

Variation of Some Fermentative Sulfur Compounds in Italian “Millesimè” Classic Sparkling Wines during Aging and Storage on Lees

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Fermentative sulfur compounds are recognized as strongly affecting wine aroma, mainly as off-flavors, but recently also as possible positive contributors to wine quality and typicity in still wines. Nevertheless, no evidence has been provided for the influence of these molecules on sparkling wine aroma, except for peculiar volatile thiols found in French Champagne. According to the traditional method, the second fermentation, occurring in sealed bottles, is the essence of the procedure. After this fermentation, sparkling wine is aged on yeast lees for a period ranging from a few months to several years so that yeast autolysis can take place. So far, no evidence is provided for the effect of yeast contact duration on the level of sulfur compounds. Following a HS-SPME/GC-MS method, 13 sulfur compounds, that is, ethylmercaptan, dimethyl sulfide, diethyl sulfide, dimethyl disulfide, diethyl disulfide, methyl thioacetate, ethyl thioacetate, 2-mercaptoethanol, 2-(methylthio)-1-ethanol, 3-(methylthio)-1-propanol, 4-(methylthio)-1-butanol, benzothiazole, and 5-(2-hydroxyethyl)-4-methylthiazole, were quantified in several Italian sparkling wines, produced according to the traditional method in two wineries from Trentino-South Tyrol, region. Additionally, the influence of winemaking technology differences and vintage effects on the evolution of the quoted sulfur compounds was considered. This investigation was carried out by coupling the HS-SPME data with those obtained by SPE method and relevant to other volatile compounds, which are considered as winemaking markers. This work furnished the first evidence of the effect of aging and lees contact at different storage temperatures on the levels of these analytes in sparkling wines. Significant increments were observed for dimethyl sulfide, diethyl sulfide, 2-(methylthio)-1-ethanol, 3-(methylthio)-1-propanol, and 4-(methylthio)-1-butanol during aging with a different variation slope possibly due to the remarkably different storage temperatures. No clear influence of lees contact duration was found for the majority of the sulfur compounds considered.

KEYWORDS: Sulfur volatiles; aroma compounds; classic sparkling wine; aging; headspace–solid phase microextraction; solid phase extraction

INTRODUCTION

The contribution of fermentative sulfur compounds to the aroma of wine is considered very important, in particular, to justify possible off-flavors (1). Recently, particular attention has been paid to understand possible positive contributions to wine quality given by these compounds when present at a concentration lower than or proximal to their sensory/preference threshold values (2). The effect of these compounds on the wine matrices is therefore an open research field. A positive effect on the aroma was already demonstrated for dimethyl sulfide (DMS) by Spedding and Raut (3) and confirmed by Segurel et al. (4, 5). For other sulfur compounds a possible connection with the varietal typicity was proved by Moreira et al. (6) and Fedrizzi et al. (7).

In sparkling wines, produced according to the Champenoise, the so-called traditional method, the second fermentation occurring in bottle, is known to deeply influence the aroma of the product (8–13). In particular, the long aging on yeast lees, as employed for producing the Millesimè products, can have a strong effect on the final quality. Remarkable changes in total thiols level as influenced by lyses have been shown by Wisser (14) with a different evolution in reductive and oxidative environment.

To date, very few studies concerning the effect of both aging and yeast lees contact on the level of fermentative sulfur compounds have been carried out, except for the varietal thiols in French Champagne (15).

Profile variations of sensory interesting volatile compounds have been investigated by Versini and Lunelli in products mostly derived from Chardonnay or Pinot noir (16); in particular, the sulfur species 3-(methylthio)propanal (i.e., methional) was taken into account for its possible contribution to “bouillon-roasted cheese” and “evolved yeast-like” scents (17, 18).

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In the present paper, several sulfur compounds using a concurrent HS-SPME/GC-MS method recently described (19), were quantified in Italian sparkling wines. To provide new information on the winemaking effects on sparkling wine aroma profile and to investigate for the first time a wide range of fermentative sulfur compounds in these products, we analyzed several samples with different aging and different lees contact.

The results permit us to show, for the first time, peculiar evolutions in the analyte concentrations, likely related to aging, storage temperature, and lees contact. Considerations about other compounds quantified with a SPE-GC-MS method and related to pressing technology, must clarification, yeast metabolism and contact aided in gaining a technical overview of these products.

