti), A.T. (Antonio Tiberini), F.A., and A.P.; visualization, A.T. (Anna Taglienti); project administration, A.T. (Antonio Tiberini); funding acquisition, A.T. (Antonio Tiberini). All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Italian Ministry of Education, University and Research—MIUR (SIR-MIUR grant number SIORTO-RBS1149LD5).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Casañas, F.; Simó, J.; Casals, J.; Prohens, J. Toward an Evolved Concept of Landrace. *Front. Plant Sci.* **2017**, *8*, 145. [[CrossRef](#)]
2. Wendin, K.; Mustafa, A.; Ortman, T.; Gerhardt, K. Consumer Awareness, Attitudes and Preferences towards Heritage Cereals. *Foods* **2020**, *9*, 742. [[CrossRef](#)] [[PubMed](#)]
3. Giupponi, L.; Pedrali, D.; Leoni, V.; Rodari, A.; Giorgi, A. The Analysis of Italian Plant Agrobiodiversity Databases Reveals That Hilly and Sub-Mountain Areas Are Hotspots of Herbaceous Landraces. *Diversity* **2021**, *13*, 70. [[CrossRef](#)]
4. Teuber, R.; Dolgoplova, I.; Nordström, J. Some like it organic, some like it purple and some like it ancient: Consumer preferences and WTP for value-added attributes in whole grain bread. *Food Qual. Prefer.* **2016**, *52*, 244–254. [[CrossRef](#)]
5. Longin, C.F.H.; Würschum, T. Back to the Future—Tapping into Ancient Grains for Food Diversity. *Trends Plant Sci.* **2016**, *21*, 731–737. [[CrossRef](#)] [[PubMed](#)]
6. Marrelli, M.; Amodeo, V.; Statti, G.; Conforti, F. Biological Properties and Bioactive Components of *Allium cepa* L.: Focus on Potential Benefits in the Treatment of Obesity and Related Comorbidities. *Molecules* **2018**, *24*, 119. [[CrossRef](#)] [[PubMed](#)]
7. Wang, A.; Luca, A.; Edelenbos, M. Emission of volatile organic compounds from yellow onion (*Allium cepa* L.) bulbs during storage. *J. Food Sci. Technol.* **2019**, *56*, 2940–2948. [[CrossRef](#)]
8. Mondy, N.; Duplat, D.; Christides, J.; Arnault, I.; Auger, J. Aroma analysis of fresh and preserved onions and leek by dual solid-phase microextraction–liquid extraction and gas chromatography–mass spectrometry. *J. Chromatogr. A* **2002**, *963*, 89–93. [[CrossRef](#)]
9. Rose, P.; Whiteman, M.; Moore, P.K.; Zhu, Y.Z. Bioactive S-alk(en)yl cysteine sulfoxide metabolites in the genus *Allium*: The chemistry of potential therapeutic agents. *Nat. Prod. Rep.* **2005**, *22*, 351–368. [[CrossRef](#)] [[PubMed](#)]
10. Imai, S.; Tsuge, N.; Tomotake, M.; Nagatome, Y.; Sawada, H.; Nagata, T.; Kumagai, H. An onion enzyme that makes the eyes water. *Nat. Cell Biol.* **2002**, *419*, 685. [[CrossRef](#)]
11. Keusgen, M. Volatile Compounds of the Genus *Allium* L. (Onions). In *Volatile Sulfur Compounds in Food*; American Chemical Society: Washington, DC, USA, 2011; Volume 1068, pp. 183–214.
12. Gîtin, L.; Dinică, R.; Neagu, C.; Dumitrascu, L. Sulfur compounds identification and quantification from *Allium* spp. fresh leaves. *J. Food Drug Anal.* **2014**, *22*, 425–430. [[CrossRef](#)]
13. Kusano, M.; Kobayashi, M.; Iizuka, Y.; Fukushima, A.; Saito, K. Unbiased profiling of volatile organic compounds in the headspace of *Allium* plants using an in-tube extraction device. *BMC Res. Notes* **2016**, *9*, 1–12. [[CrossRef](#)] [[PubMed](#)]
14. Shen, C.; Parkin, K.L. In vitro biogenesis of pure thiosulfinates and propanethial-S-oxide. *J. Agric. Food Chem.* **2000**, *48*, 6254–6260. [[CrossRef](#)] [[PubMed](#)]
15. Lanzotti, V. The analysis of onion and garlic. *J. Chromatogr. A* **2006**, *1112*, 3–22. [[CrossRef](#)] [[PubMed](#)]
16. Saviano, G.; Paris, D.; Melck, D.; Fantasma, F.; Motta, A.; Iorizzi, M. Metabolite variation in three edible Italian *Allium cepa* L. by NMR-based metabolomics: A comparative study in fresh and stored bulbs. *Metabolomics* **2019**, *15*, 105. [[CrossRef](#)]
17. Kallio, H.; Salorinne, L. Comparison of onion varieties by headspace gas chromatography-mass spectrometry. *J. Agric. Food Chem.* **1990**, *38*, 1560–1564. [[CrossRef](#)]
18. Liguori, L.; Adiletta, G.; Nazzaro, F.; Fratianni, F.; di Matteo, M.; Albanese, D. Biochemical, Antioxidant Properties and Antimicrobial Activity of Different Onion Varieties in the Mediterranean Area. *J. Food Meas. Charact.* **2019**, *13*, 1232–1241.
