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Characterization of Berry Aromatic Profile of cv. Trebbiano Romagnolo Grapes and Effects of Intercropping with *Salvia officinalis* L.

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Citation: Rodríguez-Declet, A.; Castro-Marín, A.; Lombini, A.; Sevindik, O.; Selli, S.; Chinnici, F.; Rombolà, A.D. Characterization of Berry Aromatic Profile of cv. Trebbiano Romagnolo Grapes and Effects of Intercropping with *Salvia officinalis* L. *Agronomy* **2022**, *12*, 344. https://doi.org/10.3390/agronomy12020344

Academic Editor: Antonios Chrysargyris

Received: 15 December 2021
Accepted: 25 January 2022
Published: 29 January 2022

Abstract: Volatile organic compounds (VOCs) are secondary metabolites responsible for the aroma of grapes and the quality of wine. Apart from genetics, agronomic practices may impact the aroma composition and the concentration of volatiles in grape berries. The possible influence of intercropping with medicinal aromatic plants (MAPs) on the VOCs in grape berries' profile has been poorly explored. Trebbiano Romagnolo is a white *Vitis vinifera* cultivar cultivated within the Italia region Emilia-Romagna. The study investigated, for the first time, the volatile organic profile of Trebbiano Romagnolo berries as well as the possible influences of intercropping with sage (*Salvia officinalis* L.) on the volatile composition of grape berries. A total of 48 free and bound aroma compounds were identified using solid phase extraction-gas chromatography-mass spectrometry (SPE-GC-MS). In the free aroma fraction, the main classes were C_6 derivatives, alcohols, and benzenes, while in the bound aroma fraction, the major classes were benzenes, C_{13} norisoprenoids, and terpenes. The results obtained in this experiment indicate that intercropping with *Salvia officinalis* may influence volatile compounds in grape berries, an interesting result in cultivars considered neutral such as Trebbiano Romagnolo, providing new insights for exploring the complexity of the terroir and the role of agroecological strategies.

Keywords: alcohols; C6 derivatives; grape aroma compounds; intercropping; terpenes; *Vitis vinifera*

1. Introduction

Volatile organic compounds (VOCs) are secondary metabolites responsible for the aroma of grapes and the quality of wine [1,2]. Depending on the cultivar, the main grape aroma compounds include terpenoids (monoterpenes, sesquiterpenes, and C_{13} norisoprenoids), shikimate pathway derivatives (volatile phenols or benzene derivatives), aliphatic C6 volatile compounds (aldehydes and alcohols), volatile thiols, and methoxypyrazines [3].

Aroma compounds are usually located both in the pulp and skin of grapes as free volatiles, which may contribute directly to odor, or in bound forms, mainly glycosides, that are non-volatile and do not contribute directly to the grape aroma [4,5]. However, the bound glycoside forms can undergo hydrolysis to odor-active forms, thereby enhancing the aromatic characteristics of grapes and wines derived from them [6]. In addition, aroma compounds are present in grape seeds [7].

The composition and concentration of grape aroma compounds largely depends on the genetics of the grapes, and each grape cultivar possesses its own distinctive secondary metabolite pattern [8].

Some classifications of grape cultivars have been proposed based on these metabolites. Terpene concentrations, for instance, have been used to categorize grapes in the following ways: (i) intensely muscat-flavored varieties with high free monoterpene concentrations; (ii) non-muscat but aromatic varieties with a medium free monoterpene concentration; and (iii) neutral varieties that do not depend upon monoterpenes for their flavor [9–11].

Apart from genetics, agronomic practices, both at the soil and the canopy level, may impact the aroma composition and the concentration of volatiles in grape berries [12,13]. Intercropping plays a main role within the agronomic practices used in sustainable agricultural systems, being able to improve the use efficiency of natural resources [14]. Intercropping with medicinal and aromatic plants (MAPs); basil, lemon balm, and sage, for example, has been recently indicated as one practice that could positively influence the accumulation of VOCs in grape berries [15].

Trebbiano Romagnolo is a white *Vitis vinifera* cultivar widely cultivated within the Italian region Emilia-Romagna (planted on 15,500 ha, 28% of the regional grapevine area), included in the protected designation of origin "Romagna" [16]. This cultivar is considered a neutral grape variety [17]; however, to the best of our knowledge, analytical data on the volatile composition of the berries are not yet available.

The aim of this study was to characterize the volatile organic profile of Trebbiano Romagnolo berries as well as the possible influences of intercropping with sage (*Salvia officinalis* L.), a MAP emitting VOCs [18], on the volatile composition of grape berries.

2. Materials and Methods

2.1. Site Description, Experimental Design, and Vineyard Management

The experiment was performed during the years 2019 and 2020, in a mature vineyard, planted in 2005, with *V. vinifera* L. cv. Trebbiano Romagnolo. The vineyard was located in Sant'Agata sul Santerno, Ravenna, Italy (44°26′11″ N, 11°52′04″ E, 11 m a.s.l.), on flat land, 50 m from the Santerno River, with a south/north row orientation. Vines were spaced 1.5 m within the row and 3.5 m between rows (planting density = 1900 plants/ha) and trained to a bilateral guyot system. The soil was clay loam, alkaline, with 2% of organic matter.

The experiment included two treatments: (i) control with spontaneous vegetation and (ii) intercropping with *Salvia officinalis* L. Treatments were established at the beginning of July 2019 and maintained until December 2020. A completely randomized experimental design, consisting of three plots (replicates) for each treatment, was adopted. Each plot consisted of three rows, with six (cv. Trebbiano Romagnolo) vines along the row. The main spontaneous plant species found in both treatments were the following: common wild oat (*Avena fatua* L.), couch grass (*Cynodon dactylon* L.), field bindweed (*Convolvulus arvensis* L.), common chicory (*Cichorium intybus* L.). Sampling was performed in four middle vines of the central row. In each intercropping plot, 2 sage plants (1 year old, obtained by seeds, selection "Giardino delle erbe di Casola Valsenio") were planted along the rows in the space between 2 grapevines with 50 cm distance, for a total of 30 sage plants per plot. After transplanting, water was supplied in the experimental plots to guarantee the establishment of sage. To control pests and diseases, the vineyard was treated using products allowed by the European Council (EC) Regulations to be used in organic agriculture (Reg (CE) 889/08). Treatments consisted mainly of copper (2019: 1.3 kg ha^{-1} year^{-1}; 2020: 1.23 kg ha^{-1} year^{-1}) and sulfur (2019: 13.55 kg ha^{-1} year^{-1}; 2020: 9.35 kg ha^{-1} year^{-1}), to control fungal pathogens (*Plasmopara viticola, Erysiphe necator*, and *Botrytis cinerea*). Soil was managed by mowing the spontaneous vegetation present in the alley (width 3.1 m) with a rotary cutter, two times (spring and summer, 2019) or once (2020) a year. The spontaneous vegetation present in the row strip (0.40 m width) was not submitted to cutting. Neither irrigation water nor fertilization was applied to the vineyard.