MATERIALS AND METHODS

Chemicals. The sulfur compounds studied were ethylmercaptan (EtSH), dimethyl sulfide (DMS), diethyl sulfide (DES), dimethyl disulfide (DMDS), diethyl disulfide (DEDS), methyl thioacetate (MTA), ethyl thioacetate (ETA), 2-mercaptoethanol (ME), 2-(methylthio)-1-ethanol (MTE), 3-(methylthio)-1-propanol (i.e., methionol; MTP), 4-(methylthio)-1-butanol (MTB), benzothiazole (BT), and 5-(2-hydroxyethyl)-4-methylthiazole (HMT). Dimethyl- d_6 sulfide (d_6 -DMS), dipropyl disulfide (DPDS), 3-(methylthio)-1-hexanol (MTH), and 4-methylthiazole (MT) were used as internal standards (I.S.). All of the purchased analytes had a purity of $\geq 98\%$ and were supplied by Sigma-Aldrich (Milan, Italy) and Lancaster (Milan, Italy). The other volatile compounds investigated were 1-hexanol, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl pyroglutamate, and ethyl 3-(methylthio)propionate; they all were purchased from Sigma-Aldrich.

Experimental Plan. The study involved products from two different leading wineries in sparkling wine production, located in Trentino-South Tyrol, region in northern Italy. In particular, one winery is based in the province of Trento (Ferrari Spumanti, Ravina di Trento, 250 m AMSL) and produces sparkling wines from Chardonnay grapes, whereas the other winery, based in the province of Bolzano (Arunda Sektkellerei, Moelten/Meltina, 1300 m AMSL), produces sparkling wines by blending 90% Chardonnay with 10% Pinot noir wines. All of the sparkling wines considered in this work (seven samples from Ferrari Co. and eight samples from Arunda Co. and the relevant replicates) were obtained by pressing 5 tons of grapes in a pneumatic press (Willmes Anlagentechnik GmbH, Lampertheim, Germany) applying pressures of 1.2 and 1.5 atm to the products from the Trento and Bolzano wine-growing areas, respectively. The juices so obtained were clarified by bentonite fining, settled, and supplemented with thiamin (30 mg/hL) for both wineries. Ferrari Co. supplemented their grape juices with ammonium phosphate and ammonium sulfate to reach a final yeast assimilable nitrogen (YAN) level of 200 mg/L as measured according to the "formol index number" method (20). The juice was fermented by Lalvin EC 1118 yeast strain (*Saccharomyces cerevisiae* Lallemant, Montreal, Canada) in the Arunda Co. products, whereas different yeast strains were employed by Ferrari Co. In both wineries the resulting cool-stored base wines showed a final free SO_2 level of approximately 15 mg/L.

In the second fermentation (i.e., occurring into the bottle), Arunda Co. employed the same yeast strain as the first fermentation, whereas Ferrari Co. used a particular selection of *S. cerevisiae* strain. As a fermentation/lees settling adjuvant, Adjuvant 85 (Station Oenotechnique de Champagne, CIVC, Epernay, France) was adopted by Arunda Co., whereas a bentonite fining was used by Ferrari Co.

Arunda Co. fermented and stored the bottles at about 8–10 °C, whereas Ferrari Co. fermented and kept their products at a temperature between 14 and 16 °C. This different environment temperature was considered as a parameter possibly affecting sulfur compound profile evolution.

The samples were mostly analyzed in 2007 except for those from the 1995 vintage, which were analyzed in early 2009.

None of the products presented reduced off-flavors at the sensory evaluation, carried out by a panel of eight trained judges (four males and four females, ages between 25 and 40 years), according to the international normative ISO 13299 (21). All of the sparkling wines, except for those indicated with the symbol ‡ in the tables, had been separated from the lees for about 6 months before analysis.

Table 1. Calibration Parameters and Detection Limits

analyte ^a	slope	SD slope	intercept	SD intercept	SD	F^2	L_D ($\mu\text{g/L}$)
DMS ^b	1.13	0.01	0.013	0.015	0.05	0.993	0.156
EtSH ^b	1.04	0.01	0.034	0.023	0.06	0.995	0.134
DES ^b	6.75	0.03	0.021	0.011	0.16	0.991	0.114
MTA ^b	0.65	0.01	0.011	0.054	0.18	0.992	0.306
DMDS ^c	0.98	0.01	0.006	0.012	0.05	0.997	0.062
ETA ^b	3.76	0.03	0.001	0.043	0.19	0.995	0.204
DEDS ^c	1.45	0.02	0.011	0.012	0.04	0.995	0.074
ME ^d	1.11	0.01	0.005	0.054	0.18	0.997	0.766
MTE ^d	8.32	0.08	0.003	0.087	0.11	0.998	0.206
MTP ^d	0.65	0.01	0.084	0.043	0.05	0.997	1.652
MTB ^d	2.21	0.05	0.021	0.052	0.16	0.991	0.54
BT ^e	3.01	0.03	0.011	0.076	0.11	0.992	0.968
HMT ^e	1.05	0.01	0.053	0.013	0.14	0.995	0.872

