# Identification of Ethyl 2-Sulfanylacetate as an Important Off-Odor Compound in White Wines

Maria Nikolantonaki and Philippe Darriet*

Unité de recherche Œnologie, EA 4577, USC 1219 INRA, Institut des Sciences de la Vigne et du Vin, Université de Bordeaux, 210 chemin de Leysotte, CS 50008, Villenave d'Ornon, F-33882, France

**ABSTRACT:** A number of Sauvignon blanc wines made from hard pressed juices in an inert atmosphere (nitrogen) or in contact with oxygen were identified as having heavy off-flavors to varying degrees. Samples were extracted and subjected to time-based HPLC fractionation. The fractions were assessed by a sensory panel and those with unpleasant, irritating, off-odors were re-extracted. The extracts evaluated by gas chromatography coupled with olfactometry revealed a number of odoriferous zones, including one with an off-odor similar to the one perceived in two HPLC fractions. The odor was less intense in fractions previously supplemented with copper sulfate, suggesting that the compound(s) responsible were possibly thiol-related. A selective thiols extraction protocol and the analysis of the extract by gas chromatography coupled with mass spectrometry identified a new potent thiol in these wines. The compound responsible for the odoriferous zone, ethyl 2-sulfanylacetate (**1**), had an odor reminiscent of baked beans and *Fritillaria meleagris* bulbs. Its perception threshold was determined and sensory studies using graduated supplementation in dry white wines demonstrated its contribution to the off-odor observed in dry white wines.

**KEYWORDS:** volatile thiol, ethyl 2-sulfanylacetate, off-odor, wine, oxygen

## ■ INTRODUCTION

Sulfur-containing molecules, especially thiols, are probably some of the most widely recognized key flavor compounds in many foods and beverages.[1] They are often characterized by a low detection threshold (on the ppt level).[2] In the 1990s, several studies aimed at characterizing the impact of wine aroma compounds demonstrated the role of certain powerful volatile thiols in the typical fruity nuances of wine varietal flavors or empyreumatic aromas acquired during aging.[2] Thus, volatile thiols are important aroma components in dry white wines, such as Sauvignon blanc, Semillon, Scheurebe, Petite Arvine, Gewürztraminer, and Muscat d'Alsace.[3−8] They have also been isolated from wines made from many different *Vitis vinifera* cultivars, including Riesling, Muscats, Albarino, Malvoisie, Parellada, Maccabeu, Verdejo, and Koshu.[2,7,9−12] The first volatile thiol identified was 4-mercapto-4-methylpentan-2-one, now called 4-methyl-4-sulfanylpentan-2-one,[3] with aroma descriptors of blackcurrant and broom as well as cat's urine (at higher concentrations).[3,9,13] Other odorous volatile thiols identified to date include 3-sulfanylhexan-1-ol or 3-mercaptohexan-1-ol, 3-sulfanylhexyl acetate or 3-mercaptohexyl acetate, and 4-methyl-4-sulfanylpentan-2-ol or 4-methyl-4-mercaptopentan-2-ol.[6,9] 3-Sulfanylhexan-1-ol, reminiscent of grapefruit and passion fruit nuances, has an olfactory threshold in a winelike solution in the vicinity of 60 ng/L [9] and is always present in Sauvignon blanc wines at concentrations of several hundred ng/L, and sometimes several micrograms per liter.[9] 3-Sulfanylhexyl acetate results from the acetylation of 3-sulfanylhexan-1-ol by yeast, which also contributes to the wine aroma and is mainly evocative of boxwood and also passion fruit.[9] Its olfactory threshold is 4 ng/L,[9] and certain Sauvignon blanc wines may contain up to several hundred nanograms per liter.[4,9] The organoleptic role of 4-methyl-4-sulfanylpentan-2-ol, which has an aroma similar to citrus zest, is more limited.[9] Concentration in wines seldom exceeds its olfactory threshold

