

Aging Effects and Grape Variety Dependence on the Content of Sulfur Volatiles in Wine

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Thirteen sulfur compounds (boiling points from 35 to 231 °C), usually considered as possible off-flavoring volatiles, were quantified by a concurrent headspace–solid phase microextraction method coupled with gas chromatography–mass spectrometry (HS-SPME/GC-MS) on 80 not off-flavoring wines of four varieties (Merlot, Marzemino, and Teroldego as red wines and Chardonnay as a white one) and of five vintages produced in the North Italian Trentino region. The results of the research, the first Italian data-bank per variety on such volatiles, allow us to make a comparison with the data of other winegrowing areas, to investigate the aging effect on the considered volatiles, and, finally, to try a variety discrimination using statistical procedures. Dimethyl sulfide, 3-(methylthio)-1-propanol, diethyl sulfide, and diethyl disulfide were found to increase with time whereas 2-mercaptoethanol and ethylmercaptan showed a decreasing trend. Furthermore, the concentration of several compounds was found to be dependent on the variety. For instance, sulfide, disulfides, benzothiazole, and thioalcohols are at higher levels in Merlot wines, whereas thiols and thioacetates are more abundant in Marzemino and Teroldego wines. Chardonnay products, well apart from the other wines, are the poorest in 3-(methylthio)-1-propanol and rather rich in dimethyl disulfide and in diethyl disulfide, mostly in the aged wines. Applying the principal component analysis to the data, it was possible to demonstrate that Chardonnay and Merlot wines are well-discriminated from the Italian native varietal wines, which on their turn are only partially distinguishable among them. A contribution of these compounds to the variety characteristics of wine is reasonable.

KEYWORDS: Sulfur volatiles; Wine; HS-SPME/GC-MS; Storage time effect; Variety effect

INTRODUCTION

Volatile sulfur compounds usually play a remarkable role on the aroma of foodstuffs and beverages, even at very low concentrations, often with typifying scents but also with off-flavors (1–5). In the fermented drinks, they were mainly investigated in beer (6–9) and wine (1, 10, 11), mostly to justify possible off-scenting resembling onion, garlic, cooked cabbage, rubber, and putrefaction, related to the presence at trace level of short-chain thiols, sulfides, disulfide, thioesters, and heterocyclic compounds. Below the sensory thresholds, summarized by Mestres et al. (10), the sulfur compounds interact with the main flavor and contribute to the wine aroma complexity (12); this statement is supported by the comparison of the wine aroma before and after a treatment with Ag salts. A positive effect of dimethyl sulfide on aroma was demonstrated by Spedding and Raut (13) and was confirmed by Segurel et al. (14); this

compound increases with aging, and its level can be influenced by the variety (15). Further, some recently identified box-tree and tropical fruit scenting long-chain polyfunctional sulfur compounds can improve the quality and support the typicality of wines of different grape varieties obtained working with particular reductive winemaking techniques and yeast strains (16–20).

Organic sulfur compounds can be generated following biological or chemical pathways, that is, enzymatic processes in yeast fermentation involving sulfates, sulfites, sulfur containing amino acids and oligopeptides (e.g., glutathione), or nonenzymatic mechanisms as chemical, photochemical, and thermal reactions during winemaking and storage (10).

By convention, the compounds usually considered off-flavor in wine are divided into “light” (bp < 90 °C) and “heavy” (bp > 90 °C) compounds (10). This difference is connected to different sampling methods for the analyses, for example, static and dynamic headspace and headspace–solid phase microextraction (HS-SPME) for the lighter species and liquid–liquid extraction for the heavier ones (10, 20–22).

In this work the content of 13 sulfur volatiles (boiling points from 35 to 231 °C) is quantified in wine using a new

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Table 1. Yeast Strains Employed for the Wine Analyzed

number	yeast strains	species and race	producer
1	Blastosel Grand Cru	<i>Saccharomyces cerevisiae</i> (r. f. <i>bayanus</i>)	Pall Filtration & Separations, Verona, Italy
2	Blastosel Kappa	<i>Saccharomyces cerevisiae</i>	Pall Filtration & Separations, Verona, Italy
3	Blastosel Terroir	<i>Saccharomyces cerevisiae</i> (r. f. <i>bayanus</i>)	Pall Filtration & Separations, Verona, Italy
4	Blastosel VS	<i>Saccharomyces cerevisiae</i> (r. f. <i>bayanus</i>)	Pall Filtration & Separations, Verona, Italy
5	CAB90	<i>Saccharomyces cerevisiae</i>	INTEC, Verona, Italy
6	Collection cepage Merlot	<i>Saccharomyces cerevisiae</i>	Gist-Brocades, Delft, The Netherlands
7	Davistart	<i>Saccharomyces cerevisiae</i>	Gist-Brocades, Delft, The Netherlands
8	EC1118	<i>Saccharomyces cerevisiae</i> (r. f. <i>bayanus</i>)	Lalvin-Lallemand, Montreal, Canada
9	Enolevure K34	<i>Saccharomyces cerevisiae</i>	INRA, Montpellier, France
10	Fermiblanco Arom	<i>Saccharomyces cerevisiae</i>	Gist-Brocades, Delft, The Netherlands
11	Fermicru LS2	<i>Saccharomyces cerevisiae</i> (r. f. <i>bayanus</i>)	Gist-Brocades, Delft, The Netherlands
12	Fermivin Cryo	<i>Saccharomyces cerevisiae</i>	Gist-Brocades, Delft, The Netherlands
13	GAR26	<i>Saccharomyces cerevisiae</i>	Lalvin-Lallemand, Montreal, Canada
14	La Claire CGC62	<i>Saccharomyces cerevisiae</i>	Pall Filtration & Separations, Verona, Italy
15	La Claire EM2	<i>Saccharomyces cerevisiae</i>	Pall Filtration & Separations, Verona, Italy
16	Premium rouge	<i>Saccharomyces cerevisiae</i>	Vason, Verona, Italy
17	Fermicru VR5	<i>Saccharomyces cerevisiae</i>	DSM Food Specialties B.V., Delft, The Netherlands

