



Article

A Deep Analytical Investigation of the Aroma Chemistry of Incrocio Bruni 54 and Its Differentiation from Italian White Varieties

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Abstract

Incrocio Bruni 54 is a little-known white grape variety developed in the Marche region (Italy) from a cross between Verdicchio and Sauvignon Blanc to combine aromatic freshness with structure. In light of the growing interest in minor and autochthonous cultivars, this study provides the first comprehensive chemical characterization of the aroma profile of Incrocio Bruni 54 wines. Seventeen commercial wines were analyzed for varietal compounds, such as terpenes, norisoprenoids, volatile thiols, methyl salicylate and its glycosides, and fermentative compounds, including esters, alcohols, acids, phenols, aldehydes, and ketones, using GC-MS/MS and LC-MS/MS. Odor activity value (OAV) calculations revealed an aroma profile dominated by ethyl esters, such as ethyl caproate and isopentyl acetate, β -damascenone, 4-vinylguaiacol, TDN, and the volatile thiols 3MH and 4MMP, imparting fruity, floral, spicy, and tropical notes. Comparison with datasets of 246 Italian monovarietal white wines and related sub-datasets composed of Verdicchio and Lugana showed significantly higher concentrations of 3MH and free methyl salicylate in Incrocio Bruni 54, but markedly lower levels of glycosylated methyl salicylate forms, suggesting a greater expression of this odorant in young wines balanced by a lower potential over aging. These findings highlight the distinctive aromatic fingerprint of Incrocio Bruni 54, combining parental traits with unique sensory potential, and support its knowledge and valorization in wine production.

Keywords: volatile organic compounds; methyl salicylate glycosides; thiols; volatile carbonyl compounds



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1. Introduction

Incrocio Bruni 54 (*Vitis vinifera* L. cv. Incrocio Bruni 54) is a little-known white grape variety developed in Italy in the early 20th century. Created in 1936 by Professor Bruno Bruni, this grape is a cross between Verdicchio, a native Italian variety, and Sauvignon Blanc, the renowned French grape [1]. The breeding objective was to combine the aromatic intensity and freshness of Sauvignon Blanc with the structure and regional characteristics of Verdicchio. Despite its promising enological potential, due to the excellent structure and distinctive sensorial profile of its wine, which differs from that of both its parents, this cultivar has received limited attention in the scientific literature and remains relatively understudied compared to other Italian hybrid varieties. In recent years, there has been a

renewed interest in minor and autochthonous grape varieties, driven by the wine industry's growing focus on biodiversity, sustainability, and the enhancement in regional identity [1,2].

A crucial step in the valorization of a grape variety dedicated for winemaking is the in-depth characterization of the aromatic profile of its monovarietal wines. Wine aroma results from a complex matrix of volatile organic compounds (VOCs), including varietal metabolites such as terpenes, norisoprenoids, and volatile thiols, as well as fermentation-derived compounds, such as higher alcohols, esters, and carbonyls. Additionally, bioactive compounds such as methyl salicylate (MeSA), found both in free and glycosylated forms, have attracted increasing interest. In plants, methyl salicylate glycosides are synthesized as storage and transport forms of salicylic acid derivatives, playing a role in defense mechanisms and signaling pathways. From a sensory viewpoint, MeSA is associated with minty and wintergreen-like notes, which can influence the aroma profile of wines. During winemaking, enzymatic or acid hydrolysis may release the aglycone from its glycosylated precursor, thereby contributing to the volatile composition of the final product. These characteristics make methyl salicylate glycosides promising markers for varietal differentiation [3]. Among the varietal compounds, volatile thiols are particularly noteworthy due to their low odor thresholds and their ability to impart intense tropical, citrus, or herbaceous notes. Although present in grapes as non-volatile precursors, these are released during alcoholic fermentation, primarily through yeast metabolism. Their expression in wine is highly dependent on grape genotype, fermentation conditions, and yeast strain selection [4]. Similarly, carbonyl compounds, including aldehydes and ketones, can contribute to both desirable and undesirable aroma nuances depending on their nature and concentration, and can serve as indicators of oxidation or microbial activity. Methyl salicylate glucoside, on the other hand, has been linked to plant stress responses and is of interest due to its potential sensory contribution and role in varietal aroma precursors [5], as already observed in Verdicchio and its genetical related Lugana [6]. Previous studies have reported notable differences among these varieties in the balance between free methyl salicylate and its glycosylated forms, as well as in the abundance of certain varietal thiols [7,8]. Such comparisons can provide valuable insights into the genetic and metabolic determinants underlying the aromatic profile of Incrocio Bruni 54.

This study aims to provide a comprehensive chemical characterization of wines produced from Incrocio Bruni 54, focusing on the quantification and profiling of volatile compounds, varietal thiols, carbonyl compounds, and methyl salicylate glucoside. This integrated analytical approach seeks to explore the aromatic complexity of this underexplored grape variety, contributing to its oenological evaluation and potential valorization within modern wine production strategies.

2. Materials and Methods

2.1. Solvents and Standards

All solvents for GC analysis (MS grade) were purchased from Merck KGaA (Darmstadt, Germany). Plastic syringes and the 0.22 µm cartridge filter were supplied by Millex-GV (Millipore, Tullagreen, Ireland).

For the analysis of major aroma compounds, all the standards were purchased at the highest purity available; ethanol 99.8%, n-heptanol 99.9%, dichloromethane 99.8%, and methanol for HPLC 99.9% were purchased from Sigma-Aldrich (St. Louis, MO, USA); and cartridges with 200 mg of stationary phase based on styrene divinylbenzene Isolute® ENV+ (Biotage, Uppsala, Sweden) were used for solid-phase extraction (SPE).

For the analysis of thiols, 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexanol (3MH), 3-mercaptohexyl acetate (3-MHA), and the internal standards (4-Methoxy- α -toluenethiol) were all bought from Merck KGaA (Darmstadt, Germany) at the highest

purity available ($\geq 95\%$). Also, the derivatizing agent (Ebselen) was purchased from Merck KGaA, Darmstadt, Germany.