2.2. Climatic Conditions

Overall, the first vegetative season (2019) was characterized by average temperatures of 20.5 °C, with maximum daily temperature of 34.9 °C on 11 August 2019, and the lowest of 2.3 °C recorded on 14 April 2019. From bud burst (first week of April) to harvest (12 September 2019), the average relative humidity (RH) varied from 75% to 86%, the highest values were observed during April (98%), and the lowest were recorded in June (36%). The total rainfall from bud burst to harvest (438.2 mm) occurred mainly in the month of May.

The second vegetative season (2020) was characterized by average temperatures of 23.4 °C, with maximum daily temperature of 37.4 °C on 1 August 2020. April was characterized by low minimum temperatures, with the lowest, −2.8 °C, recorded on 3 April 2020, causing damages to 15–20% of the buds. From bud burst (end of March) to harvest (10 September 2020), the average relative humidity (RH) varied from 55% to 73%, the highest values were observed during April (95%), with the lowest value also registered in April (34%). The total rainfall from bud burst to harvest (253 mm) occurred mainly in the period of June–July.

2.3. Productive Parameters

In 2020, at harvest (10 September 2020), the number of clusters and yield per vine (kg) (Wunder Digital Dynamometer, Wunder SA-Bi S.r.l, Milan, Italy) were determined.

2.4. Sampling

In both years, at harvest 400 berries per experimental plot were randomly collected. The samples were brought on ice to the laboratory and stored at −80 °C to preserve their composition until extraction.

2.5. Grape Composition

2.5.1. Technological Parameters

The following parameters were analyzed: berry weight, expressed as g per berry, total soluble solids (TSS; °Brix, Digital Refractometer HI 96811, Hanna instruments, Milan, Italy), titratable acidity (TA; expressed as g L^{-1} of tartaric acid), and pH (Mettler Toledo pH meter, Sigma-Aldrich, Milan, Italy).

2.5.2. Volatile Compounds

For each replicate, volatile compounds were determined on 100 berries following Di Stefano [19] after some modifications [20]. Briefly, for each sample, 20 mL of methanol was added to the obtained berry peels in order to deactivate the enzymes and facilitate the extraction of compounds of interest. To the pulp, 60 mg of sodium metabisulfite was added to avoid oxidation. Since the analyses were limited to peels and pulp, the seeds were discarded. After one hour, peels and pulps were combined, and 50 mL of buffer solution pH 3.2 (2 gL^{-1} sodium metabisulfite, 5 gL^{-1} tartaric acid, and 22 mL L^{-1} NaOH 1N) was added to the mixture. Mixtures were homogenized by using an immersion blender (Ultra-Turrax, Germany) and centrifuged for 10 min at 2500 *g*. The supernatants were collected, and the procedure was performed twice, washing with an additional 40 mL pH 3.2 tartaric buffer solution. The supernatant obtained was clarified by adding 1.0 mL of a pectolytic enzyme solution (Vinozym, FCE) at 2.5 gL^{-1}, left at room temperature overnight, and subsequently frozen at −20 °C.

Before the solid phase extraction (SPE), cartridges (2-g C18 Sep Pack, Waters, Milford, MA, USA) were activated by adding 5 mL methanol and then washed with 10 mL of water. Samples added to internal standard (i.s.) (100 µL 2-octanol at 500 µg mL^{-1}) were deposed in the cartridges and washed with 20 mL of distilled water to remove acids and sugars. Free volatiles were eluted with 10 mL dichloromethane, recovered in vials containing anhydrous sodium sulfate, and concentrated to about 300 µL before analysis. Glycosylated compounds were then recovered with 6 mL methanol. The eluted fraction was dried in a rotary evaporator to eliminate methanol. The residues were dissolved in 6 mL of phosphate–citrate buffer (0.1 M Na_2HPO_4 and 50 mM citric acid: pH 5). To hydrolyze the glycosylated fraction, 2.6 mL of β-glycosidase enzyme (cytolase, 2000 U/g) was added. Samples were kept in the incubator overnight at 40 °C.

The day after, samples were centrifuged for 10 min (2500 *g*), added to i.s., and deposed on the cartridges (1-g C18 Sep Pak, Waters) previously activated with 5 mL methanol followed by 10 mL water. The glycosylated compounds were recovered with 5 mL dichloromethane and concentrated up to 200 μL before analysis.

2.6. Gas Chromatography–Mass Spectrometry (GC-MS)

Gas chromatographic analysis of volatile compounds was carried out according to Castro Marin et al. [21]. The Trace GC ultra-apparatus coupled with a Trace DSQ mass selective detector (Thermo Fisher Scientific, Milan, Italy) was equipped with a fused silica capillary column Stabilwax DA (Restek, Bellefonte, PA, USA; 30 m, 0.25 mm i.d., and 0.25 μm film thickness). The carrier gas was helium at a constant flow of 1.0 mL/min. The GC programmed temperature was: 45 °C (held for 3 min) to 100 °C (held for 1 min) at 3 °C/min, then to 240 °C (held for 10 min) at 5 °C/min. Injection was performed at 250 °C in splitless mode, and the injection volume was 1 μL. Detection was carried out by ion electron ionization (EI) mass spectrometry in full scan mode, using an ionization energy of 70 eV. The transfer line interface was set at 220 °C and the ion source at 260 °C. The mass acquisition range was *m/z* 30–400, and the scanning rate was 5.9 scans s^{-1}.

Compounds were identified by a triple criterion: (i) by comparing their mass spectra and retention time with those of authentic standards, (ii) compounds lacking standards were identified after matching their respective mass spectra with ones present in the commercial libraries NIST 08 and Wiley 7, (iii) matching the linear retention index (LRI) obtained under our conditions, with already published LRIs on comparable polar columns. The quantification of compounds was carried out from total ion current peak areas according to the internal standard method. Analyses were done in duplicate, and data were collected by means of the Xcalibur software (Thermo Fisher Scientific, Milan, Italy).

2.7. Statistical Analysis

One-way ANOVA was performed using SAS 6.04 software (SAS Institute, Cary, NC, USA) software package. All statistics were performed with significance at $p = 0.05$.

3. Results

3.1. Plant Productive Parameters

Plant yield, determined in 2020, was not influenced by intercropping with sage (2020: Control: 4.9 kg/vine, Intercropped: 5.4 kg/vine).