HS-SPME/GC-MS concurrent method (23), and the obtained data are submitted to statistical procedures. Possible aging and variety effects on such compounds were investigated on 80 wines of 5 vintages and of 4 varieties typical of Trentino (Northern Italy). The varieties considered were the red berry native Teroldego (T) and Marzemino (Ma), the international Merlot (M), and the white berry Chardonnay (C), treated with standard vinification conditions in the different vintages and stored at the same constant temperature. For the first time, the capability of the content of the sulfur volatiles to discriminate different wine types, differently aged, was demonstrated.

MATERIALS AND METHODS

Chemicals and Reagents. The quantified sulfur compounds were ethyl mercaptan (EtSH) [75–08–1], dimethyl sulfide (DMS) [75–18–3], diethyl sulfide (DES) [352–93–2], dimethyl disulfide (DMDS) [624–92–0], diethyl disulfide (DEDS) [110–81–6], methyl thioacetate (MTA) [1534–08–3], ethyl thioacetate (ETA) [625–60–5], 2-mercaptoethanol (ME) [60–24–2], 2-(methylthio)-1-ethanol (MTE) [5271–38–5], 3-(methylthio)-1-propanol (MTP) [505–10–2], 4-(methylthio)-1-butanol (MTB) [20582–85–8], benzothiazole (BT) [95–16–9], and 5-(2-hydroxyethyl)-4-methylthiazole (HMT) [137–00–8]. Dimethyl sulfide-*d*₆ [926–09–0], dipropyl disulfide [629–19–6], 3-(methylthio)-1-hexanol [51755–66–9], and 4-methylthiazole [693–95–8] were considered as possible internal standards (IS). All purchased standards had a purity of >98% and were supplied by Sigma-Aldrich (Milan, Italy) and

Table 2. Main Basic Data and Standard Deviation (in Brackets) for the Four Wines Analyzed

variety	alcohol concentration (% vol.)	pH	titratable acidity ^a
Teroldego	12.25 (0.69)	3.74 (0.18)	5.10 (0.84)
Marzemino	12.05 (0.47)	3.78 (0.11)	5.01 (0.32)
Chardonnay	12.23 (1.30)	3.27 (0.14)	6.91 (1.39)
Merlot	12.78 (0.80)	3.70 (0.18)	4.82 (0.57)

^a Titratable acidity is expressed in g/L of tartaric acid.

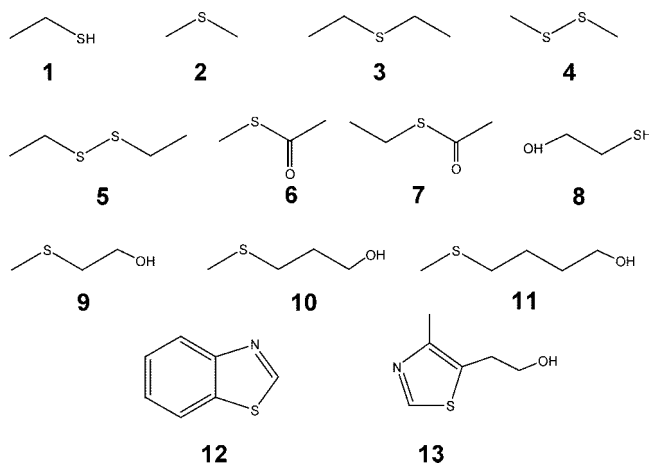


Figure 1. Sulfur compounds analyzed. 1, ethyl mercaptan; 2, dimethyl sulfide; 3, diethyl sulfide; 4, dimethyl disulfide; 5, diethyl disulfide; 6, methyl thioacetate; 7, ethyl thioacetate; 8, 2-mercaptoethanol; 9, 2-(methylthio)-1-ethanol; 10, 3-(methylthio)-1-propanol; 11, 4-(methylthio)-1-butanol; 12, benzothiazole; and 13, 5-(2-hydroxyethyl)-4-methylthiazole.