19. Cranshaw, W.; Crowe, F.; Davis, R.; Dugan, F. Infections/Biotic Diseases. In *Compendium of Onion and Garlic Diseases and Pests*; Schwartz, H., Mohan, S., Eds.; The American Phytopathological Society: St. Paul, MN, USA, 2007.
20. Manglli, A.; Tomassoli, L.; Tiberini, A.; Agosteo, G.E.; Fontana, A.; Pappu, H.R.; Albanese, G. A survey on the infection of Onion yellow dwarf virus and Iris yellow spot tospovirus in seed and bulb productions systems of onion in Calabria, Italy. *Eur. J. Plant Pathol.* **2020**, *156*, 767–778. [[CrossRef](#)]
21. Mishra, J.; Srivastava, R.; Trivedi, P.K.; Verma, P.C. Effect of virus infection on the secondary metabolite production and phytohormone biosynthesis in plants. *3 Biotech* **2020**, *10*, 1–16. [[CrossRef](#)]
22. Tungadi, T.; Groen, S.C.; Murphy, A.M.; Pate, A.E.; Iqbal, J.; Bruce, T.J.A.; Cunniffe, N.J.; Carr, J.P. Cucumber mosaic virus and its 2b protein alter emission of host volatile organic compounds but not aphid vector settling in tobacco. *Viol. J.* **2017**, *14*, 91. [[CrossRef](#)]
23. Mwando, N.L.; Tamiru, A.; Nyasani, J.O.; Obonyo, M.A.O.; Caulfield, J.C.; Bruce, T.J.A.; Subramanian, S. Maize Chlorotic Mottle Virus Induces Changes in Host Plant Volatiles that Attract Vector Thrips Species. *J. Chem. Ecol.* **2018**, *44*, 681–689. [[CrossRef](#)] [[PubMed](#)]
24. Fleming-Jones, M.E.; Smith, R.E. Volatile Organic Compounds in Foods: A Five Year Study. *J. Agric. Food Chem.* **2003**, *51*, 8120–8127. [[CrossRef](#)] [[PubMed](#)]
25. Gouinguéné, S.P.; Turlings, T.C. The Effects of Abiotic Factors on Induced Volatile Emissions in Corn Plants. *Plant Physiol.* **2002**, *129*, 1296–1307. [[CrossRef](#)] [[PubMed](#)]
26. Li, C.; Schmidt, N.E.; Gitaitis, R. Detection of onion postharvest diseases by analyses of headspace volatiles using a gas sensor array and GC-MS. *LWT* **2011**, *44*, 1019–1025. [[CrossRef](#)]
27. De Lange, E.S.; Rodriguez-Saona, C. Does enhanced nutrient availability increase volatile emissions in cranberry? *Plant Signal. Behav.* **2019**, *14*, 1616517. [[CrossRef](#)] [[PubMed](#)]
28. Villière, A.; Le Roy, S.; Fillonneau, C.; Guillet, F.; Falquerho, H.; Bousely, S.; Prost, C. Evaluation of aroma profile differences between sué, sautéed, and pan-fried onions using an innovative olfactometric approach. *Flavour* **2015**, *4*, 24. [[CrossRef](#)]

29. Choi, S.M.; Lee, N.-J.; Kim, J.-Y.; Lim, S.-T. Volatile composition and sensory characteristics of onion powders prepared by convective drying. *Food Chem.* **2017**, *231*, 386–392. [[CrossRef](#)]
30. Prithiviraj, B.; Vikram, A.; Kushalappa, A.; Yaylayan, V. Volatile Metabolite Profiling for the Discrimination of Onion Bulbs Infected by *Erwinia carotovora* ssp. *carotovora*, *Fusarium oxysporum* and *Botrytis allii*. *Eur. J. Plant Pathol.* **2004**, *110*, 371–377. [[CrossRef](#)]
31. Vikram, A.; Hamzehzarghani, H.; Kushalappa, A. Volatile metabolites from the headspace of onion bulbs inoculated with postharvest pathogens as a tool for disease discrimination. *Can. J. Plant Pathol.* **2005**, *27*, 194–203. [[CrossRef](#)]
32. Wang, A.; Islam, N.; Johansen, A.; Haapalainen, M.; Latvala, S.; Edelenbos, M. Pathogenic *Fusarium oxysporum* f. sp. *cepae* growing inside onion bulbs emits volatile organic compounds that correlate with the extent of infection. *Postharvest Biol. Technol.* **2019**, *152*, 19–28. [[CrossRef](#)]
33. Tiberini, A.; Mangano, R.; Micali, G.; Leo, G.; Mangli, A.; Tomassoli, L.; Albanese, G. Onion yellow dwarf virus $\Delta\Delta$ Ct-based relative quantification obtained by using real-time polymerase chain reaction in ‘Rossa di Tropea’ onion. *Eur. J. Plant Pathol.* **2018**, *153*, 251–264. [[CrossRef](#)]
34. Araniti, F.; Pantò, S.; Lupini, A.; Sunseri, F.; Abenavoli, M.R. Chemical Characterization of Volatile Organic Compounds (VOCs) Through Headspace Solid Phase Micro Extraction (SPME). In *Advances in Plant Ecophysiology Techniques*; Metzler, J.B., Ed.; Springer: Cham, Switzerland, 2018; pp. 401–417.