For the analysis of volatile carbonyl compounds, linear aldehydes (propanal, butanal, pentanal, hexanal, heptanal, octanal, nonanal, methional), E-2-unsaturated aldehydes (2-propenal, E-2-butenal, E-2-pentenal, E-2-hexenal, E-2-heptenal, E-2-octenal, E-2-nonenal, E-2-decenal), Strecker aldehydes (2-methylpropanal, 2-methylbutanal, 2-methylpentanal, 3-methyl-2-butenal, 3-methylbutanal, Benzaldehyde, Phenylacetaldehyde), ketones (2-butanone, 3-methyl-2-butanone, 2-pentanone, 3-pentanone, 3-penten-2-one, 2-hexanone, 3-hexanone, 2-methyl-3-pentanone, 2-cyclohexen-1-one, 2-heptanone, 4-heptanone, 2-octanone, 6-methyl-5-hepten-2-one, 2-nonanone, 2-decanone, 2-undecanone), and furans (2-furfural, 5-methyl-2-furfural) were purchased from Merck KGaA (Darmstadt, Germany). 3-methylthio-2-butanone, 4-(methylthio)-2-butanone, 4-methyl-2-pentanone, and 4-methyl-4-methylthio-2-pentanone came from abcr GmbH (Karlsruhe, Germany). All standards were purchased at the highest purity available. A 1 g/L ethanol solution of every compound was freshly prepared, and various mixtures of all analytes were prepared at lower concentrations (10, 1, 0.1, and 0.01 mg/L) to allow every operation related to method optimization, calibration, and validation to be performed. A separate mixture of internal standards (acetone d6, 4-methyl-3-penten-2-one d10, octanal d16, and 4-fluorobenzaldehyde) was prepared in ethanol at 25 mg/L. The derivatizing solution was prepared at 40 g/L daily by dissolving solid O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) in water. SPME fibers (65 μm , bonded PDMS/DVB) came from Supelco/Merck KGaA (Darmstadt, Germany).

For the analysis of methyl salicylate glycosides, methyl salicylate 2-O- β -d-glucoside (MeSAG) was purchased from Sigma Aldrich, whereas Methyl salicylate 2-O- β -d-xylopyranosyl (1-6)-d-glucopyranoside (MeSA-primeveroside or Gaultherin) was supplied from iChemical Technology (Shanghai, China).

2.2. Samples

A set of 3 bottles of Incrocio Bruni 54 wines from the 2019 or 2018 harvest was collected directly from the producers within the commercial bottles. All wines were produced in the Marche region (Italy) and were kindly provided as gifts for research purposes only. The wineries sampled represented the entirety of current winemakers of Incrocio Bruni 54. Details are reported in Table 1. In addition, a commercial white wine (Tavernello bianco) was treated with Geosorb (Laffort, Bordeaux, France) at 100 g/L for volatile compound removal and used as a matrix for calibration curve acquisition. The bottles were stored in a cellar at 4 °C until analysis and opened on the same day under an inert atmosphere inside a sealed hood provided by Captair Pyramid, fed with a continuous stream of nitrogen to ensure the absence of oxygen. Calibration was carried out using 20 concentration levels for VOCs, 18 levels for thiols, and 11 levels for carbonyl compounds.

Table 1. Detailed list of Incrocio Bruni 54 wines analyzed.

| Producer | Sample | Vintage | Closure | Altitude | Province, Country |
|--------------------|--------|---------|-----------|----------|----------------------|
| I 3 filari | IB_1 | 2019 | Cork | 200 m | Macerata, Italy |
| Cantina Polenta | IB_2 | 2019 | Synthetic | 130 m | Ancona, Italy |
| Terracruda | IB_3 | 2019 | Synthetic | 400 m | Pesaro Urbino, Italy |
| Strologo | IB_4 | 2019 | Synthetic | 230 m | Ancona, Italy |
| Terrargillosa | IB_5 | 2019 | Synthetic | 150 m | Ascoli Piceno, Italy |
| Fontezoppa | IB_6 | 2019 | Cork | 150 m | Macerata, Italy |
| Conventino | IB_7 | 2018 | Synthetic | 270 m | Pesaro Urbino, Italy |
| La Montata | IB_8 | 2019 | Synthetic | 400 m | Pesaro Urbino, Italy |
| Bruscia | IB_9 | 2019 | Synthetic | 200 m | Pesaro Urbino, Italy |
| Villa Lazzarini | IB_10 | 2019 | Cork | 180 m | Macerata, Italy |
| Podere Santa Lucia | IB_11 | 2018 | Cork | 140 m | Ancona, Italy |

Table 1. Cont.

| Producer | Sample | Vintage | Closure | Altitude | Province, Country |
|--------------------------------|--------|---------|-----------|----------|----------------------|
| Cantina Mezzanotte | IB_12 | 2019 | Cork | 40 m | Ancona, Italy |
| Finocchi | IB_13 | 2019 | Cork | 450 m | Ancona, Italy |
| Muraro | IB_14 | 2019 | Cork | 170 m | Pesaro Urbino, Italy |
| Ca' Le Suore | IB_15 | 2019 | Synthetic | 300 m | Pesaro Urbino, Italy |
| Tenuta Santi Giacomo e Filippo | IB_16 | 2018 | Cork | 110 m | Pesaro Urbino, Italy |
| Vignamato | IB_17 | 2019 | Cork | 250 m | Ancona, Italy |

2.3. Analysis of Major Aroma Compounds

The analysis of volatile thiols was performed according to the procedure developed by our research group [3]. The solid-phase extraction (SPE) protocol was based on the extraction of 50 mL of wine fortified with 100 µL of the internal standard (n-heptanol 250 mg/L) using pre-conditioned Isolute[®] ENV+ cartridges (Biotage, Uppsala, Sweden). After a washing step (3 mL of water), the stationary phase was air-dried for 10 min and eluted with 2 mL of dichloromethane directly into the injection vials.

Instrumental analyses were carried out by GC-MS/MS using an Agilent Intuvo 9000 system for fast GC coupled with an Agilent 7010B triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) equipped with an electron ionization source operating at 70 eV. Signals were acquired in MRM mode. Further details regarding method development, sample preparation, chromatography, source tuning, and MRM transitions, and quantification are reported in the related article [3]. Data acquisition and signal processing were performed using Mass Hunter v. 10.

2.4. Analysis of Volatile Thiols

The analysis of volatile thiols (VTs) was performed according to the procedure developed by our research group [7]. The method used is based on a targeted approach involving derivatization, which plays a pivotal role in obtaining LOQs suitable for measuring VTs in fermented beverages [8]. In brief, 35 mL of wines, previously fortified with 35 µL of internal standard solution (4-methoxy- α -toluenethiol 100 µg/L) and 5 mL of acetonitrile, was extracted using 12 g of anhydrous magnesium sulfate, 4 g of sodium chloride, 1.5 g of sodium citrate dibasic sesquihydrate, and 3 g of dehydrate tribasic sodium citrate. After 10 min of stirring in an orbital shaker at 60 rpm, the samples were centrifuged (4500 rpm, 5 °C, 5 min), and 2 mL of the organic solution was derivatized by adding 150 µL of Ebselen ethanol solution (600 mg/L), making the reaction proceed for 5 min. The samples were finally filtered before injection.