(55 ng/L),[9] but this level can be reached in some wines.[9] More recently, volatile thiols associated with noble botrytized grapes were described; these compounds, such as 3-sulfanylhexan-1-ol, 4-methyl-4-sulfanylpentan-2-one, 3-sulfanylheptanol and 3-sulfanylpentanol, can contribute toward the citrus nuances of Sauternes wines.[6,14,15] Other thiols such as 2-furanmethanethiol (furfurylthiol), 2-methyl-3-furanthiol, and benzenemethanethiol can contribute to the empyreumatic nuances in wine "bouquet".[16−18] In general, concentrations of varietal volatile thiols decrease during wine aging.[19−22] However, the kinetics of empyreumatic thiol formation in aged Champagne wines followed a reverse trend, as reported by Tominaga et al.[18]

However, sulfur compounds are also considered in enology as responsible for off-flavors. The presence of certain low molecular weight (light) sulfur compounds, such as hydrogen sulfide, methanethiol, ethanethiol, 2-sulfanylethanol, and 3-methylsulfanylpropan-1-ol, characterized by unpleasant smells (rotten egg, garlic, sewage, rubber, and cooked cauliflower), results in off-flavors in wine.[1,23−25] Even at low concentrations (on the order of micrograms per liter) these odors are likely to ruin a wine's aroma.[26] The production of light sulfur compounds is mainly related to yeast metabolic activity during alcoholic fermentation,[27−30] which is modulated by must nutritionals (nitrogen, vitamins) [31] and exposure to oxygen during the prefermentative operations,[32] turbidity proportion in the must during alcoholic fermentation,[33] and the strain of *Saccharomyces cerevisiae* yeast.[27−30]

The main purpose of this study was to investigate the olfactory profile of dry white wines made from grape juice obtained by

**Table 1. Origin of White Wine Samples (2009) Elaborated from Hard Pressed Juices Extracted during Grape Pressing under Nitrogen Gas Atmosphere (Inert) or with Conventional Vinification Process, in Contact with Oxygen (Oxidation)**

| samples | appellation | elaboration conditions |
|---|---|---|
| N−I | Graves | inert |
| O−I | | oxidation |
| N−II | | inert |
| O−II | | oxidation |
| | | |
| N−III | Pessac-Léognan | inert |
| O−III | | oxidation |
| N−IV | | inert |
| O−IV | | oxidation |
| | | |
| N−V | Entre Deux Mers | inert |
| O−V | | oxidation |

pressing in an inert atmosphere or using the conventional vinification process, in contact with oxygen. As, these wines expressed heavy flavors to varying degrees depending on the wine-making conditions, sensory and analytical approaches were combined to identify trace compounds likely to impact the wines' aromatic finesse. This resulted in the identification of a new odoriferous thiol, associated with unpleasant odors. The contribution of this compound to the aromatic expression of several wines was then examined, particularly in relation to oxygen management during prefermentative operations of vinification and bottle aging.

## ■ MATERIALS AND METHODS

**Chemicals and Reference Compounds.** Water was purified through a Milli-Q system (Milipore, France). Dichloromethane (Chromasolv grade), sodium acetate (99%), sodium *p*-hydroxymercuribenzoate, 5,5′-dithiobis(2-nitrobenzoic acid), copper(II) sulfate (99.9%), L-(+)-tartaric acid (≥99.5%, puriss.), and ethyl acetate for HPLC (99.9%, Chromasolv Plus) were obtained from Sigma-Aldrich (St. Quentin Fallavier, France). Absolute ethanol (≥99.9%, LiChrosolv quality) was obtained from Merck (Paris, France). Ethyl 2-sulfanylacetate (98%) and aliphatic hydrocarbon standards (alkanes) were supplied by Interchim (Montluçon, France). 4-Methoxy-2-methylbutane-2-thiol (≥98%) was purchased from Oxford Chemicals (Hartepool, England).