^aDMS, dimethyl sulfide; EtSH, ethylmercaptan; DES, diethyl sulfide; MTA, methyl thioacetate; DMDS, dimethyl disulfide; ETA, ethyl thioacetate; DEDS, diethyl disulfide; MTE, 2-mercaptoethanol; MTE, 2-(methylthio)-1-ethanol; MTP, 3-(methylthio)-1-propanol; MTB, 4-(methylthio)-1-butanol; BT, benzothiazole; HMT, 5-(2-hydroxyethyl)-4-methylthiazole. ^bDimethyl- d_6 sulfide (d_6 -DMS) as I.S. ^cDipropyl disulfide (DPS) as I.S. ^d3-(Methylthio)-1-hexanol (MTH) as I.S. ^e4-Methylthiazole (MT) as I.S.

The vintage ranges considered were 1989–1995 and 1979–1998 for the Trento and Bolzano province products, respectively. To reduce any wine-making influence due to the second fermentation, we took three bottles for each year, pooled their contents, and submitted the mixed contents to both HS-SPME and SPE analyses.

The lees contact effect was evaluated by performing a control on the same sparkling wine (vintage 1995) kept on the lees for shorter (6 years) and longer times (14 years). Also in this case, three bottles were collected, pooled, and submitted to analyses, which were carried out in 2009.

All of the HS-SPME and SPE analyses were performed in triplicate, and standard errors similar to those obtained in previous investigations regarding the applied methods were found (22).

HS-SPME/GC-MS Method. The analytical method used for the quantification of the quoted sulfur compounds has already been described elsewhere by Fedrizzi et al. (19).

The SPME holder for manual sampling and fibers were purchased from Supelco (Bellefonte, PA). The fibers were conditioned before use according to the producer's instructions. The fiber adopted was a Carboxen-polydimethylsiloxane-divinylbenzene (CAR/PDMS/DVB, 2 cm). The sample (20 mL) was put in a 30 mL vial, and 5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ was added. HS-SPME sampling was carried out at 35 °C with a sampling time of 30 min.

The GC-MS apparatus was an Autosystem XL gas chromatograph coupled with a TurboMass Gold mass spectrometer (Perkin-Elmer, Boston, MA) equipped with a 30 m \times 0.32 mm i.d. \times 0.25 μm film thickness Innnowax (PEG) fused-silica capillary column (Agilent Technologies, Milano, Italy). Gas chromatography conditions were as follows: GC injector temperature, 250 °C; injection in splitless mode for 1 min; oven temperature program, 35 °C (5 min), raised at 1 °C/min to 40 °C, and raised at 10 °C/min to 250 °C.

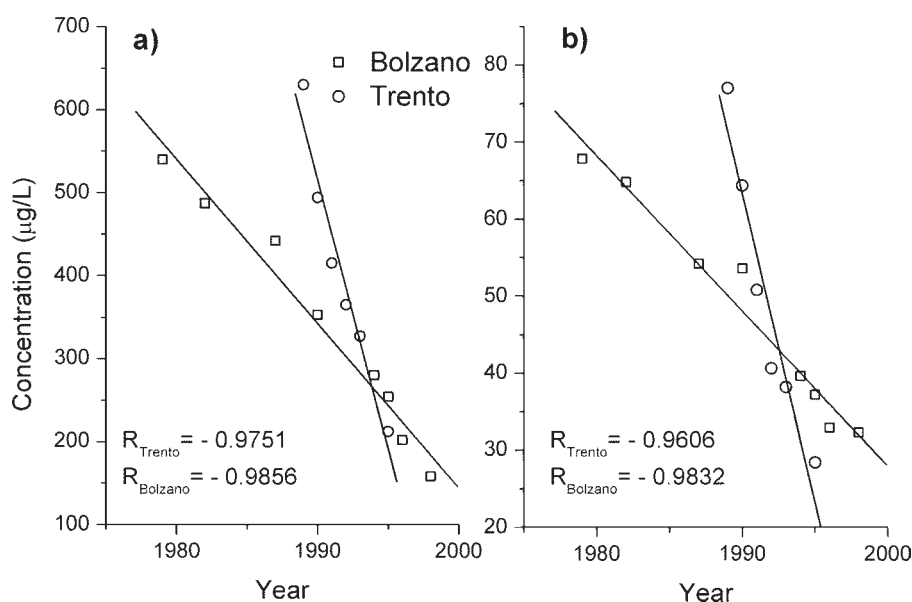
The chromatographic analyses were carried out in single ion recording (SIR) mode following the working conditions already reported (19). Identification of analytes and internal standards was achieved by means of co-injection of the pure reference compounds and comparison of the mass fragments with those reported in the NIST library.