3.2. Technological Parameters of Grapes

Intercropping with sage did not influence technological parameters (Table 1).

Table 1. Berry weight (g) and technological parameters (total soluble solids, pH, titratable acidity) of samples at harvest for the two vintages. ns: not significant at $p \leq 0.05$.

	Berry Weight (g)		**Total Soluble Solids (°Brix)**		**pH**		**Titratable Acidity (gL^{-1})**	
Treatments	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
Control	2.31 ± 0.12	2.15 ± 0.12	19.13 ± 1.43	16.50 ± 1.07	3.34 ± 0.11	3.23 ± 0.09	7.94 ± 1.33	8.89 ± 1.73
Sage	2.18 ± 0.11	2.11 ± 0.10	18.89 ± 0.84	17.08 ± 1.17	3.36 ± 0.09	3.28 ± 0.10	7.81 ± 1.00	8.47 ± 1.60
Significance	ns	ns	ns	ns	ns	ns	ns	ns

3.3. Volatile Compounds Identified in cv. Trebbiano Romagnolo Berries

The volatile composition of the berries of cv. Trebbiano Romagnolo examined at harvest, for two years, are presented in free and glycosylated form in Tables 2–4. A total of 48 compounds were identified and quantified (Table 2).

Table 2. Free and glycosylated volatile compounds identified in berries of cv. Trebbiano Romagnolo. For each compound (both free and glycosylated forms), minimum (min.) and maximum (max.) refer to values of all replicates (n = 12), regardless of treatment and year.

LRI	**Compounds**	**Identification Method°**	**Free Compounds (μg kg^{-1} fw)**			**Glycosylated Compounds (μg kg^{-1} fw)**		
	Acids and Esters		**min.**	**max.**	**average**	**min.**	**max.**	**average**
1918	**Hexanoic acid**	Std, MS, LRI	3.57	97.09	30.93	n.d.	n.d.	-
1974	**(*E*)-2-Hexenoic acid**	Std, MS, LRI	11.80	38.32	24.63	n.d.	n.d.	-
2114	**Octanoic acid**	Std, MS, LRI	5.08	9.56	7.32 **	0.83	2.49	1.66 *
2286	**Decanoic acid**	Std, MS, LRI	2.79	14.65	8.72 *	6.35	16.18	10.01
1651	Ethyl decanoate	Std, MS, LRI	n.d.	n.d.	-	0.81	0.98	0.90 **
		Sum	**23.24**	**159.62**	**71.60**	**7.99**	**19.65**	**12.57**
	Alcohols							
1111	**3-Methyl-3-pentanol**	Std, MS, LRI	1.44	4.23	2.84 *	0.81	10.71	4.53
1168	**3-Penten-2-ol**	Std, MS, LRI	0.38	102.59	36.69	0.65	15.07	6.16
1206	**Isoamyl alcohol**	Std, MS, LRI	0.90	8.65	4.70	1.03	2.39	1.74
1250	**1-Pentanol**	Std, MS, LRI	1.83	4.70	3.19	n.d.	n.d.	-
1310	**2-Hexanol**	Std, MS, LRI	31.63	2.69	12.88	1.75	11.15	5.27
1350	**(*Z*)-2-Penten-1-ol**	MS, LRI	2.14	4.28	3.34	1.64	2.20	1.92 *
1446	**1-Octen-3-ol**	Std, MS, LRI	1.01	2.33	1.67 **	5.26	6.39	5.83 *
1479	**2-Ethylhexanol**	MS, LRI	1.54	35.01	16.49	1.06	6.47	2.86
1557	**1-Octanol**	MS, LRI	1.47	5.29	3.57	2.97	6.17	4.40
1627	(*E*)-2-Octen-1-ol	MS, LRI	n.d.	n.d.	-	0.67	1.68	1.32
1683	**1-Nonanol**	MS, LRI	0.46	6.43	3.22	1.33	4.52	2.71
		Sum	**42.80**	**176.20**	**88.59**	**17.17**	**66.75**	**36.74**
	Benzenes							
1821	**1-Phenylethanol**	Std, MS, LRI	0.02	4.55	2.60	n.d.	n.d.	-
2180	**2-Phenoxy ethanol**	Std, MS, LRI	2.23	150.98	60.64	2.96	47.14	17.42
1525	**Benzaldehyde**	Std, MS, LRI	n.d.	n.d.	-	0.70	6.22	2.97
1671	**Acetophenone**	Std, MS, LRI	0.02	3.28	1.57	0.06	0.84	0.60
1913	**Benzyl alcohol**	Std, MS, LRI	2.68	32.91	15.58	12.05	23.57	17.02
1939	**Phenethyl alcohol**	Std, MS, LRI	31.26	120.38	67.28	16.21	64.33	37.04
2401	**Benzoic acid**	Std, MS, LRI	8.45	56.30	35.42	6.91	15.87	11.20
		Sum	**44.66**	**368.40**	**183.09**	**38.89**	**157.87**	**86.25**
	C6 Derivatives							
1355	**n-Hexanol**	Std, MS, LRI	20.17	143.54	70.43	4.20	9.41	6.99
1386	**(*Z*)-3-Hexen-1-ol**	Std, MS, LRI	3.09	12.14	7.62 *	1.39	3.66	2.53 *
1409	**(*E*)-2-Hexen-1-ol**	Std, MS, LRI	30.90	106.14	71.43	0.45	1.43	0.95
1085	**Hexanal**	Std, MS, LRI	0.49	7.15	3.22	1.06	7.06	2.94
1248	**2-Hexenal**	Std, MS, LRI	51.88	209.45	145.33	0.04	2.60	1.17