Table 3. Variability Range Indexes of the 13 Sulfur Volatiles Analyzed in the 80 Wines and Relevant Ranges in the Literature

analyte	mean (μg/L)	min (μg/L)	max (μg/L)	std. dev.	literature values (μg/L)	reference
1	1.6	0.1	10.5	1.9	0–12	(11, 33, 43)
2	26.9	2.4	78.2	18.8	0–480	(31, 32, 37, 43)
3	6.1	0.9	17.2	3.4	1–2	(31–33)
4	5.2	0.2	31.0	6.9	0–22	(11, 31)
5	3.9	2.0	13.7	2.9	0–80	(11, 31)
6	9.8	1.4	29.0	5.4	0–20	(31, 44)
7	2.6	0.6	7.1	1.4	0–56	(11, 31)
8	15.6	0.8	47.5	11.6	0–180	(21, 39)
9	23.0	3.8	61.9	15.6	0–70	(32, 40)
10	2551	862	4914	866	0–4500	(29, 45)
11	38.7	5.3	107.6	24.9	0–180	(29, 32)
12	5.2	1.0	14.1	3.5	0–14	(32, 46, 47)
13	2.6	0.9	6.1	1.1	5–50	(21)

Lancaster (Milan, Italy). Charcoal was supplied by Merck (Milan, Italy); inorganic compounds and anhydrous ethanol (>99%) were supplied by Carlo Erba (Milan, Italy).

From individual standard solutions in pure ethanol of each sulfur compound, a working ethanolic solution containing all of the analytes was prepared; all solutions were stored at –16 °C. The same procedure was followed to prepare a solution at similar concentration, containing all the ISs proposed.

HS-SPME Equipment. The SPME holder for manual sampling and the carboxen-polydimethylsiloxane-divinylbenzene (CAR-PDMS-DVB; 50/30 μm × 2 cm) fibers were purchased from Supelco (Bellefonte, PA, USA). The fibers were conditioned before the use according to the producer's instructions.

Analytical Determination of the Sulfur Compounds Considered. The analytical conditions used to prepare the calibration curves and to quantify the analytes are reported elsewhere (23).

Table 4. Mean Values and Standard Deviation per Variety of the Sulfur Volatiles Analyzed and Tukey's Test Results to Test Variety Effects*

analyte ($\mu\text{g/L}$)	Merlot		Teroldego		Marzemino		Chardonnay	
	mean	std. dev.	mean	std. dev.	mean	std. dev.	mean	std. dev.
1	0.7 b	0.35	3.2 a	2.00	1.5 b	1.05	0.8 b	0.32
2	39.9 a	17.67	22.1 b	14.83	21.9 b	14.20	23.8 b	15.62
3	7.2 ab	3.43	5.2 bc	1.95	7.5 a	4.68	4.3 c	2.05
4	9.0 a	6.96	0.7 b	0.18	1.1 b	0.49	10 a	8.35
5	4.9 a	2.40	2.2 b	0.13	2.3 b	0.22	6.0 a	4.08
6	8.5 b	3.21	13.7 a	6.57	7.6 b	5.01	9.2 b	2.94
7	2.1 n.s.	0.66	2.7 n.s.	2.03	2.7 n.s.	1.58	2.7 n.s.	1.10
8	11.5 b	6.27	20.7 a	12.91	22.3 a	12.82	8.0 b	5.61
9	44.7 a	8.90	11.5 c	3.70	24.2 b	9.40	11.4 c	7.05
10	3024 a	493	2569 b	755	2861 ab	1041	1749 c	453
11	75.1 a	20.02	28.9 b	7.27	26.0 b	7.85	24.9 b	13.58
12	9.0 a	3.29	2.9 c	2.40	3.2 c	2.14	5.5 b	1.82
13	2.3 b	0.83	2.0 b	0.56	2.4 b	1.08	3.6 a	1.16

*Values with the same letter do not differ significantly in the Tukey's test, $p < 0.05$. n.s., not significant.

HS-SPME Sampling. A 20 mL portion of wine, 100 μL of IS solution, and 5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ were put into a 30 mL vial at room temperature with a magnetic stir bar, and then the vial was sealed with a screw-top cap with a Teflon-faced septum. The solution was stirred at 500 rpm for 5 min at 35 °C. The SPME fiber (CAR-PDMS-DVB; 50/30 $\mu\text{m} \times 2$ cm) inserted into the headspace was allowed to equilibrate for 30 min and was then removed from the vial and immediately desorbed into the injector of the GC for 5 min at 250 °C.

GC-MS Analyses. The instrumental apparatus was an Autosystem XL gas chromatograph coupled with a TurboMass Gold mass spectrometer (Perkin-Elmer; Boston, MA, USA) equipped with a 30 m \times 0.32 mm I.D. \times 0.25 μm film thickness Innowax (PEG) fused-silica capillary column (Agilent Technologies; Palo Alto, CA, USA). The chromatographic analyses were carried out following the working conditions already reported and in single ion recording (SIR) mode (23).

Calibration Curves and Performance Evaluation. According to the quoted method (23), calibration curves for each analyte were prepared using the following compounds as internal standard: dimethyl sulfide- d_6 , 25 $\mu\text{g/L}$; dipropyl disulfide, 25 $\mu\text{g/L}$; 4-methylthiazole, 10 $\mu\text{g/L}$; and 3-(methylthio)-1-hexanol, 50 $\mu\text{g/L}$. The matrix used was a white wine (WW; 10% alcohol strength v/v.; <4 g/L sugar content; 5.4 g/L total acidity as tartaric acid, and pH 3.1) treated twice with charcoal (3 g/L) to remove any sulfur compounds detectable by the proposed headspace SPME/GC-MS method and other main volatile compounds, except the most polar ones such as the higher alcohols. Linearity and precision were verified in the concentration ranges typical of wines for each compound using 7 concentration levels and 5 replicate solutions per level. The detection (L_D) limit was calculated following the Hubaux-Vos method (24, 25).