According to the quoted method (19), calibration curves for each analyte were prepared using the following compounds as internal standard: dimethyl- d_6 sulfide, 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 dry white wine (10% alcohol strength v/v) treated twice with charcoal (3 g/L) to remove any sulfur compounds detectable by the proposed HS-SPME/GC-MS method as reported elsewhere (19). Having no information about sulfur compound concentration in sparkling wines, we explored for each compound the concentration ranges typical of still wines using seven concentration levels and five replicate solutions per level. The detection limits (L_D) calculated following the Hubaux–Vos method (23) were in agreement with those found previously (19). Calibration parameters and detection limits are reported in **Table 1**.

Table 2. Sulfur Compound Concentrations in the Sparkling Wine Analyzed^a

vintage	ppb													origin
	DMS	EtSH	DES	MTA	DMDS	ETA	DEDS	ME	MTE	MTP	MTB	BT	HMT	
1989	77.03	nd	10.56	7.28	3.54	3.80	2.87	1.84	6.27	630	7.83	5.46	4.98	Trentino
1990	64.35	nd	7.60	12.04	1.86	3.00	3.02	3.82	6.29	494	5.48	5.58	4.75	Trentino
1991	50.81	nd	4.20	5.06	1.08	1.33	2.74	3.95	5.10	415	4.64	4.31	6.10	Trentino
1992	40.64	nd	3.62	7.58	1.06	0.81	2.68	5.08	4.35	365	4.36	5.48	6.10	Trentino
1993	38.22	nd	3.41	6.54	1.02	0.86	2.61	5.83	4.21	327	4.25	4.18	4.30	Trentino
1995	28.43	nd	3.25	6.03	1.54	1.01	2.34	6.34	4.01	212	2.4	3.92	4.44	Trentino
1995 (01) [‡]	24.53	nd	2.01	3.10	1.23	1.12	1.01	4.44	4.06	193	2.07	3.21	4.01	Trentino
1979	67.85	nd	9.11	5.27	3.59	3.11	2.70	1.03	4.83	540	8.18	5.10	3.91	South Tyrol
1982	64.81	nd	8.94	6.25	3.29	2.74	2.46	1.20	4.65	487	7.89	4.30	4.71	South Tyrol
1987	54.21	nd	8.64	4.84	1.99	1.88	2.57	1.66	4.04	442	7.80	4.62	6.24	South Tyrol
1990	53.61	nd	6.73	6.30	2.21	1.78	2.56	3.40	3.71	353	7.17	4.34	4.56	South Tyrol
1994	39.65	nd	4.91	8.18	1.56	1.46	2.53	5.34	3.66	280	6.23	3.54	5.18	South Tyrol
1995	37.21	0.10	4.71	7.99	1.80	1.73	2.57	10.40	2.92	254	6.52	3.25	6.18	South Tyrol
1996	32.94	0.13	4.50	7.91	1.41	1.18	2.48	13.84	3.60	202	5.82	3.20	5.46	South Tyrol
1998	32.31	0.26	4.32	7.49	1.59	1.48	2.45	15.98	2.88	158	6.05	4.40	5.67	South Tyrol

^a All of the sparkling wines, except for the one indicated with the symbol [‡], were disgorged about 6 months before analysis. DMS, dimethyl sulfide; EtSH, ethylmercaptan; DES, diethyl sulfide; MTA, methyl thioacetate; DMDS, dimethyl disulfide; ETA, ethyl thioacetate; DEDS, diethyl disulfide; ME, 2-mercaptoethanol; MTE, 2-(methylthio)-1-ethanol; MTP, 3-(methylthio)-1-propanol; MTB, 4-(methylthio)-1-butanol; BT, benzothiazole; HMT, 5-(2-hydroxyethyl)-4-methylthiazole.

**Figure 1.** Evolution profile with aging for (a) MTP and (b) DMS. The figure reports the correlation coefficients of the linear fittings.