35. Araniti, F.; Lupini, A.; Sunseri, F.; Abenavoli, M.R. Allelopathic Potential of *Dittrichia viscosa* (L.) W. Greuter Mediated by VOCs: A Physiological and Metabolomic Approach. *PLoS ONE* **2017**, *12*, e0170161. [[CrossRef](#)] [[PubMed](#)]
36. Wold, S.; Sjöström, M.; Eriksson, L. PLS-regression: A basic tool of chemometrics. *Chemom. Intell. Lab. Syst.* **2001**, *58*, 109–130. [[CrossRef](#)]
37. Szymańska, E.; Saccenti, E.; Smilde, A.K.; Westerhuis, J.A. Double-check: Validation of diagnostic statistics for PLS-DA models in metabolomics studies. *Metabolomics* **2011**, *8*, 3–16. [[CrossRef](#)]
38. Brereton, R.G. Consequences of sample size, variable selection, and model validation and optimisation, for predicting classification ability from analytical data. *TrAC Trends Anal. Chem.* **2006**, *25*, 1103–1111. [[CrossRef](#)]
39. Westerhuis, J.A.; Hoefsloot, H.C.J.; Smit, S.; Vis, D.J.; Smilde, A.K.; Van Velzen, E.J.J.; Van Duijnhoven, J.P.M.; Van Dorsten, F.A. Assessment of PLS-DA cross validation. *Metabolomics* **2008**, *4*, 81–89. [[CrossRef](#)]
40. Brereton, R.G. Pattern recognition in chemometrics. *Chemom. Intell. Lab. Syst.* **2015**, *149*, 90–96. [[CrossRef](#)]
41. Lee, I.; Yang, J. Common Clustering Algorithms. In *Comprehensive Chemometrics*; Elsevier: Amsterdam, The Netherlands, 2009; pp. 577–618.
42. Xia, J.; Wishart, D.S. Using MetaboAnalyst 3.0 for Comprehensive Metabolomics Data Analysis. *Curr. Protoc. Bioinform.* **2016**, *55*, 14.10.1–14.10.91. [[CrossRef](#)]
43. *ISO 8586:2012 Sensory Analysis—General Guidelines for the Selection, Training and Monitoring of Selected Assessors and Expert Sensory Assessors*; ISO: Geneva, Switzerland, 2012.