Instrumental analysis was performed by LC-MS/MS using an Exion LC system provided by AB Sciex LLC (Framingham, MA, USA) with an Acquity UPLC BEH C18 (1.7 µm, 2.1 mm × 50 mm) column (Waters corporation, Milford, MA, USA) at 40 °C, coupled to an AB Sciex LLC QTRAP 6500+ (Framingham, MA, USA) operated in positive ion multiple reaction monitoring (MRM) mode. MultiQuant and Analyst from AB Sciex LLC (Framingham, MA, USA) were used for data acquisition and elaboration, respectively. Details about sample preparation, chromatographic gradient, source tuning, and MRM are reported in the related article [7].

Calibration curves were acquired by assessing the whole analytical process' spiked samples prepared using the deodorized white wine described in Section 2.2.

2.5. Analysis of Volatile Carbonyl Compounds

The analysis of volatile carbonyl compounds was performed according to the procedure developed by our research group [5]. Sample preparation was conducted by using a CTC-PAL3 autosampler whose workflow was handled using TriPlus RSH Sampling

Workflow Editor software v. 1.1.18277.858. A total of 2 mL of wine was first transferred into a 20 mL vial, then spiked with 20 μ L of 10 μ g/L internal standard solution and 100 μ L of 40 g/L PFBHA solution. After 10 min at 45 °C and 300 rpm to let the reaction take place, the headspace was extracted using a DVB/PDMS fiber for 20 min at 40 °C. Finally, the SPME fiber was moved into the injector (250 °C for 4 min) of a TSQ Quantum XLS Ultra Triple Quadrupole GC-MS/MS (Thermo Scientific, Austin, TX, USA) equipped with a 30 m \times 0.25 mm ID \times 0.25 μ m Restek Rx Sil MS w/Integra-Guard[®] column (Restek corporation, Bellefonte, PA, USA). A signal was acquired at 70 eV in multiple reaction monitoring (MRM) mode. Further details about sample preparation, time-programmed temperature chromatography, source tuning, and MRM transitions are reported in the related article [5].

Calibration curves were acquired by assessing the whole analytical process' spiked samples prepared using the deodorized white wine described in Section 2.2. Data acquisition was conducted using Xcalibur v. 4.0.27.10, whereas signal processing was performed using TraceFinder v. 5.1, both provided by Thermo Scientific (Austin, TX, USA).

2.6. Analysis of Methyl Salicylate Glycosides

The analysis of methyl salicylate (MeSA) glycosides was performed according to the procedure developed by our research group [9]. Samples were filtered at 0.22 μ m and directly injected into an LC-MS/MS system. Separation was performed at 40 °C in reverse phase with an Exion LC system provided by AB Sciex LLC (Framingham, MA, USA) using an Acquity UPLC[®] C18 HSS-T3 (1.8 μ m, 2.1 mm \times 150 mm) column from Waters (Milford, MA, USA). The signal was acquired in electrospray mode (ESI) using an AB Sciex LLC QTRAP 6500+ (Framingham, MA, USA) collecting the [M+Na⁺]-based transitions in positive ion multiple reaction monitoring (MRM) mode. MultiQuant 3.0.3 and Analyst 1.7.3 from AB Sciex LLC (Framingham, MA, USA) were used for data acquisition and elaboration, respectively. Further details about the chromatographic conditions, source tuning, and MRM transitions are reported in the related article [9].

2.7. Comparison with a Reference Dataset of Italian White Wines

Concentrations of VTs and free and bound methyl glycosides in Incrocio Bruni 54 wines were compared with data previously collected by our research group on other monovarietal Italian white wines using the same instruments and analytical methods. The comparative dataset included 246 monovarietal white wines (vintage 2019) from 18 Italian grape cultivars across 9 Italian regions, as previously published [7,9]. This dataset also contained concentrations measured for Verdicchio (11 samples from Marche) and Lugana (21 samples from Veneto), with Verdicchio being cultivated in the same geographical area and vintage as the samples investigated in this study. The comparison was performed first against the entire dataset of Italian white wines and then specifically with Verdicchio and with Lugana wines.

2.8. Statistical Analysis

All data were processed and analyzed using Microsoft Excel, MATLAB R2017a (MathWorks Inc., Natick, MA, USA), and R software (v. 4.3.1, R Foundation for Statistical Computing, Vienna, Austria) for Windows. For the comparison between Verdicchio and Lugana wines, data were first assessed using an F-test for homogeneity of variances, followed by a T-test ($\alpha = 0.05$) to determine statistically significant differences in compound concentrations.

3. Results

A total of 17 commercial wines produced from Incrocio Bruni 54 were analyzed (Table 1), originating from different wineries across four different provinces in the Marche region (Italy), located at altitudes ranging from 40 to 450 m above sea level. The wines

spanned two vintages (2018 and 2019) and were sealed with either cork or synthetic closures. This sampling strategy ensured a total representative overview of the enological expression of Incrocio Bruni 54 wines across different production conditions. The distribution of VOC classes identified in Incrocio Bruni 54 wines is shown in Figure 1.