SPE-GC-MS Method. To enforce the control on some technological situations and to support the HS-SPME data, some volatile compounds were investigated via a SPE-GC-MS procedure. The ENV+ cartridges (6 mL volume, 1 g sorbent) for the SPE extraction were supplied by Isolute (IST Ltd., Mid Glamorgan, U.K.). The analysis of 1-hexanol, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl pyroglutamate, and ethyl 3-(methylthio)propionate was performed by slightly modifying the procedure reported by Fedrizzi et al. (24). *N*-(3-Methylbutyl)acetamide was tentatively identified using the NIST library, and the quantification was carried out using the same SPE-GC-MS method.

The extraction was carried out with an automated solid phase extraction apparatus (Aspec XL, Gilson Inc., Middleton, WI). The sample (58 mL, wine/distilled water, 1:1 v/v) was first percolated through the cartridge. After loading the sample, the cartridge was washed with 10 mL of distilled water, and then the analytes were recovered with dichloromethane (9 mL), dried with sodium sulfate, and concentrated to 200 μ L under a gentle stream of nitrogen.

The analyses were carried out on a GC 6890N Network GC System equipped with a 60 m \times 320 μ m i.d. \times 0.25 mm film thickness HP-Wax capillary column (Agilent Technologies, Milano, Italy) and coupled with a mass spectrometer MS 5975B inert XL EI/CI (Agilent Technologies).

The oven temperature program adopted was 50 $^{\circ}$ C (4 min), raised at 4 $^{\circ}$ C/min to 240 $^{\circ}$ C, 240 $^{\circ}$ C (16 min); injection volume was 2 μ L. Helium

was used as carrier gas at a flow rate of 1.5 mL/min. The temperatures of the transfer line and of the GC injector were 200 and 280 $^{\circ}$ C, respectively. The electron impact energy was 70 eV, and the MS source was set at 230 $^{\circ}$ C. All of the analyses were carried out in SCAN mode, using the NIST library to confirm the identification and adopting a response factor equal to 1 toward the internal standard (1-heptanol), as commonly performed in the analysis of flavor compounds.

The data were statistically evaluated and plotted using Statistica v7.1 (Statsoft Italia S.r.l., Padova, Italy) and Origin v7.0 (OriginLab Corp., Northampton, MA).

RESULTS AND DISCUSSION

HS-SPME/GC-MS Results. Sulfur compound concentrations (Table 2) are mostly within the ranges reported in the literature for still wines (7, 18, 25).

The lack of details on the effect of the employed technological parameters on the evolution profile of sulfur compounds in sparkling wines drove our attention on this class of molecules.

Interesting increasing evolutions for MTP (Figure 1a) and DMS (Figure 1b) levels during aging are evident; the different profiles for the two wineries considered are likely due to the

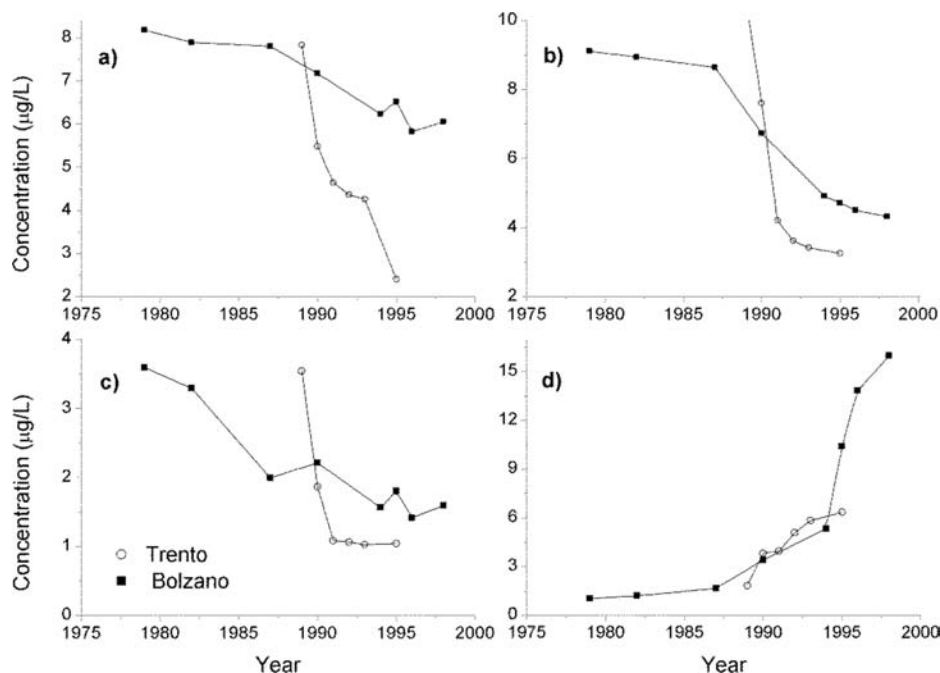


Figure 2. Interpolation of the evolution profile of other sulfur compounds with aging: (a) MTB; (b) DES; (c) DMDS; (d) ME.