Table 2. *Cont.*

LRI	Compounds	Identification Method°	Free Compounds (μg kg^{-1} fw)			Glycosylated Compounds (μg kg^{-1} fw)		
		Sum	**106.53**	**478.42**	**298.03**	**7.14**	**24.16**	**14.58**
	Norisoprenoids							
2600	**Dihydro-3-oxo-β-ionol**	MS, LRI	n.d.	n.d.	-	3.00	25.69	12.89
2610	Dihydro-β-ionone	MS, LRI	n.d.	n.d.	-	4.90	16.02	10.46 *
2642	**3-Oxo-α-ionol**	MS, LRI	n.d.	n.d.	-	11.56	45.12	28.71
2708	**3-Oxo-7,8-dihydro-α-ionol**	MS, LRI	n.d.	n.d.	-	8.57	25.69	16.80
		Sum	**-**	**-**	**-**	**28.03**	**112.52**	**68.86**
	Phenols							
2196	**Eugenol**	Std, MS, LRI	0.93	7.06	3.20	0.16	1.66	0.91 **
2342	**Isoeugenol**	Std, MS, LRI	n.d.	n.d.	-	3.39	9.21	4.44
		Sum	**0.93**	**7.06**	**3.20**	**3.55**	**10.87**	**5.35**
	Terpenes							
1549	**Linalool**	Std, MS, LRI	0.04	0.24	0.14 *	0.21	1.27	0.71
1715	**α-Terpineol**	Std, MS, LRI	0.04	0.14	0.09 *	0.33	4.53	1.99
1718	Methyl geraniate	MS, LRI	n.d.	n.d.	-	2.29	3.08	2.69 **
1748	Citral	Std, MS, LRI	n.d.	n.d.	-	0.59	1.53	1.06 **
1775	β-Citronellol	Std, MS, LRI	3.03	4.43	3.73 **	0.69	1.34	1.02 **
1791	**Isogeraniol**	Std, MS, LRI	0.52	2.55	1.42	0.61	1.70	1.06
1877	**Geraniol**	Std, MS, LRI	3.31	21.50	9.81	17.92	31.83	24.20
2315	**8-Hydroxylinalool**	Std, MS, LRI	n.d.	n.d.	-	2.11	10.87	5.93
2334	**Geranic acid**	Std, MS, LRI	7.09	21.34	14.22 *	6.76	21.46	12.76
1803	Nerol	MS, LRI	1.01	2.35	1.68 *	1.64	4.37	3.01 *
		Sum	**15.04**	**52.55**	**31.09**	**33.15**	**81.98**	**54.43**
	Vanillins							
2554	Vanillin	Std, MS, LRI	0.54	6.17	2.95	1.12	8.98	3.90
2645	**Acetovanillone**	Std, MS, LRI	0.76	7.77	3.37	4.00	9.20	6.60 *
2878	3,4,5-Trimethoxybenzyl methyl ether	MS, LRI	n.d.	n.d.	-	10.13	24.68	17.41 *
		Sum	**1.30**	**13.94**	**6.32**	**15.25**	**42.86**	**27.91**
	Miscellaneous							
1986	**2-Acetylpyrrole**	MS, LRI	n.d.	n.d.	-	1.12	3.45	2.41

Compounds in bold were identified in both years; n.d.: not detected; * only identified in vintage 2019; ** only identified in vintage 2020. °Identification method: Std = comparing mass spectra, LRI, and retention times with pure compounds; MS = by comparing mass spectra with NIST08 and Wiley 7 spectral database; LRI = matching LRI on comparable polar columns taken from the following publicly available databases: pubchem.ncbi.nih.gov; nist.gov/srd; flavornet.org/flavornet.html (accessed on 1 December 2021).

Table 3. Concentration (μg kg^{-1} fw) and percentage of free volatile organic compounds detected in cv. Trebbiano Romagnolo berries.

	2019				**2020**			
Compound	**Control**	**Sage**	**%C**	**%S**	**Control**	**Sage**	**%C**	**%S**
Acids								
Hexanoic acid	72.76 ± 23.76 ^{a}	20.46 ± 9.94 ^{b,*}	13.5	4.3	97.75 ± 9.71	102.72 ± 18.63	10.4	12.4
(*E*)-2-Hexenoic acid	22.48 ± 9.13	23.47 ± 13.54	4.2	4.9	23.87 ± 8.63	20.35 ± 3.83	2.5	2.5
Octanoic acid	n.d.	n.d.			6.09 ± 0.71	7.32 ± 3.17	0.6	0.9
Decanoic acid	6.70 ± 3.29	6.47 ± 4.08	1.2	1.4	n.d.	n.d.		
Total	**101.94 ± 36.18**	**50.4 ± 27.56**	**18.9**	**10.6**	**127.71 ± 19.05**	**130.39 ± 25.63**	**13.5**	**15.8**
Alcohols								
3-Methyl-3-pentanol	3.34 ± 0.79	2.42 ± 0.85	0.6	0.5	n.d.	n.d.		
3-Penten-2-ol	88.71 ± 13.09	73.77 ± 26.81	16.4	15.5	0.65 ± 0.06	0.56 ± 0.26	0.1	0.1
Isoamyl alcohol	1.45 ± 0.45	2.02 ± 1.61	0.3	0.4	7.86 ± 1.30 ^{a}	5.61 ± 0.31 ^{b,*}	0.8	0.7
1-Pentanol	2.46 ± 1.01	2.42 ± 1.24	0.5	0.5	4.52 ± 0.20 ^{a}	2.60 ± 0.26 ^{b,**}	0.5	0.3
2-Hexanol	27.12 ± 4.64	21.70 ± 8.19	5.0	4.6	3.24 ± 0.55	3.81 ± 1.21	0.3	0.5
(*Z*)-2-Penten-1-ol	2.84 ± 1.12	2.64 ± 0.47	0.5	0.6	3.88 ± 0.41 ^{a}	2.88 ± 0.13 ^{b,*}	0.4	0.3
1-Octen-3-ol	n.d.	n.d.			1.96 ± 0.39	1.26 ± 0.36	0.2	0.2
2-Ethylhexanol	2.09 ± 0.69	1.89 ± 0.29	0.4	0.4	27.26 ± 0.65	31.41 ± 5.09	2.9	3.8
1-Octanol	1.26 ± 0.60	2.51 ± 1.31	0.2	0.5	4.72 ± 0.82	4.24 ± 1.02	0.5	0.5
1-Nonanol	n.d.	1.37 ± 1.29		0.3	4.92 ± 1.40	5.31 ± 0.46	0.5	0.6
Total	**129.27 ± 22.39**	**110.74 ± 42.06**	**23.9**	**23.3**	**59.01 ± 5.78**	**57.68 ± 9.10**	**6.2**	**7.0**
Benzenes								
1-Phenylethanol	1.21 ± 1.81	4.55 ± 1.55	0.2	1.0	2.35 ± 0.48	2.90 ± 2.03	0.2	0.4
2-Phenoxy ethanol	0.89 ± 0.50	1.97 ± 1.46	0.2	0.4	114.56 ± 17.50	118.32 ± 46.19	12.1	14.3
Acetophenone	1.27 ± 1.01	1.43 ± 1.67	0.2	0.3	1.69 ± 0.23	1.87 ± 0.41	0.2	0.2
Benzyl alcohol	3.64 ± 0.41	4.49 ± 1.91	0.7	0.9	27.70 ± 6.64	22.04 ± 0.22	2.9	2.7
Phenethyl alcohol	46.00 ± 14.92	54.20 ± 20.41	8.5	11.4	102.99 ± 17.90 ^{a}	52.69 ± 7.88 ^{b,*}	10.9	6.4
Benzoic acid	11.59 ± 3.51	28.22 ± 14.20	2.1	5.9	35.56 ± 6.77	45.40 ± 15.42	3.8	5.5
Total	**64.60 ± 22.16**	**94.86 ± 41.20**	**12.0**	**20.0**	**284.85 ± 49.52**	**243.22 ± 72.15**	**30.2**	**29.5**
C6 Derivatives								
n-Hexanol	38.54 ± 12.00	42.85 ± 31.96	7.1	9.0	118.49 ± 24.68 ^{a}	43.22 ± 6.52 ^{b,*}	12.5	5.2
(*Z*)-3-Hexen-1-ol	8.47 ± 2.42	6.74 ± 3.01	1.6	1.4	27.62 ± 3.29	29.11 ± 6.18	2.9	3.5
(*E*)-2-Hexen-1-ol	67.90 ± 3.23	57.63 ± 18.88	12.6	12.1	81.97 ± 21.08	62.60 ± 3.13	8.7	7.6
Hexanal	1.82 ± 0.33 ^{a}	0.64 ± 0.14 ^{b,**}	0.3	0.1	5.56 ± 1.85	3.42 ± 0.42	0.6	0.4
2-Hexenal	102.63 ± 32.09	77.23 ± 24.20	19.0	16.3	191.77 ± 10.29	206.36 ± 4.38	20.3	25.0
Total	**219.36 ± 50.07**	**185.09 ± 78.19**	**40.6**	**39.0**	**425.41 ± 61.19**	**344.71 ± 20.63**	**45.1**	**41.7**
Phenols								
Eugenol	2.69 ± 0.52	1.78 ± 0.75	0.5	0.4	5.13 ± 2.02	1.99 ± 0.47	0.5	0.2
Total	**2.69 ± 0.52**	**1.78 ± 0.75**	**0.5**	**0.4**	**5.13 ± 2.02**	**1.99 ± 0.47**	**0.5**	**0.2**