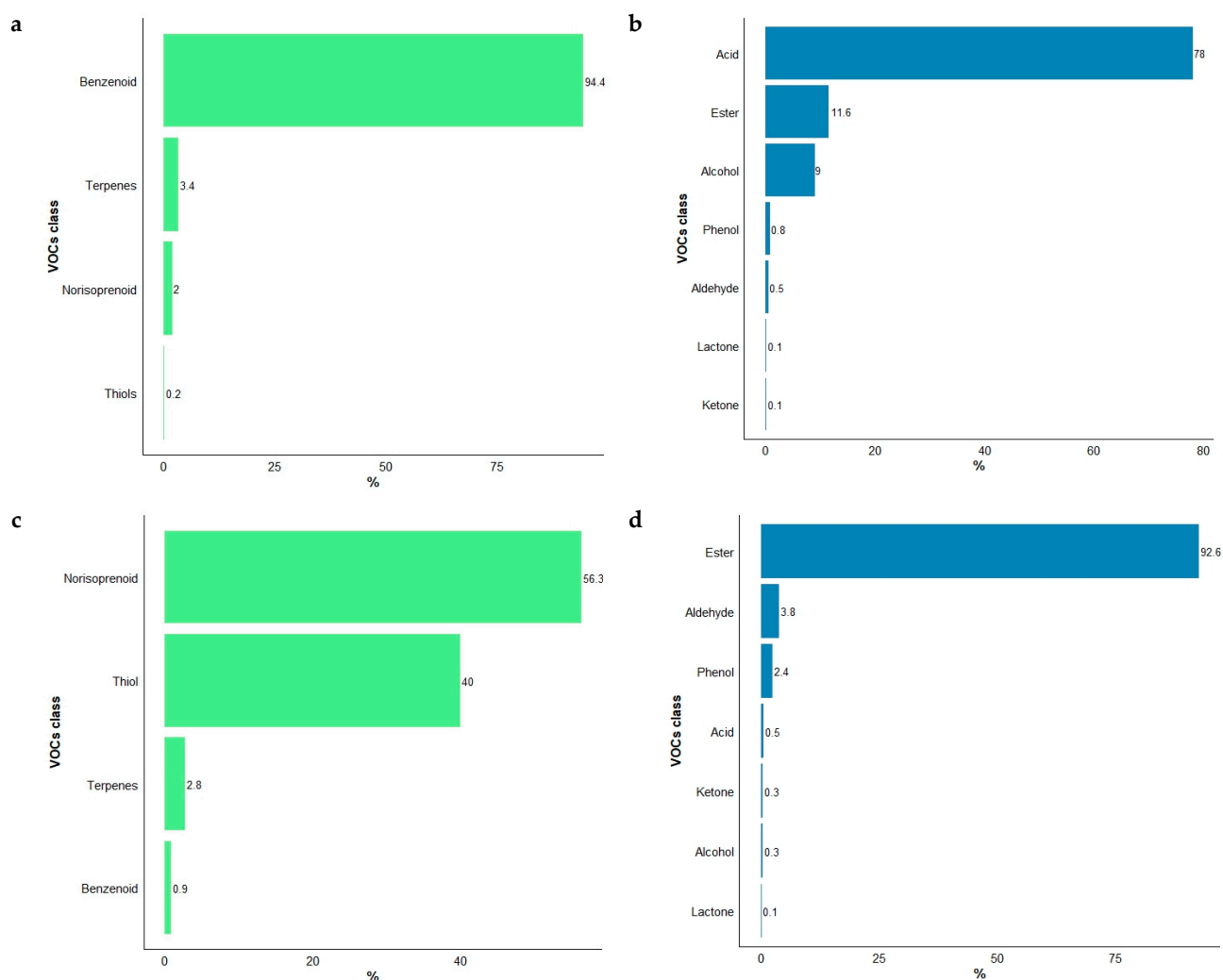


Figure 1. Relative distribution (%) of VOC classes calculated as concentrations ((a) varietal and (b) fermentative) and as OAVs ((c) varietal and (d) fermentative) identified in Incrocio Bruni 54.

Among the varietal compounds detected (Figure 1a), benzenoids accounted for the majority, representing more than 90% of the total varietal fraction. Terpenes and norisoprenoids were present in considerably lower amounts, comprising 3.4% and 2.0%, respectively. Thiols, known for their high odor activity despite their typically low concentrations, represented only 0.2% of the total fraction. Among the fermentative compounds (Figure 1b), medium-chain fatty acids were the dominant contributors, accounting for 78% of the total fermentative fraction, highlighting their central role in the post-fermentative aroma profile. Esters and alcohols, which contributed 11.6% and 9.0%, respectively, are associated with fruity and floral sensory notes, originating from yeast metabolism during alcoholic fermentation. Minor contributions, such as phenols (0.8%), aldehydes (0.5%), ketones (0.1%), and lactones (0.1%), were also observed. Although present in small amounts, these compounds may still influence the overall aroma due to their low perception thresholds or specific sensory descriptors. More than 100 VOCs were quantified in Incrocio Bruni 54 wines. For each VOC, the mean concentration, odor threshold (ODT), and odor activity value (OAV) were calculated to evaluate their potential impact on wine aroma (Table 2).

Table 2. Concentration (Mean, Min, Max), odor threshold (ODT), and odor activity value (OAV) of VOCs and methyl salicylate glycosides in Incrocio Bruni 54 wines.