different winemaking conditions adopted, among which storage temperatures might play a pivotal role in.

The circumstances that for both species the evolution profiles appear linear, but steeper for Trento products, suggests a possible dominant effect of the storage temperature. This hypothesis is supported by the fact that winemaking procedures applied within each group (i.e., winery) remained the same for all of the samples analyzed over the years considered, except for the yeast employed in the basic wine fermentation by Ferrari Co. This coincidence limits a possible influence of other factors on the final content of the measured sulfur compounds. Our hypothesis is also in agreement with that found in still wines for the yeast effect (7).

It can be recalled that Seeber et al. (26), considering a large number of young base sparkling wines obtained following rigorously the same technology, were not able to discriminate among different vintages by ANOVA, PCA, and LDA data treatment using 3-(methylthio)-1-propanol as one of the target analytes.

The approximately linear evolution tendency for both DMS and MTP was also confirmed by plotting the natural logarithm of the concentration ($\ln C$) versus vintage year (data not shown); this situation permits us to hypothesize the presence of only one precursor reaction according to a first-order kinetics. The differences between the two wineries (i.e., slopes) may be ascribed to the different storage temperatures according to Arrhenius temperature dependence theory (27).

According to the data shown in **Figure 2**, other sulfur compounds appear to be significantly affected by aging; in particular, MTB (**Figure 2a**), DES (**Figure 2b**), and DMDS (**Figure 2c**) show an increment with wine aging, whereas ME (**Figure 2d**) shows an opposite behavior.

MTB data for both wineries show a linear evolution profile similar to that for MTP. This result supports the hypothesis of an analogous formation mechanism originating from homomethionine (28).

Panels **b** and **c** of **Figure 2** show the evolution for DES and DMDS, respectively. For the Ferrari Co. products profile it is possible to observe complex kinetics (27), whereas for Arunda Co. products the evolution appears linear with a slower rate. The different evolution profile is likely connected with storage condi-

tions, and a pivotal role might have been played by storage temperature (27).

Figure 2d reports the evolution profile for ME during aging: Ferrari Co. products show a linear decrement during aging, whereas Arunda Co. sparkling wines display a different evolution, reasonably connected with complex kinetics (27).

Comparing the sparkling wine data with those found in still wines, we note that DMS, DES, and DMDS increase during aging in both matrices, whereas DEDS remains constant in sparkling wines unlike that found in still wines (7).

According to Vasserot et al. (29), variation concerning DMDS could be connected to lees contact and the presence of methanethiol. Furthermore, MTA appears rather constant, which is likely due to the equilibrium with methanethiol sequestered by lees. The other *S*-thioacetate instead, that is, ETA, which should undergo an equilibrium similar to that of EtSH or DEDS, appears to slightly increase with aging. In this complex equilibrium the binding capability of methanethiol toward yeast lees (29) and the efficiency of the bottle closure in preventing DMDS formation by oxidation (30) must be considered.

To achieve a better picture on the connection between the different variables considered (i.e., sulfur compounds concentration, winery, vintage), experimental data were submitted to principal component analysis (PCA). The first two components (PC1, PC2) collected 71.91% of the total variability of the system, and the biplot scattering of the scores indicates a trend following two ideal axis relevant to the two wineries (**Figure 3a**). Furthermore, it is possible to note that the products from the vintages from 1991 onward create a cluster in the top left part of the scores biplot.

Taking into account the loadings plot (**Figure 3b**), it can be observed that Bolzano province products are correlated with aging, being all parallel to the relevant loading, whereas Trento province products are mostly not correlated with this parameter as they are perpendicular to the relevant loading.

The loadings biplot (**Figure 3b**) shows that the first component is positively related mainly with DMS, DES, DMDS, DEDS, ETA, MTE, MTP, MTB, and BT and negatively with HMT and ME; also, the vintage year shows a negative correlation with PC1.