Table 3. *Cont.*

	2019				2020			
Compound	**Control**	**Sage**	**%C**	**%S**	**Control**	**Sage**	**%C**	**%S**
Terpenes								
Linalool	0.12 ± 0.01	0.13 ± 0.10	0	0	n.d.	n.d.		
α-Terpineol	0.11 ± 0.00	0.09 ± 0.05	0	0	n.d.	n.d.		
β-Citronellol	n.d.	n.d.			4.03 ± 0.57	3.54 ± 0.72	0.4	0.4
Isogeraniol	0.17 ± 0.20	0.41 ± 0.36	0	0.1	2.26 ± 0.26	2.07 ± 0.21	0.2	0.3
Geraniol	8.89 ± 2.47	12.45 ± 9.10	1.6	2.6	7.24 ± 2.87	7.21 ± 5.16	0.8	0.9
Geranic acid	8.51 ± 2.16	13.23 ± 7.02	1.6	2.8	19.41 ± 2.83	26.54 ± 14.19	2.1	3.2
Nerol	1.90 ± 0.65	1.43 ± 0.55	0.4	0.3	n.d.	n.d.		
Total	**19.70 ± 5.49**	**27.74 ± 17.18**	**3.6**	**5.8**	**32.94 ± 6.53**	**39.36 ± 20.28**	**3.5**	**4.8**
Vanillins								
Vanillin	1.13 ± 0.27	0.91 ± 0.22	0.2	0.2	4.06 ± 0.68	5.17 ± 1.42	0.4	0.6
Acetovanillone	1.16 ± 0.40	3.45 ± 2.12	0.2	0.7	5.12 ± 2.42	3.14 ± 0.79	0.5	0.4
Total	**2.29 ± 0.67**	**4.36 ± 2.34**	**0.4**	**0.9**	**9.18 ± 3.1**	**8.31 ± 2.21**	**1.0**	**1.0**
Total Sum	**539.85 ± 137.48**	**474.97 ± 209.28**			**944.23 ± 147.19**	**825.66 ± 150.47**		

n.d.: not detected. In the same row, and for each vintage, different letters indicate significant differences; *, significant (p < 0.05); **, significant (p < 0.01). %C and %S indicate the percentage of the specific volatile compound to the total sum of volatile compounds in the control (C) and in the sage-intercropped (S) treatment, respectively.

Table 4. Concentration (µg kg^{-1} fw) and percentage of glycosylated volatile organic compounds detected in cv. Trebbiano Romagnolo berries.

	2019				2020			
Compound	**Control**	**Sage**	**%C**	**%S**	**Control**	**Sage**	**%C**	**%S**
Acids and Esters								
Octanoic acid	1.23 ± 0.35	1.76 ± 1.04	0.4	0.6	6.14 ± 0.41	4.91 ± 1.31	2.2	2.1
Decanoic acid	9.10 ± 2.03	11.66 ± 6.40	3.3	3.8	10.02 ± 0.65	7.96 ± 2.17	3.6	3.4
Ethyl decanoate	n.d.	n.d.			0.97 ± 0.02 ^{a}	0.83 ± 0.02 ^{b,*}	0.3	0.4
Total	**10.33 ± 2.38**	**13.42 ± 7.44**	**3.7**	**4.3**	**17.13 ± 1.08**	**13.70 ± 3.50**	**6.2**	**5.8**
Alcohols								
3-Methyl-3-pentanol	7.27 ± 2.98	6.37 ± 1.50	2.6	2.1	0.96 ± 0.19	1.01 ± 0.25	0.3	0.4
3-Penten-2-ol	11.84 ± 2.8	10.14 ± 3.19	4.3	3.3	0.66 ± 0.01 ^{b}	0.91 ± 0.10 ^{b,*}	0.2	0.4
Isoamyl alcohol	1.91 ± 0.47	1.61 ± 0.29	0.7	0.5	1.86 ± 0.40	1.51 ± 0.53	0.7	0.6
1-Pentanol	n.d.	n.d.			0.27 ± 0.04	0.26 ± 0.20	0.1	0.1
2-Hexanol	9.32 ± 2.57	8.2 ± 3.08	3.4	2.7	1.91 ± 0.05	1.99 ± 0.22	0.7	0.8
1-Octen-3-ol	5.55 ± 0.29 ^{b}	6.27 ± 0.18 ^{a,*}	2.0	2.0	9.37 ± 0.75	9.18 ± 0.96	3.4	3.9
2-Ethylhexanol	1.51 ± 0.47	1.65 ± 0.08	0.5	0.5	4.08 ± 2.06	2.67 ± 1.24	1.5	1.1