| VOC | Mean | Min | Max | ODT | OAV |
|----------------------------|------|-------|------|--------------|-------|
| Acid (µg/L) | | | | | |
| Decanoic acid | 2200 | 1110 | 4240 | 10,000 [10] | 0.22 |
| Octanoic acid | 3940 | 3170 | 4930 | 3000 [10] | 1.31 |
| Valeric acid | 17.7 | 11.6 | 33.0 | 11,000 [11] | 0.001 |
| Alcohol (µg/L) | | | | | |
| 1-hexanol | 617 | 190 | 1170 | 2500 [12] | 0.08 |
| <i>cis</i> -3-hexen-1-ol | 60.4 | 24.9 | 130 | 70 [13] | 0.86 |
| <i>trans</i> -3-hexen-1-ol | 35.1 | 3.43 | 84.5 | 600 [14] | 0.06 |
| Aldehyde (µg/L) | | | | | |
| 2-furfural | 194 | 49.5 | 877 | 14,100 [10] | 0.01 |
| 2-methylpentanal | 0.10 | 0.05 | 0.39 | | |
| 2-propenal | 7.14 | 3.41 | 17.1 | | |
| 3-methyl-2-butenal | 0.59 | 0.55 | 0.69 | | |
| 5-methyl-2-furfural | 6.77 | 2.28 | 24.0 | 1100 [15] | 0.01 |
| Benzaldehyde | 8.35 | 0.01 | 107 | 2000 [16] | 0.004 |
| Butanal | 7.48 | 1.60 | 16.5 | 9.00 [17] | 0.83 |
| Decanal | 8.22 | 3.02 | 28.8 | 5.00 [18] | 1.34 |
| <i>trans</i> -2-butenal | 1.77 | 1.52 | 2.11 | | |
| <i>trans</i> -2-decenal | 2.03 | 1.97 | 2.13 | 1.00 [17] | 2.03 |
| <i>trans</i> -2-heptenal | 0.11 | 0.09 | 0.32 | 4.6 [18] | 0.02 |
| <i>trans</i> -2-hexenal | n.d. | n.d. | n.d. | 4.00 [18] | |
| <i>trans</i> -2-nonenal | n.d. | n.d. | n.d. | 0.60 [18] | |
| <i>trans</i> -2-octenal | n.d. | n.d. | n.d. | 3.00 [18] | |
| <i>trans</i> -2-pentenal | 0.19 | 0.18 | 0.23 | 1500 [19] | 0.00 |
| Heptanal | 1.28 | 0.54 | 4.10 | 3.00 [12] | 0.43 |
| Hexanal | 2.10 | 0.02 | 16.1 | 4.50 [13] | 0.42 |
| Nonanal | 7.97 | 1.39 | 69.3 | 15.0 [17] | 0.53 |
| Octanal | 1.34 | 0.76 | 5.10 | 0.70 [17] | 1.91 |
| Pentanal | 1.37 | 0.41 | 6.15 | 12.0 [20] | 0.11 |
| Propanal | 25.1 | 7.06 | 72.6 | 9.50 [20] | 2.64 |
| Undecanal | 7.69 | 3.53 | 23.5 | 5.00 [20] | 1.54 |
| Benzenoid (µg/L) | | | | | |
| Benzyl alcohol | 161 | 53.9 | 334 | 200,000 [21] | 0.00 |
| Methyl salicylate | 16.9 | 1.53 | 41.9 | 38 [9] | 0.42 |
| Ester (µg/L) | | | | | |
| Butyl acetate | 0.57 | 0.01 | 3.61 | 1800 [21] | 0.00 |
| Diethyl succinate | 2040 | 1560 | 2490 | 200 [10] | 10.2 |
| Ethyl 2-methylbutyrate | 29.5 | 10.9 | 70.6 | 18.0 [10] | 1.64 |
| Ethyl butyrate | 357 | 186 | 629 | 20.0 [10] | 17.9 |
| Ethyl caprate | 269 | 84.5 | 596 | 200 [10] | 1.35 |
| Ethyl capronate | 724 | 427 | 1290 | 14.0 [10] | 51.7 |
| Ethyl caprylate | 881 | 384 | 1450 | 5.00 [21] | 176 |
| Ethyl cinnamate | 0.55 | 0.15 | 1.48 | 1.10 [21] | 0.50 |
| Ethyl heptanoate | 0.52 | 0.33 | 0.71 | 18.0 [22] | 0.03 |
| Ethyl isovalerate | 54.4 | 25.9 | 95.8 | 3.00 [22] | 18.1 |
| Ethyl leucate | 117 | 55.8 | 209 | 400 [23] | 0.29 |
| Ethyl phenylacetate | 5.10 | 3.48 | 7.40 | 73.0 [24] | 0.07 |
| Ethyl valerate | 1.16 | 0.44 | 2.28 | | |
| Hexyl acetate | 14.3 | 0.60 | 59.1 | 1500 [10] | 0.01 |
| Isobutyl acetate | 22.2 | 9.00 | 39.2 | 1600 [21] | 0.01 |
| Isopentyl acetate | 585 | 113 | 2112 | 30.0 [10] | 19.5 |
| Phenylethyl acetate | 89.7 | 15.50 | 337 | 250 [10] | 0.36 |
| Furan (µg/L) | | | | | |
| Furfurylthiol | n.d. | n.d. | n.d. | 0.002 [25] | |
| Heterocycle (µg/L) | | | | | |
| Benzothiazole | 0.75 | n.d. | 2.11 | 50.0 [26] | 0.02 |
| Ketone (µg/L) | | | | | |
| 2-aminoacetophenone | 0.12 | 0.07 | 0.17 | 0.50 [27] | 0.23 |
| 2-butanone | 14.2 | 0.26 | 102 | 50,000 [5] | 0.00 |
| 2-cyclohexen-1-one | 0.69 | n.d. | 1.34 | | |
| 2-decanone | 0.11 | 0.09 | 0.19 | | |
| 2-heptanone | 1.67 | 0.30 | 4.42 | | |
| 2-hexanone | 0.16 | 0.11 | 0.23 | | |
| 2-methyl-3-pentanone | 0.31 | 0.24 | 0.60 | | |
| 2-nonanone | 2.02 | 1.38 | 3.32 | 5.00 [5] | 0.40 |
| 2-octanone | 0.72 | 0.68 | 0.78 | 50.0 [5] | 0.01 |
| 2-pentanone | n.d. | n.d. | 2.86 | 1.38 [15] | |
| 2-undecanone | 1.51 | 1.33 | 2.74 | 7.00 [5] | 0.22 |

Table 2. Cont.