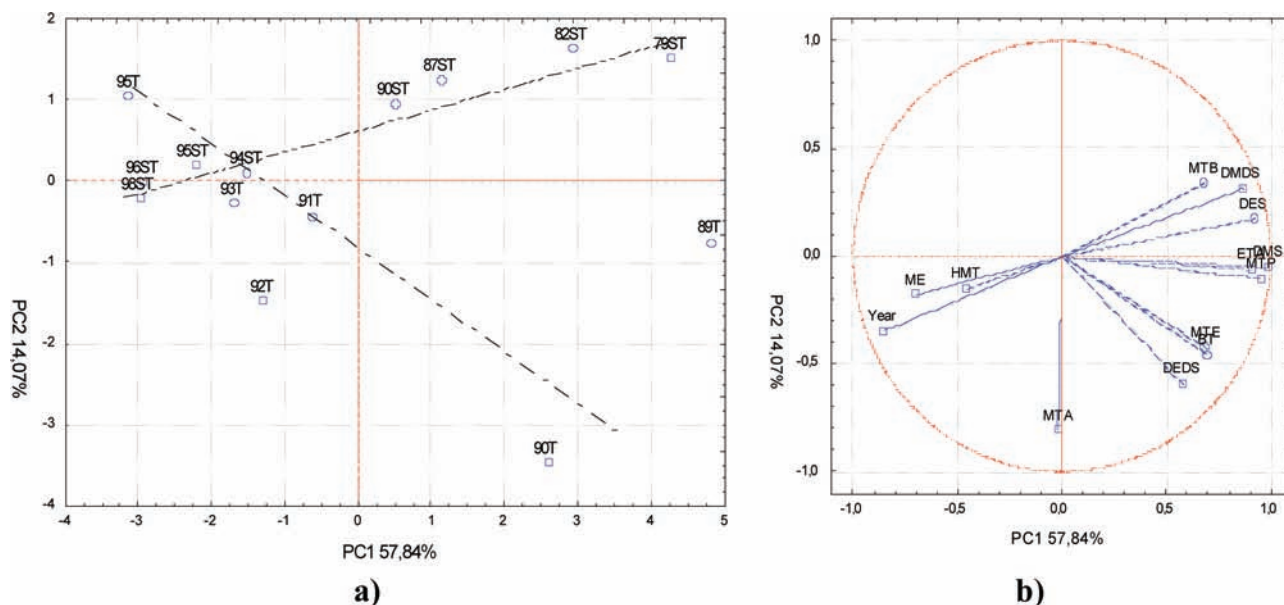


Figure 3. PCA data treatment of the studied sulfur compounds: (a) biplot of the scores; (b) biplot of the loadings for the sparkling wines analyzed. T, Trentino; ST, South Tyrol.

Table 3. Yeast Lees Contact Effect on Considered Sulfur Compounds^a

vintage	ppb											
	DMS*	DES*	MTA	DMDS*	ETA	DEDS*	ME*	MTE	MTP	MTB	BT*	HMT
1995	29.28	3.63	5.95	1.18	0.98	2.10	2.68	4.40	212.13	2.43	3.71	4.73
SD	1.20	0.54	0.12	0.23	0.05	0.35	0.47	0.55	0.71	0.01	0.30	0.40
1995 (01) [†]	23.48	2.4	4.06	1.50	0.89	1.17	4.16	4.35	169.74	2.72	3.11	4.34
SD	1.48	0.61	1.35	0.37	0.33	0.22	0.40	0.41	33.45	0.92	0.15	0.47
<i>t</i>	3.19	3.61	2.06	3.28	1.59	11.06	7.65	0.22	0.08	0.68	10.48	2.02

^a Analytes indicated with an asterisk (*) appeared to be affected by yeast lees contact according to *t* test ($\alpha \leq 0.05$, $t_c = t_{\alpha/2, V=4} = 2.78$). SD, standard deviation. DMS, dimethyl sulfide; DES, diethyl sulfide; MTA, methyl thioacetate; DMDS, dimethyl disulfide; ETA, ethyl thioacetate; DEDS, diethyl disulfide; MTE, 2-mercaptoethanol; MTE, 2-(methylthio)-1-ethanol; MTP, 3-(methylthio)-1-propanol; MTB, 4-(methylthio)-1-butanol; BT, benzothiazole; HMT, 5-(2-hydroxyethyl)-4-methylthiazole.

Analysis of the loadings plot (**Figure 3b**) permits us also to observe that ME and HMT are negatively correlated with aging; DMS, DES, and DMDS are positively correlated with aging; and DEDS shows no correlation with aging.