Table 4. *Cont.*

	2019				2020			
Compound	**Control**	**Sage**	**%C**	**%S**	**Control**	**Sage**	**%C**	**%S**
1-Octanol	3.38 ± 0.69	3.94 ± 0.41	1.2	1.3	5.37 ± 0.09	5.41 ± 1.03	1.9	2.3
(*E*)-2-Octen-1-ol	1.27 ± 0.31	0.96 ± 0.40	0.5	0.3	1.61 ± 0.10	1.45 ± 0.12	0.6	0.6
1-Nonanol	1.62 ± 0.29	2.12 ± 0.59	0.6	0.7	3.24 ± 0.44	3.57 ± 1.06	1.2	1.5
Total	**43.67 ± 10.87**	**41.26 ± 9.72**	**15.7**	**13.3**	**29.33 ± 4.13**	**27.96 ± 5.71**	**10.5**	**11.8**
Benzenes								
2-Phenoxy ethanol	0.15 ± 0.08	0.24 ± 0.10	0.1	0.1	29.03 ± 20.61	32.96 ± 12.29	10.4	13.9
Benzaldehyde	1.43 ± 0.23	0.95 ± 0.35	0.5	0.3	3.30 ± 0.01	4.45 ± 1.54	1.2	1.9
Acetophenone	0.11 ± 0.03	0.09 ± 0.03	0	0	0.55 ± 0.18	0.45 ± 0.34	0.2	0.2
Benzyl alcohol	16.15 ± 6.38	15.56 ± 11.33	5.8	5.0	18.54 ± 1.37 ^{a}	14.09 ± 1.26 ^{b,*}	6.7	5.9
Phenethyl alcohol	54.97 ± 12.07	49.62 ± 16.42	19.8	16.0	24.53 ± 7.16	19.40 ± 2.84	8.8	8.2
Benzoic acid	7.86 ± 1.01	10.21 ± 4.36	2.8	3.3	12.41 ± 2.36	14.32 ± 3.69	4.5	6.0
Total	**80.67 ± 19.80**	**76.67 ± 32.59**	**29.1**	**24.8**	**88.36 ± 31.69**	**85.67 ± 21.96**	**31.7**	**36.1**
C6 Compounds								
n-Hexanol	6.46 ± 2.38	5.73 ± 1.77	2.3	1.9	8.74 ± 0.95	7.48 ± 1.84	3.1	3.2
(Z)-3-Hexen-1-ol	2.46 ± 1.14	2.01 ± 0.82	0.9	0.6	4.43 ± 0.26	4.24 ± 1.04	1.6	1.8
(*E*)-2-Hexen-1-ol	1.37 ± 0.52 ^{a}	0.77 ± 0.23 ^{b,*}	0.5	0.2	1.03 ± 0.09	0.79 ± 0.46	0.4	0.3
Hexanal	1.88 ± 0.24 ^{a}	1.31 ± 0.35 ^{b,*}	0.7	0.4	6.86 ± 0.29 ^{a}	2.12 ± 0.70 ^{b,*}	2.5	0.9
2-Hexenal	0.01 ± 0.02 ^{b}	1.27 ± 0.04 ^{a,***}	0	0.4	2.40 ± 0.29 ^{a}	1.22 ± 0.36 ^{b,*}	0.9	0.5
Total	**12.18 ± 4.30**	**11.09 ± 3.21**	**4.4**	**3.6**	**23.46 ± 1.88**	**15.85 ± 4.40**	**8.4**	**6.7**
C13 Norisoprenoids								
Dihydro-3-oxo-β-ionol	4.00 ± 1.63	9.77 ± 3.38 ^{a,*}	1.4	3.2	1.22 ± 0.86	0.77 ± 0.88	0.4	0.3
Dihydro-β-ionone	9.03 ± 1.68	10.46 ± 7.86	3.3	3.4				
3-Oxo-α-ionol	36.98 ± 2.36	39.69 ± 7.68	13.3	12.8	23.60 ± 0.44 ^{a}	14.92 ± 3.54 ^{b,*}	8.5	6.3
3-Oxo-7,8-dihydro-α-ionol	13.46 ± 4.82	19.03 ± 4.53	4.9	6.2	22.64 ± 4.32	15.09 ± 6.22	8.1	6.4
Total	**63.47 ± 10.49**	**78.95 ± 23.45**	**22.9**	**25.5**	**47.46 ± 5.62**	**30.78 ± 10.64**	**17**	**13**
Phenols								
Eugenol	3.26 ± 0.67 ^{b}	4.61 ± 0.59 ^{a,*}	1.2	1.5	0.43 ± 0.24 ^{b}	1.29 ± 0.41 ^{a,*}	0.2	0.5
Isoeugenol	5.59 ± 3.14	4.99 ± 1.04	2.0	1.6	3.02 ± 0.52	2.21 ± 0.65	1.1	0.9
Total	**8.85 ± 3.81**	**9.60 ± 1.63**	**3.2**	**3.1**	**3.45 ± 0.76**	**3.50 ± 1.06**	**1.2**	**1.5**
Terpenes								
Linalool	0.40 ± 0.16	0.45 ± 0.12	0.1	0.1	1.05 ± 0.30	0.88 ± 0.15	0.4	0.4
α-Terpineol	0.61 ± 0.46	0.67 ± 0.34	0.2	0.2	3.74 ± 1.11	2.46 ± 0.68	1.3	1.0
Methyl geraniate	n.d.	n.d.			2.54 ± 0.00	2.68 ± 0.39	0.9	1.1
Citral	n.d.	n.d.			0.90 ± 0.44	1.41 ± 0.12	0.3	0.6
β-Citronellol	n.d.	n.d.			1.02 ± 0.46	0.95 ± 0.16	0.4	0.4