**Wine Samples.** The analyzed dry white and rosé wines were from various appellations. For the purpose of HPLC fractionation, Sauvignon blanc wines were produced from grapes harvested at 2009 vintage from three vineyards from the Bordeaux region (Graves, Pessac-Léognan, and Entre Deux Mers, France) (Table 1). They were all elaborated with different degrees of juice antioxidant protection following the vinification protocol described subsequently. The quantitative analysis of **1** was also carried out on 18 vintages of Sauvignon blanc and Semillon wine blends from the same winery in the Bordeaux appellation (Pauillac, France), as well as in several young white wines (2008 and 2009 vintages) made from Riesling and Sauvignon blanc grapes from different worldwide appellations (Table 4). Rosé wines were from Bordeaux (Graves, 2009 vintage) and Provence (Bandol and Côtes de Provence, 2009 vintages) areas (Table 4). Perception and rejection thresholds of **1** were determined in two Sauvignon blanc wines from the Bordeaux area (Pessac-Léognan and Entre Deux Mers, 2009 vintages).

**Vinification.** *Production of Wines Made from Juices with Different Antioxidant Protection.* Wines were produced with *Vitis vinifera* L.

cv. Sauvignon blanc grapes from three vineyards from the Bordeaux region (Graves, Pessac-Léognan, and Entre Deux Mers, France, 2009 vintage). The wineries were selected because they had the same pneumatic press tank (XPlus 40 Inertys, Bucher Vaslin, France) with the possibility to process the total press cycle under neutral gas (nitrogen) or not. The press tank has a double bottom side, secured to the tank, permitting one to gather the grape juice under an atmosphere of inert gas (nitrogen). The inerting of the grapes and juice during the pressing cycle is provided by a flexible hanging container (2.5 m³ nitrogen, 30 mbar) which is located near the press tank. In this configuration, during the pressing cycle, nitrogen is transferred between the press tank and the flexible container. Diagrammatically, the press tank is connected to a gas flexible container via the juice trough. The "tank and juice trough" and "juice trough and flexible container" are connected together or disconnected according to the pressing phases. The juice is discharged by a pump using a system of must recovery pumping control in the juice trough.

All grapes were harvested at maturity and transferred to each winery in perforated plastic boxes. Each time, the grapes were divided in two homogeneous batches and each of them was pressed under nitrogen-saturated environment (inert) or using the conventional vinification process, in contact with oxygen (oxidation). The same press cycle was applied in the three wineries. All juices were drained into an intermediate holding tank under a $CO_2$ atmosphere and pumped into a tank for settling with 30 mg/L $SO_2$ for 24 h at 12 °C. Last pressed juices or hard juices (corresponding to the last 20% of remaining juice) were separated from free run juices and treated in the same way. Grape juices were stored in stainless steel vats at low temperature (12 °C) to enable them to reach the desired level of clarification. When the same degree of turbidity [180 NTU (nephelometric turbidity unit)], measured by a nephelometer (Hach 2100P, Hach Co., Loveland, CO) was reached, the juices were racked and transferred to the laboratory. The assimilable nitrogen content in grapes juices was measured by using the Sörensen method [34] and corrected to 200 mg/L in all juice samples by adding ammonium sulfate (Laffort Œnologie, France) before alcoholic fermentation. Juices were then inoculated with *S. cerevisiae* (strain X5, Laffort Œnologie, France) precultured for 24 h according the protocol proposed by Bely et al.[35] and fermented in 750 mL sterile bottles. Bottles were sealed with a rubber bung with a thin hole, into which was inserted a 100 $\mu$L plastic pipet tip filled with glass wool to release $CO_2$ produced during fermentation. Fermentation took place in a temperature-controlled environment at 22 °C and was monitored by $CO_2$ release.[35] When alcoholic fermentation was completed, 30 mg/L $SO_2$ was added and wines samples were stored at 12 °C for analysis of volatile thiols by GC−MS at a later date. Fermentations were carried out in triplicate.