Sampling Plan and Basic Quality Data. Eighty single-variety wines produced in stainless steel tanks on a semi-industrial scale from about 100 kg of grape using traditional winemaking protocols were analyzed. All grapes were vinified in the experimental winery of the IASMA Research Center (Italy). Four wines per four variety (three red-fruited, Teroldego, Marzemino, and Merlot and a white-fruited Chardonnay) and per vintage year (1998, 2001, 2002, 2003, and 2004) were sampled among the wines produced with several *Saccharomyces cerevisiae* yeast strains and drawn from different grape-growing areas of Trentino. The yeast strains employed in this work are listed in **Table 1**. They were chosen among the most commonly yeasts marketed in the relevant vintages.

The red wines were produced with a 7 day skin-contact and complete malolactic fermentation, whereas Chardonnay was produced without skin-contact and malolactic fermentation. All sterile-filtered, screw-topped bottled wines were stored at the same constant temperature of 16 °C in the dark and were analyzed in the fall of 2006. The minimal two years aging of the products should ensure the achievement of a sensorial typicality, as well as the equilibria of some reactions, in particular those among thioacetates and the relevant thiols (11, 12). No wine chosen had sulfur compounds off-flavoring at the sensory

analysis, performed by a panel of five oenologists. Furthermore, no silver or copper fining was carried out.

The main basic parameters are reported in **Table 2** for each wine type at the bottling. Similar variation ranges are present among the red wines, but they are different from those of the white products, in particular for pH and titratable acidity.

The data were statistically evaluated and plotted using STATISTICA v7.1 (Statsoft Italia S.r.l., Padova, Italy).

RESULTS AND DISCUSSION

Variation Ranges of Sulfur Volatiles. The chemical formula of the analytes are shown in **Figure 1**. The sulfur compounds level in the wines analyzed together with their usual concentration ranges are reported in **Table 3**.

Yeast strain effect coupled with different yeast assimilable nitrogen (YAN) level in musts could have a remarkable importance in different aroma compounds formation (26, 27), including some sulfur metabolites of yeast (11). To evidenciate a possible connection with the investigated sulfur volatiles, we considered the different YAN level of musts for the grape varieties taken into account (28). It was shown that Marzemino and Merlot musts have a similar mean YAN level (ca. 90 mg/L), Teroldego musts are about 50% richer, and Chardonnay musts present a content at about 180 mg/L. However, considering the data per variety in **Table 4**, no clear connection of the YAN level with the relevant mean varietal sulfur compounds profile here reported seems to be possible.

Inspection of the data gives rise to some considerations. The contents found are, in general, in the ranges of German (11, 29), French (14, 30), Spanish (31–33), Swiss (34), and Greek (35) wines, even if these belong to subgroups classified for country and wine type (22, 45). Only diethyl sulfide presents higher levels than those quantified by Mestres et al. (31) and Lopez et al. (33) in Spanish wines. The disulfides (dimethyl- and diethyl disulfide) contents are higher than those reported by several authors (11, 31–33). The 2-(methylthio)-1-ethanol level is higher than that found in Spanish and Greek wines (31, 35). Also, the benzothiazole content found by us is remarkably higher than that present in other Italian wines (47). On the other hand, the 5-(2-hydroxyethyl)-4-methylthiazole level is, on average, about $1/10$ of that found by Rapp et al. (21).

Variety Effects. To date, no evidence of the dependence of sulfur compound concentration on grape variety has been reported in the literature, with the exception of the dimethyl sulfide (14, 15) and some tropical fruit scenting thiols (16, 17), which are not considered here.

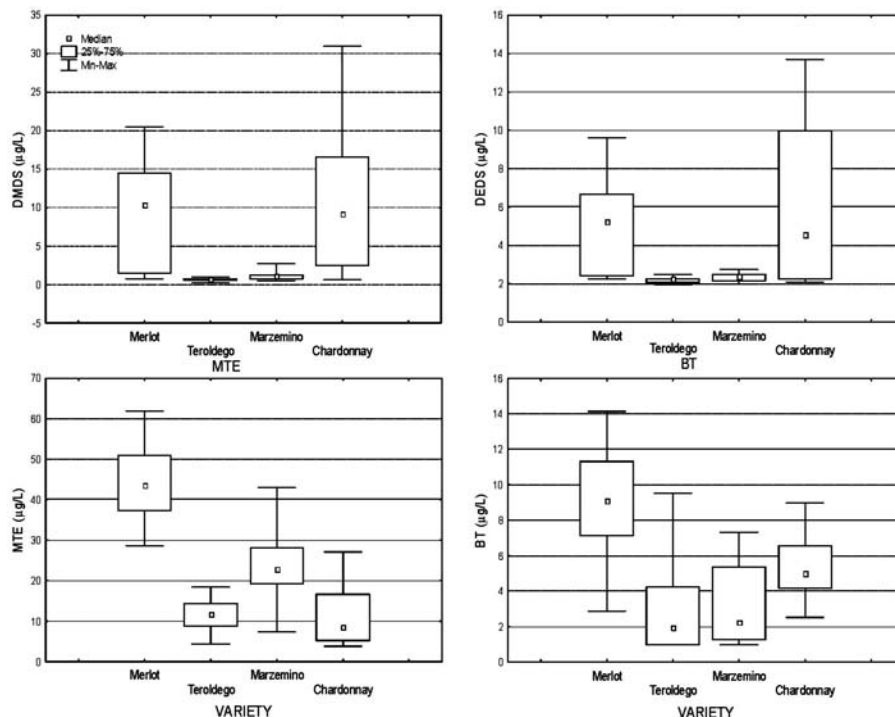


Figure 2. Box-plots of the DMDS, DEDS, MTE, and BT content in the wines analyzed.