The effect of the yeast lees contact was investigated for six samples of Ferrari Co. produced in 2005: three sparkling wines kept on lees until the analysis (lees contact of 14 years) and three sparkling wines disgorged from the lees in 2001 (lees contact of 6 years) were analyzed. **Table 3** shows the results of this study; the data of the two groups were submitted to *t* test to check for lees contact time effects.

Lees contact duration does not seem to affect the evolution of thioalcohols (ME, MTP, MTB, and MTE), *S*-thioacetates (MTA and ETA), and heterocyclic compounds (HMT and BT). On the other hand, sulfides and disulfides slightly increase with longer lees contact. This finding agrees with the report of Vasserot et al. (29), who suggested a possible involvement of yeast lees in the methanethiol and ethanethiol oxidation, producing the relevant disulfides.

According to this preliminary experiment, the effect of different lengths of lees contact does not show a significant effect on many sulfur compounds. Nonetheless, more intensive research on sulfur compounds in sparkling wines is needed to gain a conclusive opinion.

SPE-GC-MS Analyses. *Other Volatile Compounds.* To better understand the influence of winemaking on the formation and evolution of sulfur compounds, we took into account some

technological indices. In particular, we checked the variation of some volatile compounds that are known to be deeply affected by these winemaking parameters. To be less restrictive, we chose the products of the Ferrari Co. in which more technological variables could have been changed over the considered years (i.e., yeast strains and nitrogen supplementation).

Following the literature, different yeast strains in must fermentation, even if at the same imposed YAN level (Ferrari Co. working conditions) and grape processing methods (e.g., soft pressing and proper clarification technique) were considered factors possible affecting aroma profiles.

In particular, it is known that the use of different yeast strains can influence the level of some fermentation markers, for example, ethyl C₆–C₁₀ fatty acid esters (31), and soft pressing systems with normal must settling conditions, similar to what was reported by Kinzer and Schreier (32), give comparable levels for the sum of ethyl hexanoate, ethyl octanoate, and ethyl decanoate.

In our investigation, the overall amount of these esters was found to range from about 1050 to 1900 $\mu\text{g/L}$, values commonly reported in the literature (26). Furthermore, with regard to the ethyl esters group, we can also note the level of ethyl 3-(methylthio)-propionate ranging from about 3 to 5 $\mu\text{g/L}$, which confirms in the sparkling wines considered the level found by Schreier et al. (33), without a remarkable effect from aging.

Following suggestions reported in the literature (34, 35) we also considered the levels of 1-hexanol and *N*-(3-methylbutyl)acetamide

as indicators of the grape juice processing (i.e., clarification intensity) and of the vintage.

In the sparkling wines examined 1-hexanol ranged from about 1500 to 1900 $\mu\text{g/L}$, in agreement with data reported for base sparkling wines (26), even if its level might have been slightly increased by yeast contact and storage time according to Postel and Adam (36).

N-(3-Methylbutyl)-acetamide in our sparkling wines was found between 60 and 220 $\mu\text{g/L}$ as expected from well-clarified musts.

Finally, the variability ranges of the quoted compounds confirmed the declared technological conditions of the industrial processing system.

A further useful indicator of the effect of the contact time of the wine with the lees is the concentration of ethyl pyroglutamate (37, 38). Its level in still wines ranges from about 300 to 1000 $\mu\text{g/L}$ (39), whereas its content is very high in all of the sparkling wines considered, ranging uncorrelated with aging from about 4000 to 9200 $\mu\text{g/L}$. This compound probably gives an important contribution to the sparkling wine aroma with a "honey" and a "bitter-umami" flavor (40).

The results shown in this paper provide for the first time information on the concentration and evolution of sulfur compounds in sparkling wines.

The significant increment during aging for MTP and MTB is in agreement with that found in still wines, even if the different evolution profiles give rise to several hypotheses on the formation and evolution of these molecules.

The evolution profile appears to be different for the two varieties; to the best of our knowledge this evidence could be mostly related to the temperature at which the sparkling wines were stored.

Sulfides showed an evolution with aging similar to that found for still wines, most likely supporting the evidence of an origin from similar precursors. The different concentration values developed during aging might be related to the supplementation of new precursors from yeast autolysis.

No clear influence of lees contact duration has been found for many sulfur compounds here considered.

Considerations drawn from the variation of the other volatile compounds as markers of the winemaking process seem to exclude a prevalent vintage effect.

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