**Nonselective Wine Extraction of Volatile Compounds for High-Performance Liquid Chromatography Fractionation.** Five hundred milliliters of wine was extracted successively using 20, 10, and 10 mL of dichloromethane, with magnetic stirring (500 rpm) for 5 min with each extraction, and the layers were separated in a funnel. The organic phases were combined and concentrated under nitrogen flow (100 mL/min) to obtain 0.5 mL of wine extract.

**High-Performance Liquid Chromatography Fractionation.** *Chromatographic Conditions.* The procedure was based on the method first described by Ferreira et al.[36] and later adapted by Pineau et al.[37] The HPLC fractionation of wine was accomplished with a Dionex (Ultimate 3000) HPLC by using an automated injector. Acquisitions were performed using Chromeleon software. The column used was a Varian Polaris C18-Ether (250 × 4.6 mm, 3 $\mu$m). The column was held at room temperature during fractionation. The chromatographic conditions included a flow rate of 1 mL/min and an injection volume of 250 $\mu$L. The linear program gradient involved phase A, water, and phase B, ethanol, 0% B reaching 100% B in 50 min, followed by washing and reconditioning of the column. An automated

fraction collector (Dionex) was connected to the end of the column to collect 1 mL of the eluted solvent every minute. The HPLC eluate was recovered in 50 separate fractions. Subsequently, all fractions were evaluated for their odor as described below. The fractions with unpleasant odors were re-extracted and analyzed by GC−olfactometry and GC−MS.

*Flavor Fraction Re-Extraction.* The two consecutive fractions of interest were mixed and extracted again as described by Pons et al.[38] and Pineau et al.[39] The alcohol content of the fractions eluted by HPLC was adjusted to 12% (v/v) by adding ultrapure water (Milli-Q, Millipore, Bedford, MA). Then the solution was extracted with dichloromethane ($3 \times 500\,\mu$L) by a Vortex mixer (Fisher Scientific) (700 rpm) for 2 min with each extraction, and the layers were separated by a Pasteur pipet. The organic phases were combined and concentrated under nitrogen flow to obtain $20\,\mu$L of extract.

**Capillary Gas Chromatography Coupled with Olfactometry and a Flame Ionization Detector (GC−O−FID).** The analysis was carried out alternately by three operators on a Hewlett-Packard HP5890 series II (Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector (FID) and a sniffing-port (ODO-1 from Scientific Glass Engineering). A $3\,\mu$L sample of each concentrated extract was injected in splitless mode (injector temperature = 230 °C, purge time = 1 min, purge flow = 50 mL/min) at oven temperature (45 °C) in a polar type BP20 capillary column (SGE, 50 m, 0.22 mm internal diameter, 0.25 $\mu$m film thickness) or a nonpolar type BPX5 fused silica capillary column (SGE, 50 m, 0.22 mm internal diameter, 0.25 $\mu$m film thickness). For all analyses, the temperature program was as follows: 45 °C for 1 min and then raised to 240 at 3 °C/min, followed by a 20 min isotherm. The carrier gas was hydrogen (Air Liquide, Bordeaux, France) with a column-head pressure of 22 psi and a flow rate of 1 mL/min. Linear retention indices (LRI) were obtained by injection of a series of alkanes ($C_7$−$C_{23}$) under the same chromatographic conditions.[39]

**Identification and Quantification of Ethyl 2-Sulfanylacetate by Gas Chromatography−Mass Spectrometry (GC−MS).** *Selective Wine Extraction of Volatile Thiols for Identification Purpose.* The volatile thiols were specifically extracted from 0.5 L of wine, by reversible combination of the thiols with sodium *p*-hydroxymercuribenzoate (*p*-HMB) as described by Tominaga et al.[17]