The data in **Table 4** show that some sulfur compounds are more abundant in some wine varieties. In particular, as proved by the Tukey's test, the concentration of dimethyl sulfide and of 4-(methylthio)-1-butanol in Merlot wines is significantly higher than in all the other varieties. This supports the important cabbage/truffle-like scents due to dimethyl sulfide (15, 30) and the earthy-like scents for 4-(methylthio)-1-butanol (41), which are descriptors commonly used for the Merlot aroma. The high levels of 2-(methylthio)-1-ethanol and benzothiazole are discriminant of the Merlot variety, as well. Ethyl mercaptan and methyl thioacetate concentrations, mainly determining sulfurous/onion-like scents, if present over the sensory threshold levels, are on the average higher in Teroldego than in the other considered wines. Furthermore, an appreciable difference for dimethyl disulfide and diethyl disulfide between the groups Merlot/Chardonnay and Teroldego/Marzemino was found, with a higher content showing in the first group; the opposite occurs for the 2-mercaptoethanol level. Diethyl sulfide is tendentially higher in Merlot and Marzemino wines than in the other two wine varieties but at levels below the sensory threshold values. Merlot is again the leading variety for 3-(methylthio)-1-propanol, which is boiled potatoes-scented, whereas Chardonnay has the lowest content, as is due in a white wine (45).

For evaluating the connections of possible off-flavor with the compounds levels, we would like to recall, instead of the threshold level, the more proper preference threshold. This is the concentration limit, possibly quite different from the threshold limit, of a compound that interacts with other molecules for a positive contribution to the whole aroma. This situation is well-exemplified in the cases reported by Chatonnet (22).

The box-plots of the content distribution of dimethyl disulfide, diethyl disulfide, 2-(methylthio)-1-ethanol, and benzothiazole connected to the wine variety is the usual way to show the spreading of the data inside each wine-type (**Figure 2**). They show a remarkable difference with variety for dimethyl disulfide and diethyl disulfide, likely due to possible different interactions with the chosen substrates.

Further, the data were submitted to the principal component analysis (PCA) to reduce the dimensionality of the system and to allow a graphic representation of the scores and loading of the variables. The first two components collect 50.9% of the total variability of the system, and the biplot scattering of the scores indicates prevalent clustering according to three variety groupings, that is, Merlot (\blacktriangle), Teroldego (\blacklozenge) and Marzemino (\circ), and Chardonnay (\square) (**Figure 3**). The first principal component (PC1) distinguishes Merlot from Marzemino and Teroldego, whereas the second principal component (PC2) separates the red wines from the white ones.

The loadings plot (**Figure 4**) shows that the first component is positively related mainly with ethyl mercaptan, 2-mercaptoethanol, methyl thioacetate, and ethyl thioacetate (group α) and negatively with 3-(methylthio)-1-propanol, diethyl sulfide, 2-(methylthio)-1-ethanol, 2-(methylthio)-1-butanol, and dimethyl sulfide (group β). Also, the vintage year shows a direct correlation with the PC1. Furthermore, the loadings analysis permits us to observe an inverse correlation between ethyl mercaptan and diethyl disulfide (group γ), thus supporting a redox correlation between these compounds. The first component seems mostly connected with the aging; a younger wine is more charged by the group α variables, whereas an aged one is charged by those of group β . It also contributes to distinguish Merlot from Marzemino and Teroldego wines. The second function is positively related with most all of the variables, and distinguishes the white wines (Chardonnay) from the red ones. The α group of variables, above all represented by the classes of thiols and thioacetates, mostly model the wines of the Teroldego (\blacklozenge) variety and in part also those of the Marzemino (\circ), whereas the β group, including sulfides, disulfides, and most thioalcohols, corresponds better to the group of Merlot wines (\blacktriangle).

Merlot wines are particularly rich in such sulfur compounds, and this fact supports the possibility that this class of aroma compounds can be responsible for important sensory differences among the red-grape varietal wines as above evidenced by the Tukey test. Moreover, it appears difficult to distinguish Marzem-