Table 4. *Cont.*

	2019				2020			
Compound	**Control**	**Sage**	**%C**	**%S**	**Control**	**Sage**	**%C**	**%S**
Isogeraniol	0.79 ± 0.17	1.09 ± 0.29	0.3	0.4	1.43 ± 0.32	1.04 ± 0.58	0.5	0.4
Geraniol	19.65 ± 2.02	24.12 ± 3.66	7.1	7.8	27.51 ± 6.11	24.04 ± 3.34	9.9	10.1
8-Hydroxylinalool	2.51 ± 0.52	3.27 ± 1.21	0.9	1.1	10.29 ± 0.82 ^{a}	6.14 ± 1.17 ^{b,*}	3.7	2.6
Geranic acid	8.18 ± 1.24	14.96 ± 9.20	2.9	4.8	9.18 ± 0.67	11.88 ± 3.95	3.3	5.0
Nerol	2.71 ± 1.45	2.27 ± 0.21	1.0	0.7	n.d.	n.d.		
Total	**34.85 ± 6.02**	**46.83 ± 15.03**	**12.6**	**15.1**	**57.66 ± 10.23**	**51.48 ± 10.54**	**20.7**	**21.7**
Vanillins								
Vanillin	1.61 ± 0.45	1.81 ± 0.05	0.6	0.6	8.24 ± 1.05	5.07 ± 1.42	3.0	2.1
Acetovanillone	6.03 ± 2.78	8.23 ± 0.36	2.2	2.7	n.d.	n.d.		
3,4,5-Trimethoxybenzyl methyl ether	14.46 ± 4.21	19.56 ± 7.24	5.2	6.3	n.d.	n.d.		
Total	**22.10 ± 7.44**	**29.60 ± 7.65**	**8.0**	**9.6**	**8.24 ± 1.05**	**5.07 ± 1.42**	**3.0**	**2.1**
Miscellaneous								
2-Acetylpyrrole	1.37 ± 0.28	1.96 ± 0.81	0.5	0.6	3.32 ± 0.19	2.98 ± 0.39	1.2	1.3
Total	**1.37 ± 0.28**	**1.96 ± 0.81**	**0.5**	**0.6**	**3.32 ± 0.19**	**2.98 ± 0.39**	**1.2**	**1.3**
Total Sum	**277.49 ± 65.39**	**309.38 ± 101.53**			**278.41 ± 56.63**	**236.99 ± 59.62**		

n.d.: not detected. In the same row, and for each vintage, different letters indicate significant differences; *, significant ($p < 0.05$); ***, significant ($p < 0.001$). %C and %S indicate the percentage of the specific volatile compound to the total sum of volatile compounds in the control (C) and in the sage-intercropped (S) treatment, respectively.

A variety of chemical classes, which included acids and esters, alcohols, benzenes, C_6 derivatives, C_{13} norisoprenoids, phenols, terpenes, vanillins, and others, were observed (Table 2).

Some compounds showed a wide range of variability both in free (i.e., n-hexanol, 2-hexanol, acetovanillone) and bound (i.e., 3-penten-2-ol, 3-methyl-3-pentanol, dihydro-3-oxo-β-ionol) forms (Table 2).

In the free aroma fraction, the main classes were C_6 derivatives, alcohols, and benzenes. The compounds showing the highest mean concentrations were 2-hexanal, 3-penten-2-ol, and phenethyl alcohol (Table 2).

In the bound aroma fraction, the major classes were benzenes, C_{13} norisoprenoids, and terpenes. The compounds showing the highest averaged concentrations were phenethyl alcohol, 3-oxo-α-ionol, and geraniol (Table 2).

3.4. Volatile Compounds Identified in Trebbiano Romagnolo Berries Intercropped with Salvia officinalis

Intercropping with sage modified the composition of volatile compounds (Tables 3 and 4). The classes of aroma compounds that contributed most to these changes were C_6 derivatives and alcohols (Tables 3 and 4).

Compounds that showed an increase by intercropping with sage were 3-penten-2-ol, 1-octen-3-ol, 2-hexenal, dihydro-3-oxo-β-ionol, and eugenol. Noteworthy, all these compounds were found in their bound form (Tables 3 and 4).

Intercropping induced a decrease in concentration in both free (phenethyl alcohol, n-hexanol, hexanoic acid, isoamyl alcohol, 1-pentanol, (*Z*)-2-penten-1-ol, and hexanal) and bound (3-oxo-α-ionol, benzyl alcohol, 8-hydroxylinalool, hexanal, 2-hexenal, (*E*)-2-hexen-1-ol, and ethyl decanoate) volatile compound forms (Tables 3 and 4).

4. Discussion

4.1. Volatile Profile of Berries of cv. Trebbiano Romagnolo

To the best of our knowledge, this is the first report on the characterization of the volatile composition of cv. Trebbiano Romagnolo berries, its distribution in the free or glycosylated form, and on the study of the effects of intercropping with sage on that composition. Trebbiano Romagnolo is considered a neutral grape cultivar [17]. Neutral grapes are characterized by non-varietal aromas, with monoterpenes and/or other typical grape volatile compounds occurring at a lower level than the odor threshold [10]. They generally lack any distinctive aroma but are largely dominated by C_6 compounds, aldehydes, and alcohols [2].

In our study, a total of 48 volatile organic compounds were identified and quantified in the berries (Table 2). Differences between vintages were recorded due in part to the different climatic conditions as the second vegetative season had higher mean temperatures (+2.9 °C) and much lower rainfall values (−222.9 mm) and experienced a spring frost.

Volatiles were found to be mainly in the free form, and their averaged total concentration was twofold that of the glycosylated compounds (Table 2).

The berry aromatic profiles of cv. Trebbiano Romagnolo showed some similarities to other cultivars that are considered neutral. C_6 derivatives constitute the major class of free volatiles in berries, representing up to 50% of that fraction (Table 2), as in other neutral varieties such as Ugni blanc [22], Chardonnay [23], Semillon [24], Falanghina, Coda di Volpe and Greco [25,26], Grenache [27], Monastrell and Tempranillo [28].

The main C_6 compounds were 2-hexenal, 2-hexen-1-ol, and n-hexanol (Table 2). Similar results were seen in berries of the cultivars Falanghina, Coda di Volpe and Greco [25], Ugni blanc [22], and Albillo [29].

As already found in Semillon and Chardonnay [24], benzene derivatives accounted for a further 25% of the free volatile fraction, due to the significant presence of phenethyl alcohol and 2-phenoxy ethanol (Table 2).

Among alcohols, 3-penten-2-ol was the highest (Table 2), as opposed to what was reported for other white neutral cultivars, in which 3-methyl-1-butanol was found to prevail [30].

As expected, terpenes represented slightly less than 5% of the free volatiles' fraction (Table 3), and this is in accordance with the finding of Sefton et al. [23] on Chardonnay. Notably, terpenes were not detected among the free volatiles found in the berries of cv. Ugni blanc (Trebbiano Toscano) [22]. The main terpenes detected were geranic acid, geraniol, and β-citronellol (Table 2). It is known that, in the grape metabolic pathway, β-citronellol is derived directly from geraniol after enzymic reduction and that the former may eventually generate rose oxide after cyclization [31]. Since no rose oxide was detected, it could be supposed that, in cv. Trebbiano Romagnolo, the enzymes presiding over this last reaction are not expressed. Further, the fate of geraniol could be its isomerization to nerol, which was also found at low amounts in our samples, or oxidation to the mentioned geranic acid, the main free terpene in Trebbiano Romagnolo grapes.

As a term of comparison, the main free terpenes detected in the berries of neutral cultivars were linalool, α-terpineol, and geraniol (cv. Falanghina) [25] or E-2,6-dimethylocta-2,7-diene-l,6-diol and geraniol in Chardonnay grapes [23].