*GC−MS Identification Conditions.* GC−MS identification analysis was carried out on a Trace GC ultra (Thermo Fisher Scientific, France) gas chromatograph coupled with an MS DSQ II (Thermo Fisher Scientific, France). A $3\,\mu$L sample of each concentrated extract was injected in splitless mode (injector temperature = 230 °C, purge time = 1 min, purge flow = 50 mL/min) at oven temperature (45 °C) on a BP20 type capillary column [(SGE, Ringwood, Australia), 50 m, 0.22 mm internal diameter, 0.25 $\mu$m film thickness]. For all analyses, the temperature program was as follows: 45 °C for 1 min, raised to 230 at 3 °C/min, followed by a 20 min isotherm. Helium (Air Liquide, Bordeaux, France) was used as carrier gas with a column-head pressure of 22 psi and a flow rate of 1 mL/min. The mass spectrometer was functioning in electron impact mode (electron energy = 70 eV), in positive mode with a source temperature at 210 °C. Mass spectra were taken over the 40−250 $m/z$ range. The mass detector was connected to the GC with a transfer line heated at 230 °C. Xcalibur software (Thermo Fisher Scientific France) was used for data acquisition. The odor active compound, **1**, was identified on the basis of the linear retention index and a comparison of MS fragmentation patterns obtained in SCAN mode with those of the reference compound and with mass spectra in the NIST library.

*GC−MS Quantification Conditions.* Quantification of **1** was performed using a standard addition procedure. Increasing quantities (50−2000 ng/L) of **1**, prepared from standard dilute alcohol solution according to Ellman's method using 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB),[40] were added to a Muscadet wine. For each concentration, the

volatile thiols were specifically extracted from wine using the method described by Tominaga et al.[41] The calibration curve of this compound was therefore corrected by subtracting the blank ratios (height of peak formed by a selected ion of this compound contained naturally in this wine/that of internal standard). In the concentration range (50−2000 ng/L), the calibration function was linear: $[\mathbf{1}]$ (ng/L) = 718.63 $H/H_{is}$ − 27.17, $R^2$ = 0.996 ($H$, height of **1** peak; $H_{is}$, height of internal standard peak). Repeatability of the measuring system was assessed over a series of five extractions of the same wine spiked with 500 ng/L of **1**. The recovery rate for the volatile thiol was calculated according to the method described by Tominaga et al.[41] and was higher than 70%, irrespective of the quantity added. The coefficient of variation was lower than 5%. The quantification limit was calculated at 11 ng/L, defined as the minimum concentration that generated a peak signal 10 times higher than the signal from background noise.

GC−MS quantification analysis was carried out on a 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA) coupled with an MS 5973 (Agilent Technologies, Palo Alto, CA) series mass-selective detector (MSD). Data were collected and processed using MSD Chemstation software. A 3 $\mu$L sample of each concentrated extract was injected in splitless mode (injector temperature = 250 °C, purge time = 1 min, purge flow = 50 mL/min) into a BP20 type capillary column (SGE, 50 m, 0.22 mm internal diameter, 0.25 $\mu$m film thickness). For all analyses, the temperature program was as follows: initial temperature at 45 °C and then 45 °C for 10 min, raised to 230 at 3 °C/min, followed by a 20 min isotherm. Helium (Air Liquide, Bordeaux, France) was the carrier gas used with a column-head pressure of 22 psi and a flow rate of 1 mL/min. The mass spectrometer, functioning in electron impact mode (electron energy = 70 eV), was connected to the GC with a transfer line heated to 250 °C. **1** and the internal standard were detected in SIM mode by selecting the following ions: $m/z$ = 120, 74, and 47 for **1** and $m/z$ = 134 and 100 for the internal standard, 4-methoxy-2-methylbutane-2-thiol. The quantification ions were $m/z$ = 120 for **1** and $m/z$ = 134 for the internal standard. All quantification assays were performed in duplicate.

**Sensory Analysis.** *On HPLC Fractions.* A 1 mL sample of each fraction collected from preparative HPLC was poured in normalized glasses from the Association Française de Normes (AFNOR) to submit for sensory evaluation by four trained panelists. After this, a comparative study between the descriptors determined in the fractionation of the various wine extracts, elaborated with different vinification protocols, was carried out. Only the fractions that were found to present an unpleasant odor were extracted, as described previously. The level of intensity of the odor was estimated on a scale from 0 (less intense) to 10 (most intense) by each panelist and then the average of their scores was calculated.