| VOC | Mean | Min | Max | ODT | OAV |
|--|-------|------|------|-----------|------|
| 3-hexanone | n.d. | n.d. | n.d. | | |
| 3-methyl-2-butanone | 1.07 | 0.03 | 2.31 | | |
| 3-methylthio-2-butanone | 0.31 | n.d. | 0.36 | | |
| 3-pentanone | 2.54 | 1.01 | 8.45 | | |
| 4-methylthio-2-butanone | 0.10 | n.d. | 0.34 | | |
| 4-methyl-2-pentanone | 0.59 | 0.50 | 0.98 | | |
| 4-methyl-4-methylthio-2-pentan | 11.36 | 3.23 | 28.0 | | |
| 6-methyl-5-hepten-2-one | 0.09 | n.d. | 0.96 | 50.0 [5] | 0.00 |
| Lactone (µg/L) | | | | | |
| <i>cis</i> -whiskey lactone | n.d. | n.d. | n.d. | 67.0 [21] | |
| δ-decalactone | 8.48 | 4.25 | 11.6 | 386 [10] | 0.02 |
| γ-decalactone | 1.08 | 0.56 | 2.17 | 10.0 [28] | 0.11 |
| γ-dodecalactone | n.d. | n.d. | n.d. | | |
| γ-nonalactone | 2.54 | 1.18 | 4.61 | 30.0 [16] | 0.08 |
| γ-octalactone | 0.50 | n.d. | 0.97 | | |
| Menthylactone | n.d. | n.d. | n.d. | | |
| <i>trans</i> -whiskey lactone | 0.05 | n.d. | 0.51 | 67.0 [12] | 0.00 |
| Methoxypyrazine (µg/L) | | | | | |
| 2-sec-butyl-3-methoxypyrazine | n.d. | n.d. | n.d. | | |
| Norisoprenoid (µg/L) | | | | | |
| β-damascenone | 1.29 | 0.37 | 2.83 | 0.05 [21] | 25.7 |
| β-damascone | n.d. | n.d. | n.d. | | |
| β-ionone | n.d. | n.d. | n.d. | 0.03 [12] | |
| Safranal | 0.23 | 0.16 | 0.34 | | |
| TDN | 5.88 | 2.24 | 9.96 | 2.00 [29] | 2.94 |
| Phenol (µg/L) | | | | | |
| 4-ethylguaiaicol | 32.7 | n.d. | 183 | 33.0 [30] | 1.00 |
| 4-vinylguaiaicol | 66.1 | 22.9 | 164 | 10.0 [30] | 6.61 |
| Eugenol | 1.25 | 0.30 | 3.49 | 6.00 [30] | 0.21 |
| Guaiaicol | 0.36 | 0.22 | 0.87 | 10.0 [30] | 0.03 |
| Zingerone | 3.51 | 0.99 | 7.49 | | |
| Terpenes (µg/L) | | | | | |
| 1,4-cineole | 0.48 | 0.25 | 1.54 | 0.63 [31] | 0.76 |
| 1,8-cineole | 0.04 | n.d. | 0.45 | 1.10 [27] | 0.04 |
| α-terpineol | 7.01 | 2.96 | 14.5 | 250 [16] | 0.03 |
| β-citronellol | 1.02 | n.d. | 3.38 | 100 [21] | 0.01 |
| Geranic acid | 16.4 | 12.2 | 33.8 | | |
| Geraniol | 1.10 | n.d. | 3.46 | 30.0 [30] | 0.04 |
| Linalool | 3.46 | 0.86 | 10.4 | 25.2 [12] | 0.01 |
| Linalool oxide A | 3.71 | 1.12 | 10.1 | | |
| Linalool oxide B | 1.96 | 0.77 | 4.52 | | |
| Nerol | n.d. | n.d. | n.d. | 400 [32] | |
| <i>cis</i> -rose oxide | 0.07 | 0.05 | 0.16 | 0.20 [32] | 0.37 |
| <i>trans</i> -rose oxide | n.d. | n.d. | 0.02 | | |
| Terpinen-4-ol | 1.79 | 1.00 | 2.88 | 340 [33] | 0.01 |
| <i>trans</i> -terpin | 4.36 | 2.20 | 14.7 | | |
| Thiols (ng/L) | | | | | |
| Benzylmercaptan | 0.87 | n.d. | 2.00 | | |
| 3MH | 845 | n.d. | 2330 | 60.0 [34] | 14.1 |
| 3MHA | 3.60 | 0.37 | 20.6 | 4.00 [34] | 0.90 |
| 4MMP | 2.84 | 0.19 | 21.1 | 0.80 [34] | 3.54 |
| Methyl salicylate glycosides (µg/L) | | | | | |
| MeSAG | 82.3 | 10.7 | 233 | | |
| MeSA-primeveroside | 10.2 | 1.33 | 30.6 | | |
| MeSA-violutoside | 47.8 | 12.6 | 110 | | |
| MeSA-canthoside A | 6.37 | n.d. | 57.3 | | |
| MeSA-rutinoside | 33.8 | 4.41 | 87.2 | | |
| MeSA-gentiobioside | 179 | 7.08 | 599 | | |
| MSTG-A | n.d. | n.d. | n.d. | | |

TDN: 1,1,6-Trimethyl-1,2-dihydronaphthalene. 3MH: 3-mercaptohexanol. 3MHA: 3-mercaptohexyl acetate. 4MMP: 4-mercapto-4-methylpentan-2-one. MeSA: methyl salicylate. MeSAG: MeSA-gentiobioside. MSTG-A: methyl salicylate glycosides A. n.d.: not detected.

Among varietal compounds, benzenoid compounds, which are typically dominant within this fraction (Figure 1a), were limited in number. Among these, benzyl alcohol was the most abundant (Table 2), but due to its relatively high odor detection threshold, its OAV was negligible. Similarly, molecules with smaller ODTs like methyl salicylate and

terpenes such as 1,4-cineole and *cis*-rose oxide also provided a limited contribution to the overall aroma, as evidenced by their low concentrations and OAVs. On the contrary, norisoprenoids, a class of compounds derived from carotenoids, showed particularly high aromatic activity (Figure 1). The most important norisoprenoid is β -damascenone, which, with its very low threshold [20], contributed to the aroma of the wine as an enhancer of the fruity note [35]. Similarly, thiols were revealed to be key contributors to wine aroma due to their extremely low ODTs. 3-mercaptohexanol (3MH) resulted in an OAV of 14.1, while 4-mercapto-4-methylpentan-2-one (4MMP) reached an OAV of 3.54. 3-mercaptohexyl acetate (3MHA) had an OAV of 0.9 (Table 2); this subthreshold level is probably attributed to its disappearance due to hydrolysis during storage. These compounds, though trace-level, are known to contribute intensely tropical, citrus, and boxwood notes characteristic of many aromatic white wines (Figure 1c) [7].

Among fermentative compounds, the acids with the highest concentrations were octanoic and decanoic, followed by valeric acid. Octanoic acid also showed the highest OAV (1.31), followed by decanoic acid (0.22), indicating a limited but perceptible sensory contribution, often associated with goat cheesy notes (Table 2). Esters represented another important group in the aromatic profile composition of Incrocio Bruni 54 (Figure 1d). Several esters showed high concentrations and high OAVs, such as ethyl capronate (51.7), isopentyl acetate (19.5), ethyl isovalerate (18.1), ethyl butyrate (17.9), and diethyl succinate (10.2), which had a significant aromatic impact (Table 2). Alcohols, which represented approximately 9% of the total fermentative compounds (Figure 1b), were present in relatively high concentrations, particularly 1-hexanol and *cis*-3-hexen-1-ol (Table 2). However, only *cis*-3-hexen-1-ol exhibited an OAV close to 1 (0.86), suggesting a potential contribution to green and herbaceous aromatic notes. Phenolic compounds showed a moderate presence, with certain molecules exhibiting a potentially significant sensory impact due to their relatively low odor detection thresholds. The most prominent phenol was 4-vinylguaiacol, with an OAV of 6.61, indicating a strong contribution to the aroma profile, likely perceived as spicy, clove-like, or smoky notes. Aldehydes, despite accounting for only 0.5% of the fermentative compounds (Figure 1b), were the class with the highest number of detected compounds (Table 2). Despite some aldehydes being present at low concentrations, their low ODTs resulted in high OAVs. Notably, *trans*-2-decenal (2.03) emerged as the most odor-active compounds in this class, contributing fatty fried citrus notes. Other aldehydes such as propanal, undecanal, decanal, and octanal also exhibited OAVs well above 1, further enriching the complexity of the aroma profile with soapy, fruit, and waxy perceptions [17,20]. Despite the high number of compounds detected, lactones and ketones were present at low concentrations and generally exhibited negligible OAVs, suggesting a limited contribution to the overall aroma profile.