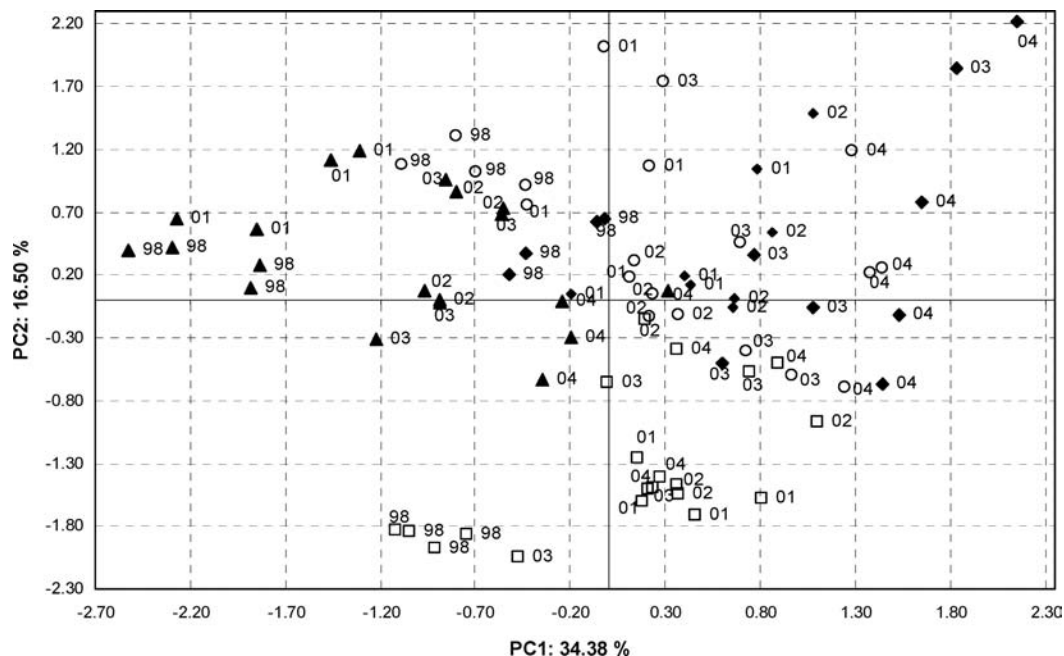


Figure 3. PCA score biplot for the wines analyzed. The varieties investigated are: Merlot (▲), Teroldego (◆), Marzemino (○), and Chardonnay (□). The number indicated represents the vintage year.

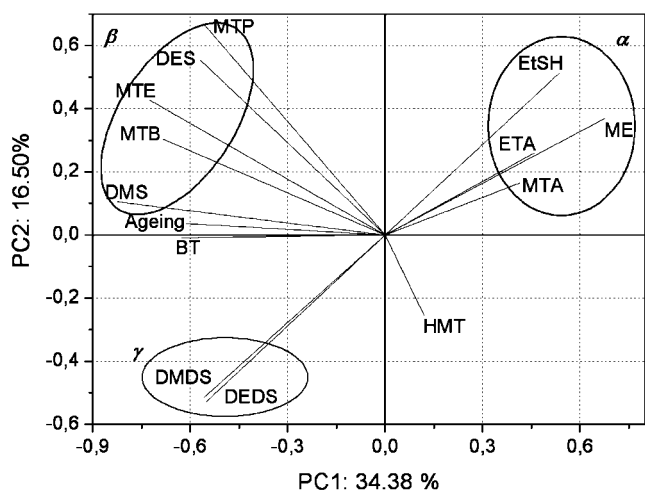


Figure 4. PCA biplot of the loadings for the wine analyzed. α : EtSH, ME, MTA and ETA; β : DMS, DES, MTE, MTP and MTB; γ : DMDS and DEDS.

ino from Teroldego wines because both are characterized by a remarkable variability of the level of thiols and thioacetates. In any case, most scores of Teroldego are better located in the space described by the variables of the α group than those referred to Marzemino wines. These experimental results represent rather well the practical sensory situation by which it is often quite difficult to distinguish such wine-types, both well distinguishable from Merlot wines.

To evaluate a difference more linked to the variety, we eliminated the temporal correlation of wines performing a centering of each variable for each variety. In particular, we shifted every cluster of scores connected to a particular vintage for a quantity resulting from the difference between the total mean and the mean of each vintage for each sulfured variable and for each variety. Repeating PCA treatment, we obtained the biplot representation of **Figure 5**. The variance explained by the first two functions increased to about 62%. We recognize better the great difference existing among the scores of Merlot,

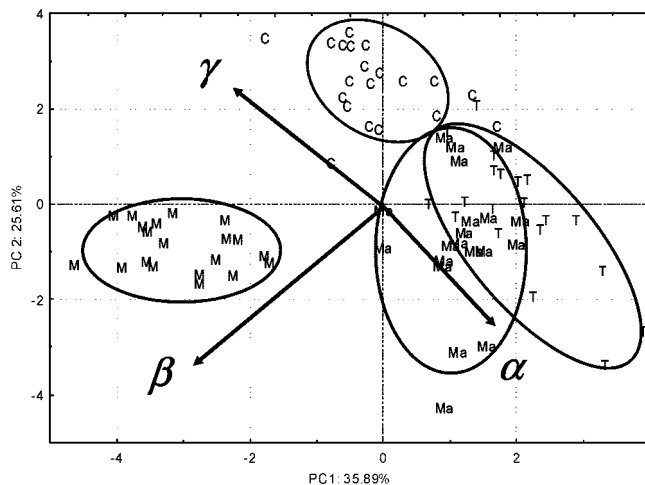


Figure 5. PCA biplot of the mean loadings and scores for the wine analyzed after values centering to eliminate vintage effect. C, Chardonnay; M, Merlot; Ma, Marzemino; T, Teroldego; α : EtSH, ME, MTA, and ETA; β : DMS, DES, MTE, MTP, and MTB; γ : DMDS and DEDS.