44. Meilgaard, M.C.; Carr, B.T. *Sensory Evaluation Techniques*; Chapman and Hall/CRC: Boca Raton, FL, USA, 2006.
45. Block, E. The Organosulfur Chemistry of the Genus *Allium*—Implications for the Organic Chemistry of Sulfur. *Angew. Chem. Int. Ed.* **1992**, *31*, 1135–1178. [[CrossRef](#)]
46. McCallum, J.; Porter, N.; Searle, B.; Shaw, M.; Bettjeman, B.; McManus, M. Sulfur and nitrogen fertility affects flavour of field-grown onions. *Plant Soil* **2005**, *269*, 151–158. [[CrossRef](#)]
47. Wang, A.; Casadei, F.; Johansen, A.; Bukman, H.; Edelenbos, M. Emission of volatile organic compounds from healthy and diseased onions. *Acta Hortic.* **2016**, *1144*, 333–340. [[CrossRef](#)]
48. Fernández-Bedmar, Z.; Demyda-Peyrás, S.; Merinas-Amo, T.; Del Río-Celestino, M. Nutraceutical Potential of Two *Allium* Species and Their Distinctive Organosulfur Compounds: A Multi-Assay Evaluation. *Foods* **2019**, *8*, 222. [[CrossRef](#)] [[PubMed](#)]
49. Sekara, A.; Pokluda, R.; Del Vacchio, L.; Somma, S.; Caruso, G.; Agnieszka, S.; Robert, P.; Del, V.L.; Silvano, S.; Gianluca, C. Interactions among genotype, environment and agronomic practices on production and quality of storage onion (*Allium cepa* L.)—A review. *Hortic. Sci.* **2017**, *44*, 21–42. [[CrossRef](#)]
50. Bernhard, R.A. Comparative Distribution of Volatile Aliphatic Disulfides Derived from Fresh and Dehydrated Onions. *J. Food Sci.* **1968**, *33*, 298–304. [[CrossRef](#)]
51. Järvenpää, E.P.; Zhang, Z.; Huopalahti, R.; King, J.W. Determination of fresh onion (*Allium cepa* L.) volatiles by solid phase microextraction combined with gas chromatography-mass spectrometry. *Z. Lebensm. Forsch. A* **1998**, *207*, 39–43. [[CrossRef](#)]
52. Løkke, M.M.; Edelenbos, M.; Larsen, E.; Feilberg, A. Investigation of Volatiles Emitted from Freshly Cut Onions (*Allium cepa* L.) by Real Time Proton-Transfer Reaction-Mass Spectrometry (PTR-MS). *Sensors* **2012**, *12*, 16060–16076. [[CrossRef](#)]
53. Block, E.; Naganathan, S.; Putman, D.; Zhao, S.H. *Allium* chemistry: HPLC analysis of thiosulfonates from onion, garlic, wild garlic (ramsoms), leek, scallion, shallot, elephant (great-headed) garlic, chive, and Chinese chive. Uniquely high allyl to methyl ratios in some garlic samples. *J. Agric. Food Chem.* **1992**, *40*, 2418–2430. [[CrossRef](#)]
54. Berger, R. *Flavours and Fragrances, Chemistry, Bioprocessing and Sustainability*; Springer: Berlin/Heidelberg, Germany, 2007.
55. Boelens, M.; De Valois, P.J.; Wobben, H.J.; Van der Gen, A. Volatile flavor compounds from onion. *J. Agric. Food Chem.* **1971**, *19*, 984–991. [[CrossRef](#)]

56. Cecchi, L.; Ieri, F.; Vignolini, P.; Mulinacci, N.; Romani, A. Characterization of Volatile and Flavonoid Composition of Different Cuts of Dried Onion (*Allium cepa* L.) by HS-SPME-GC-MS, HS-SPME-GC×GC-TOF and HPLC-DAD. *Molecules* **2020**, *25*, 408. [[CrossRef](#)]
57. Block, E.; Shu, H.Z. Onion essential oil chemistry. Cis-and trans-2-mercapto-3,4-dimethyl 2,3-dihydrothiophene from pyrolysis of bis(1-propenyl) disulfide. *Tetrahedron Lett.* **1990**, *31*, 4999–5002. [[CrossRef](#)]
58. Eady, C.C.; Kamoi, T.; Kato, M.; Porter, N.G.; Davis, S.; Shaw, M.; Kamoi, A.; Imai, S. Silencing Onion Lachrymatory Factor Synthase Causes a Significant Change in the Sulfur Secondary Metabolite Profile. *Plant Physiol.* **2008**, *147*, 2096–2106. [[CrossRef](#)]
59. Liu, C.; Zhang, J.; Zhou, Z.; Hua, Z.; Wan, H.; Xie, Y.; Wang, Z.; Deng, L. Analysis of Volatile Compounds and Identification of Characteristic Aroma Components of *Toona sinensis* (A. Juss.) Roem. Using GC-MS and GC-O. *Food Nutr. Sci.* **2013**, *4*, 305–314. [[CrossRef](#)]
60. Petropoulos, S.A.; Ntatsi, G.; Ferreira, I.C.F.R. Long-term storage of onion and the factors that affect its quality: A critical review. *Food Rev. Int.* **2016**, *33*, 62–83. [[CrossRef](#)]
61. Sharma, K.; Assefa, A.D.; Ko, E.Y.; Lee, E.T.; Park, S.W. Quantitative analysis of flavonoids, sugars, phenylalanine and tryptophan in onion scales during storage under ambient conditions. *J. Food Sci. Technol.* **2013**, *52*, 2157–2165. [[CrossRef](#)]
62. Lesemann, D.-E. Specific Cytological Alterations in Virus-Infected Plant Cells. In *Electron Microscopy of Plant Pathogens*; Springer: Berlin/Heidelberg, Germany, 1991; pp. 147–159.
63. Elena, S.F.; Rodrigo, G. Towards an integrated molecular model of plant–virus interactions. *Curr. Opin. Virol.* **2012**, *2*, 719–724. [[CrossRef](#)]