In their bound form, terpenes represented one of the major classes found in berries of cv. Trebbiano Romagnolo (13–22%) (Table 4) highlighting the potential relevance of enological practices, including the use of selected yeast, aimed to promote the release of volatile compounds from their precursors [17]. Again, as found for free volatiles, geranic acid and geraniol were the main terpenes present as glycosides (Table 2).

Norisoprenoids were only detected as bound compounds (Table 2). This is in substantial accordance with other published data confirming their almost complete glycosylation in grape tissues [2,20,23,32,33]. In our samples, they were dihydro-3-oxo-β-ionol, dihydro-β-ionone, 3-oxo-α-ionol, and 3-oxo-7,8-dihydro-α-ionol and represented about 20% of the glycosylated molecules (Table 4). The C_{13} norisoprenoids are of high importance for the aroma of grapes and wines due to their very low detection thresholds. Norisoprenoids provide floral and fruity aromas in the grapes and wine [34].

Aroma depends mainly on cultivar, but it is also influenced by altitude, soil, climate, and viticulture practices [1,12,13,15].

In the case of neutral grapes, wine aroma will be expressed only through a complex array of biochemical reactions that take place during the wine-making process [35].

Terpenes and norisoprenoids are considered varietal volatiles [1,36]. The major terpenes observed in Trebbiano Romagnolo berries (Table 2) were also detected, by other authors, in Trebbiano wines [17,37] whereas norisoprenoids were not.

4.2. Berry Volatile Composition of cv. Trebbiano Romagnolo Intercropped with Salvia officinalis

Intercropping is an agroecological strategy, the application of which in viticultural systems is increasing [38]. Recent evidence demonstrates that intercropping with a mixture of three medicinal and aromatic plants (basil, lemon balm, and sage) may influence the accumulation of VOCs in berries of cv. Sangiovese [15].

In the present study, the introduction of sage did not modify the productivity parameters of the vines or the technological parameters of the berries at harvest (Table 1).

The grape and sage produce a range of volatile compounds that make up their characteristic aroma and impact their flavor. According to our GC-MS results, intercropping appeared to influence the accumulation of volatile compounds in Trebbiano Romagnolo berries, which may contribute to their aroma and flavor diversity. The concentration and the relative percentages of volatile components varied following vineyard intercropping (Tables 3 and 4).

It has been reported that grapevines are able to absorb volatile phenols contained in the smoke [39] or that the concentration levels in berries could be influenced by proximity to high volatile emitters [40]. Eucalyptus plants, which emit high amounts of 1–8 cineole, were found to influence the concentration of that volatile compound in the berries and corresponding wine [40].

Based on these studies and on the data obtained in this experiment (Tables 3 and 4), it could be hypothesized that the volatiles emitted by sage interacted with the grapevines.

Regarding alcohols and aldehydes, C_6 derivatives such as n-hexanol, (*E*)-2-hexen-1-ol, hexanal, and 2-hexenal stood out in both control and sage-intercropped grape samples. Most of these compounds are formed in plants by the degradation of fatty acids (linoleic and linolenic acid) via lipoxygenase, 13-hydroperoxide lyases, alcohol dehydrogenase, isomerization factors, and acylases [41] and the expression of these enzymes was claimed to be closely related to the degree of ripening of the berry [28]. Noteworthy, sage essential oil can inhibit the activity of lipoxygenases, key enzymes for the first steps in the conversion of fatty acids into aldehydes [42]. The lower concentration of hexanal (Tables 3 and 4) could be explained by the likely low activity of the hydroperoxide lyase or by the higher activity of alcohol dehydrogenase that converts these aldehydes to the corresponding alcohols [43]. The intercropping application had a decreasing effect on the concentration of some alcohols (isoamyl alcohol, 1-pentanol, (*Z*)-2-penten-1-ol, n-hexanol, (*E*)-2-hexen-1-ol) and an aldehyde (hexanal) (Tables 3 and 4). Aldehydes are of particular importance from the sensory point of view due to their low threshold of perception, significantly lower than that of the corresponding alcohols [2].

Among phenols, eugenol, in its bound form, was significantly increased in the berries of intercropped grapevines in both years (Table 4). Eugenol derives from ferulic acid or related metabolites [34]. It brings about a pleasant spicy aroma [2]. Eugenol has been identified in the essential oils extracted from *Salvia officinalis* [44]. The increase of eugenol in grape berries could be, in part, related to compounds possibly released in the air by sage plants.

Changes observed on eugenol (phenol), phenethyl alcohol, and benzyl alcohol (benzenes), and the reduction of the terpene linalool isomer 8-hydroxy-linalool (bound form)

(Tables 3 and 4), suggest an influence of VOCs emitted by sage on the shikimic and terpene pathways.

Norisoprenoids showed significant changes in the bound volatiles of grapes of the intercropped treatment, with dihydro-3-oxo-β-ionol increasing and 3-oxo-α-ionol decreasing (Tables 3 and 4).

In addition to intercropping, the vintage (years 2019 and 2020) also impacted both the free and the bound volatile profile of Trebbiano Romagnolo berries (Tables 3 and 4). This result displays the important effect of climatic conditions on the vulnerable volatile compounds.

5. Conclusions

To the best of our knowledge, this is the first report to characterize the volatile compounds of cv. Trebbiano Romagnolo berries. A total of 48 free and bound aroma compounds were identified using SPE-GC-MS. In the free aroma fraction, the main classes were C_6 derivatives, alcohols, and benzenes, while in the bound aroma fraction, the major classes were benzenes, C_{13} norisoprenoids, and terpenes. The data highlight the potential of enological practices aimed at promoting the release of volatile compounds from their precursors.

The results obtained in this experiment indicate that intercropping with *Salvia officinalis* may influence volatile compounds in grape berries, a result deserving further attention in cultivars considered to be neutral such as Trebbiano Romagnolo, providing new insights for exploring the complexity of the terroir and the role of agroecological strategies.

Author Contributions: Conceptualization, A.D.R.; methodology, A.D.R. and F.C.; investigation, A.R.-D., A.C.-M. and A.L., writing—original draft preparation, A.R.-D. and A.L.; writing—review and editing, A.R.-D., A.L., O.S., F.C. and A.D.R.; supervision, S.S., F.C. and A.D.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The authors declare that the data supporting this study are available from the corresponding author on reasonable request.

Acknowledgments: The authors gratefully acknowledge the farm Podere Casetta for hosting the experiment and the precious cooperation and the students Parya Soltanmahi, Andrea Tazzari and Chiara Barchiesi for their valuable contribution in the field and laboratory analysis.

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

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