*Determination of the Olfactory Perception Threshold.* Ascending forced-choice methods were used to measure the olfactory detection threshold of **1**.[42] The stimulus intensity followed a geometric concentration series for **1** (100, 200, 300, 400, 500 ng/L) in two different wine samples, water, and a winelike hydroalcoholic solution [12% v/v, 4 g/L tartaric acid, pH 3.5 (NaOH, 1 N)]. The wines selected for purpose of determination of the olfactory perception threshold were two Sauvignon blanc wines from the Bordeaux area (Entre Deux Mers and Pessac Léognan, 2009 vintages) containing low concentrations of **1** (125 ± 14 and 234 ± 25 ng/L, respectively). In wines, final concentrations of **1** were therefore corrected by subtracting the blank **1** concentration contained naturally in this wine. The stimulus was increased in a series of triangle tests, in an ascending way, to find points when each individual panelist's responses changed from not correctly identifying the spiked sample to correctly identifying it. The samples were provided to each panelist as a series of five blind-coded sets of three samples per set. The first set was the wine without added **1** (containing the lowest concentration of **1**). The panelist had to make a choice about which sample was different before receiving their next try and so on for five sets. Forty-six

**Table 2. Free Choice Profiling Test of Fractions Obtained by Preparative HPLC Method from Sauvignon Blanc Wines Elaborated from Hard Pressed Juices Extracted under Nitrogen Gas Atmosphere (Inert) or using Conventional Vinification Process, in Contact with Oxygen (Oxidation)**

| fraction (min) | inert | oxidation |
|---|---|---|
| 10 | roasted coffee | coffee grounds |
| 11 | celery | crystallized onion |
| 12 | cheese | cheese |
| 13 | —[a] | solvent |
| 14 | caramel | caramel |
| 15 | vanilla | — |
| 16 | isoamyl alcohol | isoamyl alcohol |
| 17 | isoamyl alcohol | isoamyl alcohol |
| 18 | rose | rose |
| 19 | rose | rose |
| 20 | — | faded rose |
| 21 | — | floral |
| 22 | — | soy sauce |
| **23** | **irritating** (5)[b] | **irritating** (7) |
| **24** | **baked beans** (8) | **irritating, herbaceous** (9) |
| 25 | clove | spicy |
| 26 | — | vegetal |
| 27 | soap | soap |
| 28 | floral | cotton candy |
| 29 | banana | banana |
| 30 | banana | banana |

[a] —, nondetected odor. [b] Fraction aromatic intensity evaluated on a scale from 0 (less intense) to 10 (more intense); —, nondetected odor.

**Table 3. Main Odoriferous Zones Perceived by GC—O of the Re-Extracted $F_{23+24}$ HPLC Fractions of a Sauvignon Blanc Wine Elaborated from Juice Pressed in Conventional Vinification Process, in Contact with Oxygen**

| no. | RT (min) | LRI[a] | odor descriptors[b] |
|---|---|---|---|
| 1 | 8.8 | 1119 | sulfurous (1)[c] |
| 2 | 12.2 | 1219 | herbaceous box tree (3) |
| 3 | 13.2 | 1244 | spicy |
| 4 | 14.4 | 1275 | vegetal, box tree (1) |
| 5 | 15.9 | 1310 | mushroom |
| 6 | 16.6 | 1327 | meaty |
| 7 | 18.0 | 1360 | vegetal (4) |
| 8 | 19.2 | 1383 | box tree, broom, cats urine (6) |
| **9** | **20.5** | **1411** | **irritating, *Fritillaria meleagris* bulb, baked beans** (7) **(OZ9)** |
| 10 | 27.7 | 1569 | roasted |
| 11 | 29.8 | 1630 | cotton |
| 12 | 30.9 | 1666 | cabbage |
| 13 | 31.8 | 1695 | cheese (2) |
| 14 | 33.6 | 1742 | roasted (1) |
| 15 | 34.2 | 1757 | box tree, complex (3) |
| 16 | 38.9 | 1881 | fruity |
| 17 | 40.5 | 1925 | spicy |