MeSA glycosides were analyzed to characterize Incrocio Bruni 54 wines (Table 2) and to compare their content with the parent “Verdicchio” and its related Lugana. Among the compounds detected, MeSA-gentiobioside (MeSAG) exhibited the highest mean concentration, with a wide concentration range, indicating high variability among the samples, followed by MeSA-Violutoside, MeSA-rutinoside, and MeSA-Primeveroside. In contrast, MeSA-Canthoside A was generally present at low levels, and MSTG-A was not detected in any of the samples. This absence is consistent with its typical occurrence being limited to other grape varieties or matrices. These glycosylated compounds may act as latent aroma precursors, especially under hydrolytic conditions during fermentation or aging [5,36].

Overall, the aroma profile of Incrocio Bruni 54 wines is shaped by a complex matrix of VOCs, in which both fermentative and varietal compounds play key sensory roles. Highly abundant as well as trace-level yet potent odorants could contribute synergistically to the perceived sensory characteristics. VOCs with OAVs higher than 3 are summarized in Figure 2.

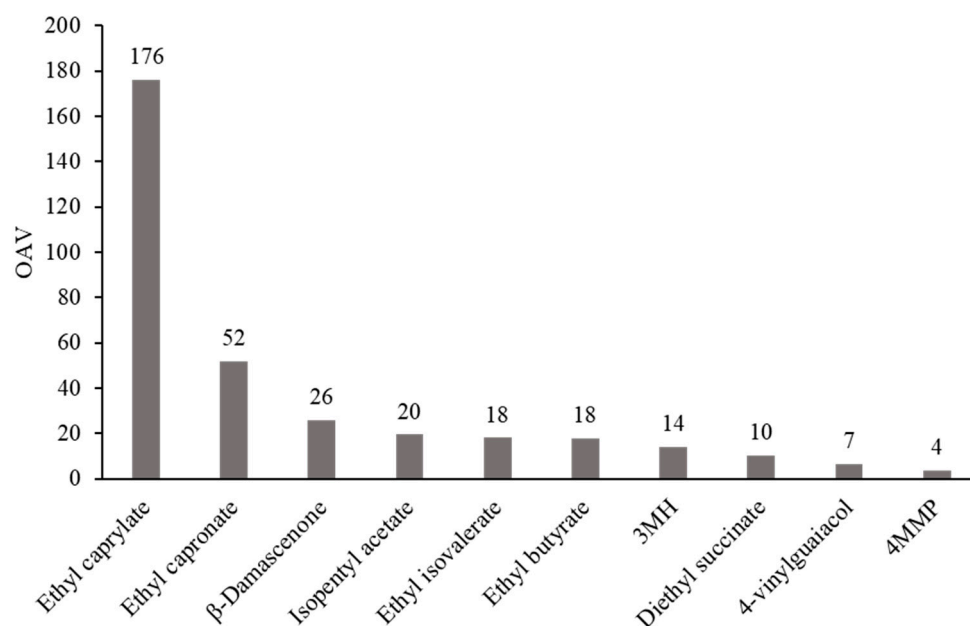


Figure 2. Selected VOCs with an OAV > 3.

Particularly notable were the roles of esters, norisoprenoids, and thiols in defining the aromatic fingerprint of this variety. Among them, ethyl caprylate and ethyl capronate exhibited the highest OAV, suggesting a dominant contribution of fruity and sweet notes, typically associated with tropical and pineapple-like aromas [10]. The third most impactful compound was β -damascenone, a norisoprenoids typically known for its potent fruity and floral notes [37], followed by esters, such as isopentyl acetate, ethyl isovalerate, and ethyl butyrate known for their intense banana and pineapple notes. 3MH and 4MMP, two potent volatile thiols, contribute grapefruit, passion fruit, and box tree-like notes. Their presence highlights the varietal aromatic potential of Incrocio Bruni 54, potentially aligning it with aromatic white grape varieties such as Verdicchio and Sauvignon Blanc [7]. Additional fruity and spicy notes are imparted by diethyl succinate and 4-vinylguaiacol.

4. Discussion

To the best of our knowledge, the results of this study provide for the first time a comprehensive overview of Incrocio Bruni 54. The samples came from a restricted geographical area and from two consecutive vintages, thus reducing the impact of external factors on the cultivar characteristics; it is worth mentioning that all winemakers producing this wine variety at the time when this study was designed have been included.

Six of the ten most relevant odor-active molecules were fermentative esters, especially ethyl esters, attributable to the samples' youth and the aromatic bouquet obtained from conventional winemaking of white wine varieties. β -damascenone and 4-vinylguaiacol are two impactful wine odorants belonging to the classes of norisoprenoids and phenols, respectively. β -damascenone in fermented beverages is formed through the acid-catalyzed hydrolyses of plant-derived apocarotenoids, in both aglycon and glycoconjugated forms [35], whereas 4-vinylguaiacol originates from the non-oxidative enzymatic decarboxylation of phenolic acids (especially ferulic acid) performed by *Saccharomyces cerevisiae* in the must [38]. Finally, two volatile thiols completed the list of the ten most impactful odorants detected in the Incrocio Bruni 54 wines; 3MH and 4MMP are sulfur-containing compounds known to provide fresh, tropical, green, and fruity nuances, and are included in the list of 35 wine aroma vectors [39]. The detectable contribution to the wine aroma of these odorants was expected considering that volatile thiols represent the most impactful

varietal compounds for Sauvignon Blanc and Verdicchio [7,40,41]. The concentration of 3MHA, which was the third analyzed thiol and represents the acetic ester of 3MH, is, on average, below the ODT, as reported in Table 2, even though some samples were strongly above the threshold. These results are in accordance with previous studies reporting that the final concentration depends on the balance between the activities of the enzymes related to esterification/hydrolysis processes, but it is also associated with the yeast strain used [42,43]. It should also be noted that these wines were analyzed two years after bottling, and consequently, the hydrolysis reaction that the acetate undergoes over time must be considered [44,45]. Within varietal compounds of parent cultivars, there exists methyl salicylate (MeSA); this molecule often stands out in the aromatic bouquet of Verdicchio and Lugana wines, but this did not happen for the analyzed Incrocio Bruni 54, as reported in Table 2 (average OAV: 0.42). Another interesting odor-active molecule above the sensory threshold was the TDN; this norisoprenoid is the molecular fingerprint of the Riesling aroma but it is also known to accumulate in white wines over time, providing pleasant scents of kerosene and hydrocarbons [29,46]. The detectable amount of TDN measured for Incrocio Bruni 54 cannot be associated with any of the originating cultivars and should be interpreted as a specific characteristic of this variety.