Chardonnay, and Marzemino plus Teroldego, these last ones resulting as two partially overlapped groups.

Because of the different utilization frequency per vintage and variety of the chosen yeasts and the rather large number of sulfur compound considered, a possible “yeast effect” on the wine aroma profile can be investigated examining the biplot of the scores labeled according to the relevant inoculated yeast (**Figure 6**). The scores of the most used strains such as, the 6 for Chardonnay, 8 for Teroldego and Marzemino, and 12 for Merlot, are spread over the whole space connected to the relevant variety (see **Figure 6**). Further, the scores related to the same yeast, but belonging to a different wine variety, are well discriminate from each other. This evidence supports the absence of yeast effects, because the variety clusters obtained in this PCA are modeled only by the content of sulfur compounds.

Storage Time Effects. To recognize possible aging and variety effects on each compound, we applied the Tukey test,

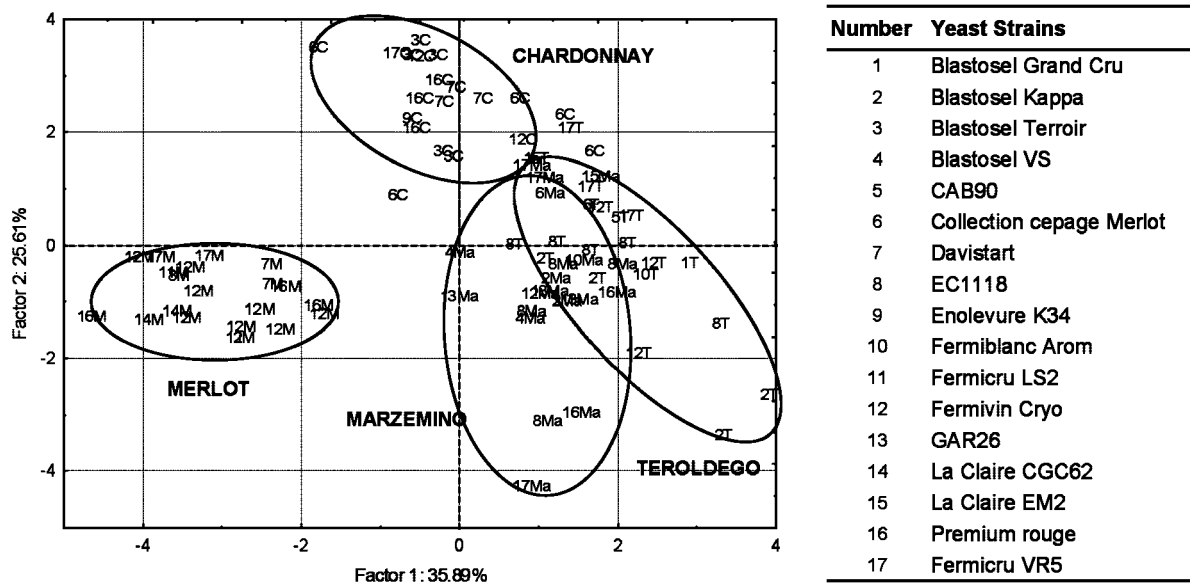


Figure 6. PCA biplot of the scores for the wine analyzed, after values centering to eliminate vintage effect, with the yeast strains employed.

Table 5. Mean Values and Standard Deviation per Vintage of the Sulfur Volatiles Analyzed and Tukey's Test Results to Test Aging Effects*

analyte (µg/L)	1998		2001		2002		2003		2004	
	mean	std. dev.	mean	std. dev.	mean	std. dev.	mean	std. dev.	mean	std. dev.
1	0.7 b	0.26	1.5 ab	0.77	1.5 ab	0.48	1.8 ab	1.09	2.5 a	1.74
2	53.4 a	9.42	35.6 b	8.86	22.5 c	7.83	15.1 d	6.61	8.0 c	5.77
3	8.9 a	2.55	7.4 ab	2.26	4.9 bc	1.86	5.7 bc	2.38	3.5 c	1.79
4	8.7 a	8.23	4.0 ab	3.62	3.5 b	3.27	5.3 ab	4.33	4.5 ab	3.60
5	6.0 a	4.22	3.4 b	2.19	3.5 b	2.12	3.7 b	2.81	2.8 b	1.15
6	8.2 n.s.	3.14	8.3 n.s.	3.55	10.4 n.s.	3.19	10.3 n.s.	4.82	11.6 n.s.	4.55
7	1.8 b	0.64	2.3 ab	0.82	2.8 ab	1.44	2.7 ab	1.49	3.2 a	2.09
8	4.8 d	2.39	10.9 c	8.30	13.8 c	4.36	21.0 b	12.04	27.7 a	11.24
9	26.4 a	14.14	25.5 ab	18.00	20.8 ab	15.58	23.2 ab	14.90	18.9 b	13.04
10	3386 a	840.5	2807 b	881.4	2543 bc	588.2	2170 cd	521.5	1850 d	596.9
11	38.7 n.s.	24.84	42.6 n.s.	32.53	41.0 n.s.	22.29	41.1 n.s.	26.81	30.2 n.s.	16.00
12	6.6 a	3.21	4.4 ab	3.16	3.8 b	2.35	5.9 ab	4.24	5.1 ab	3.69
13	2.1 n.s.	0.95	3.0 n.s.	1.29	2.5 n.s.	1.14	2.6 n.s.	0.99	2.6 n.s.	1.01

* Values with the same letter do not differ significantly with the Tukey's test, $p < 0.05$. n.s., not significant.