For comparison, the concentrations of VTs and methyl salicylate glycosides measured in Incrocio Bruni 54 wines were evaluated against a reference dataset of Italian monovarietal white wines previously characterized by our research group [7,8]. In particular, comparisons focused on Verdicchio and Lugana wines, given their genetic and geographical proximity. The results are reported in Table 3.

Table 3. Concentrations (µg/L or * ng/L) and *p*-values for the T-test performed for volatile thiols and free and bound methyl glycosides between Incrocio Bruni 54 wines and the Italian white wine dataset and its subset for Verdicchio and Lugana. Bold *p*-values indicate a value lower than α .

| Compound | Incrocio Bruni 54 | Italian Whites | | Verdicchio | | Lugana | |
|--------------------|-------------------|----------------|-----------------|------------|-----------------|--------|-----------------|
| Parameter | Conc. | Conc. | <i>p</i> -Value | Conc. | <i>p</i> -Value | Conc. | <i>p</i> -Value |
| 4MMP * | 2.84 | 2.32 | 0.3445 | 1.16 | 0.1072 | 2.45 | 0.3865 |
| 3MH * | 846 | 448 | 0.0011 | 355 | 0.0093 | 399 | 0.0114 |
| 3MHA * | 3.60 | 3.04 | 0.3247 | 1.49 | 0.0663 | 2.64 | 0.2488 |
| Methyl salicylate | 16.9 | 1.80 | 0.0000 | 5.26 | 0.0021 | 4.96 | 0.0017 |
| MeSAG | 82.3 | 108 | 0.0991 | 452 | 0.0000 | 504 | 0.0000 |
| MeSA-primeveroside | 10.2 | 9.02 | 0.3511 | 31.3 | 0.0014 | 34.5 | 0.0000 |
| MeSA-violutoside | 47.8 | 47.3 | 0.4843 | 115 | 0.0005 | 145 | 0.0000 |
| MeSA-canthoside A | 6.37 | 2.05 | 0.1341 | 4.75 | 0.3446 | 2.73 | 0.1801 |
| MeSA-rutinoside | 33.9 | 10.1 | 2.9038 | 36.8 | 0.3848 | 38.6 | 0.3189 |
| MeSA-gentiobioside | 179 | 115 | 0.1271 | 461 | 0.0262 | 561 | 0.0000 |

* ng/L. 4MMP: 4-mercapto-4-methylpentan-2-one. 3MH: 3-mercaptohexanol. 3MHA: 3-mercaptohexyl acetate. MeSA: methyl salicylate. MeSAG: MeSA-gentiobioside.

When analyzing *p*-values, immediately, Incrocio Bruni 54 emerges due to its significant richness in terms of 3MH and methyl salicylate compared to each dataset; the genetics of Sauvignon Blanc play a key role in determining 3MH abundance, whereas for methyl salicylate a relevant difference in the comparison with Verdicchio and Lugana was not expected. However, this abundance is paired with a significantly lower concentration of glycosylated forms; MeSAG, MeSA-Primeveroside, MeSA-Violutoside, MeSA-Canthoside A, and MeSA-gentiobioside were present at higher concentrations in Verdicchio and Lugana compared to Incrocio Bruni 54, suggesting a reduced capability of accumulating bound forms. Interestingly, MeSA-rutinoside did not show a significant difference in any case, even though the means of Incrocio Bruni 54, Verdicchio, and Lugana are very close to each other, and the full Italian white wine dataset was much lower; in this case, the heterogeneous distribution of the concentration values due to the presence of Verdicchio and Lugana

within the samples prevented the detection of differences. A complete overview of the three supplementary datasets is reported in Table S1.

5. Conclusions

In this study, wines from all current producers of Incrocio Bruni 54 were chemically characterized with respect to key odor-active compounds and selected MeSA glycosides with the aim of elucidating both the expressed and potential aroma profile of this little-known cultivar. The results revealed a central role for ethyl esters (ethyl caproate, isopentyl acetate, ethyl isovalerate, ethyl butyrate, and diethyl succinate), which exhibited OAVs above 3 and contributed predominantly fresh and fruity notes, in line with the recent literature. In addition to esters, compounds such as β -damascenone, 4-vinylguaiacol, and TDN were identified at relevant concentrations, while volatile thiols 3MH and 4MMP reflected the varietal imprint of the parental cultivars Verdicchio and Sauvignon Blanc.

Comparisons with a comprehensive dataset of 246 Italian monovarietal wines, and with reduced subsets of Verdicchio and Lugana, further highlighted distinctive features of *Incrocio Bruni 54*. 3MH and free MeSA concentrations were consistently higher than in all reference groups, suggesting an intrinsic propensity of this cultivar to synthesize these molecules. Conversely, MeSA glycosides were comparable to the Italian average and lower than those in Verdicchio and Lugana, indicating a reduced potential for odor-active MeSA accumulation over time. Finally, despite its genetic link to Sauvignon Blanc and an average OAV > 3, *Incrocio Bruni 54* did not accumulate higher concentrations of 4MMP relative to any of the datasets considered. A further investigation about similarities between *Incrocio Bruni 54* and Sauvignon Blanc was not possible because of the limited availability of Sauvignon Blanc samples from the Marche region that precluded direct comparisons under comparable pedoclimatic conditions.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/fermentation11100590/s1>, Table S1: Concentration (Mean, Min, Max), of thiols, free and bound methyl salicylate in the Italian varietal white wines, Verdicchio, and Lugana sample set.

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Abbreviations

The following abbreviations are used in this manuscript:

| | |
|------|--|
| IB | Incrocio Bruni 54 |
| VT | Volatile thiol |
| VOC | Volatile organic compound |
| MeSA | Methyl salicylate |
| 4MMP | 4-mercapto-4-methylpentan-2-one |
| 3MH | 3-mercaptohexanol |
| 3MHA | 3-mercaptohexyl acetate |
| TDN | 1,1,6-Trimethyl-1,2-dihydronaphthalene |
| ODT | Odor detection threshold |
| OAV | Odor activity value |

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