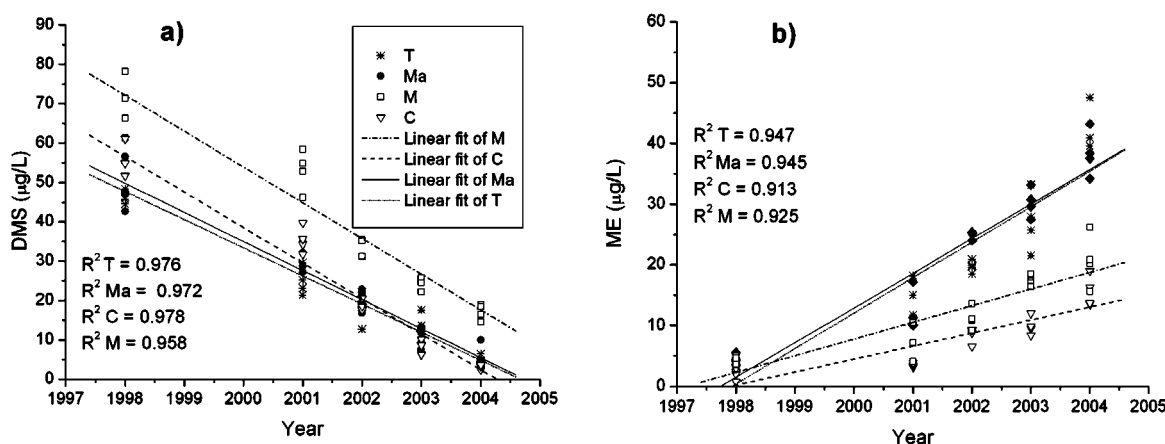


Figure 7. Dependence of the evolution profile on aging and variety for dimethylsulfide (DMS) and 2-mercaptoethanol (ME) and linear fitting (R^2). T, Teroldego; Ma, Marzemino; C, Chardonnay; M, Merlot.

a pairwise comparison of the means, to the data of Table 5 to verify the presence of significant differences (36).

A change in the concentration of sulfur volatiles with the storage time in a rather large experimental time is reported in few papers (15, 30). It is well-known, in fact, that the dimethyl sulfide level increases with the aging (15, 30, 37) and that methyl- and ethyl thioacetates hydrolyze in the first

months of storage with the concomitant increase of the relevant thiols and disulfides (11). The present research, on the basis of the balanced sampling plan and of the statistical approach adopted, indicates that the level of some sulfur compounds is without a doubt affected by aging, a fact quite important and useful in the definition and in the understanding of the wine aroma evolution.

Besides dimethyl sulfide, 3-(methylthio)-1-propanol and 2-mercaptoethanol contents also change with time, increasing and decreasing, respectively. **Figure 7** shows the opposite evolution of dimethyl sulfide and 2-mercaptoethanol for each variety, using a straight line model, which shows a high prediction capability according to the R^2 values. Therefore, mainly for such variables, a possible vineyard/yeast per vintage effect seems rather negligible in this experimentation.

It appears that, for both the species considered, Merlot and Chardonnay show a parallel evolution profile with the storage time. Analogously, according to the results in **Table 4**, ethyl mercaptan and diethyl sulfide vary inversely with time. Diethyl disulfide follows the increasing tendency with time shown by diethyl sulfide. This fact confirms the findings of Bobet et al. (38), concerning the shift with time toward the oxidized form in the redox equilibrium between thiols and disulfide.

The behaviors of the examined species with time can be rationalized on the basis of different arguments: (i) the level of dimethyl sulfide increases due to the S-methyl methionine degradation (30); (ii) the decrease of 2-mercaptoethanol is due to the its oxidation (39, 40); and (iii) the increase of 3-(methylthio)-1-propanol, not evidenced until now, is supposed to derive either from the degradation of methionine via the Strecker mechanism (41) or by decarboxylation of the 2-oxo-derivative of the amino acid obtained via the Ehrlich mechanism to methional (42) and subsequent reduction.

Finally, it was found that, besides the dimethyl sulfide already considered, the level of some sulfur volatiles among the 13 analyzed can depend on the aging; this fact increases the knowledge of compounds connected to the aroma development in wine. Interestingly, some of the 13 resulted also correlated, this fact improves the knowledge on the intervariability of sulfured compounds. On the basis of all volatiles investigated, it was also possible, for the first time, to discriminate different variety pure wines produced in several years, mostly from red grapes that were characterized by chemically different groups of volatiles. The relevant contents, when related to the variety, gave useful information for justifying possible particular scents associable to the variety itself, even in connection with the aging. These compounds belonging to the groups of substances usually considered as wine off-flavoring were found almost at levels not much higher than their commonly accepted sensory or preference thresholds in wine. At the same time, it should be recalled that occasional higher levels of some sulfur volatiles basically induced either by particular nutritional situations for the yeast (11, 12, 19) or by a wrong technological conduction of the vinification (35, 40, 41), could easily transform a typical wine in an off-flavoring product.

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