[a] Retention index (LRI) of odor peak on a BP20 (50 m × 0.25 mm, 0.25 μm) column by GC—O. [b] Odor descriptors generated by the two assessors during GC—O. [c] Odoriferous zone intensity evaluated in increasing mode on a scale from 0 (less intense) to 10 (more intense).

trained panelists participated in this sensory analysis. The odor perception threshold corresponded to the minimum concentration below which 50% of the testers statistically failed to detect the difference from the control.

*Paired Comparison Preference Test.* Paired comparison preference tests were performed for the determination of the **1** rejection threshold in two different Sauvignon blanc wines samples.[43] The wines selected for purpose of determination of the olfactory rejection threshold were two Sauvignon blanc wines from the Bordeaux area [Entre Deux Mers (wine 1) and Pessac Léognan (wine 2), 2009 vintages]. A series of paired comparison tests were used: each pair consisted of one sample of wine and one sample of wine spiked with increasing concentrations of **1** (50, 100, 200, 300, 600 ng/L). In wine, final concentrations of **1** were therefore corrected by subtracting the blank **1** concentration contained naturally in these wines. The assessors were asked to choose the sample they preferred in terms of varietal typicality, from the pairs presented. Eleven trained panelists participated in the sensory analysis. The criteria used for the significant rejection, as a function of the **1** concentrations, were based on binomial distribution tables. Significance was considered at the 5% and 1% level for the number of assessors ($N$) participating in each test performed.

## ■ RESULTS AND DISCUSSION

This research investigated the aromatic composition of Sauvignon Blanc wines made from hard pressed juice that had heavy off-flavors to varying degrees, depending on the pressing conditions (in the presence of oxygen or an inert nitrogen atmosphere). Selected white wine extracts (see Materials and Methods) (Table 1) were subjected to direct semipreparative HPLC fractionation, using the protocol described by Pineau et al.[37] This method uses water and ethanol as eluents for fractionation of the wine extract, making it possible to assess the aromatic characteristics of each fraction by direct olfaction. Comparative semipreparative HPLC was applied to all the extracts of wines made from juice maintained in an inert atmosphere or allowed contact with oxygen during prefermentative vinification operations (Table 1). Doing so, two consecutive wine fractions ($F_{23}$ and $F_{24}$) were isolated and described as having an unpleasant odor of baked beans, as well as an irritating, pungent, herbaceous odor (Table 2). These fractions were globally present in all samples but their intensity varied depending on the origin of the wines and the winemaking methods used. More precisely, the unpleasant $F_{23}$ and $F_{24}$ odor was most intense in wine samples made from hard pressed juices obtained in contact with oxygen and less marked into samples pressed under inert conditions (Table 2). These off-aromas were generally less intense or even absent in wines made from free-run juice (data not shown).

Isolated for their off-odor character, $F_{23}$ and $F_{24}$ fractions of each wine sample were then mixed ($F_{23+24}$) and extracted again, as described in Materials and Methods. A GC—O—FID analysis of the extract $F_{23+24}$ revealed 17 main odoriferous zones on a BP20 column (Table 3). One particularly intense odoriferous zone (OZ9), presenting on two different capillaries with the linear retention indices (LRI) $LRI_{BP20} = 1411$ and $LRI_{BPX5} = 847$, had an off-odor reminiscent of the $F_{23}$ and $F_{24}$ fractions off-odor.

**Identifying Ethyl 2-Sulfanylacetate in Wine Using GC—MS.** Direct analysis of the $F_{23+24}$ extract using GC—MS with a BP20 capillary column only gave a poor mass spectrum (data not
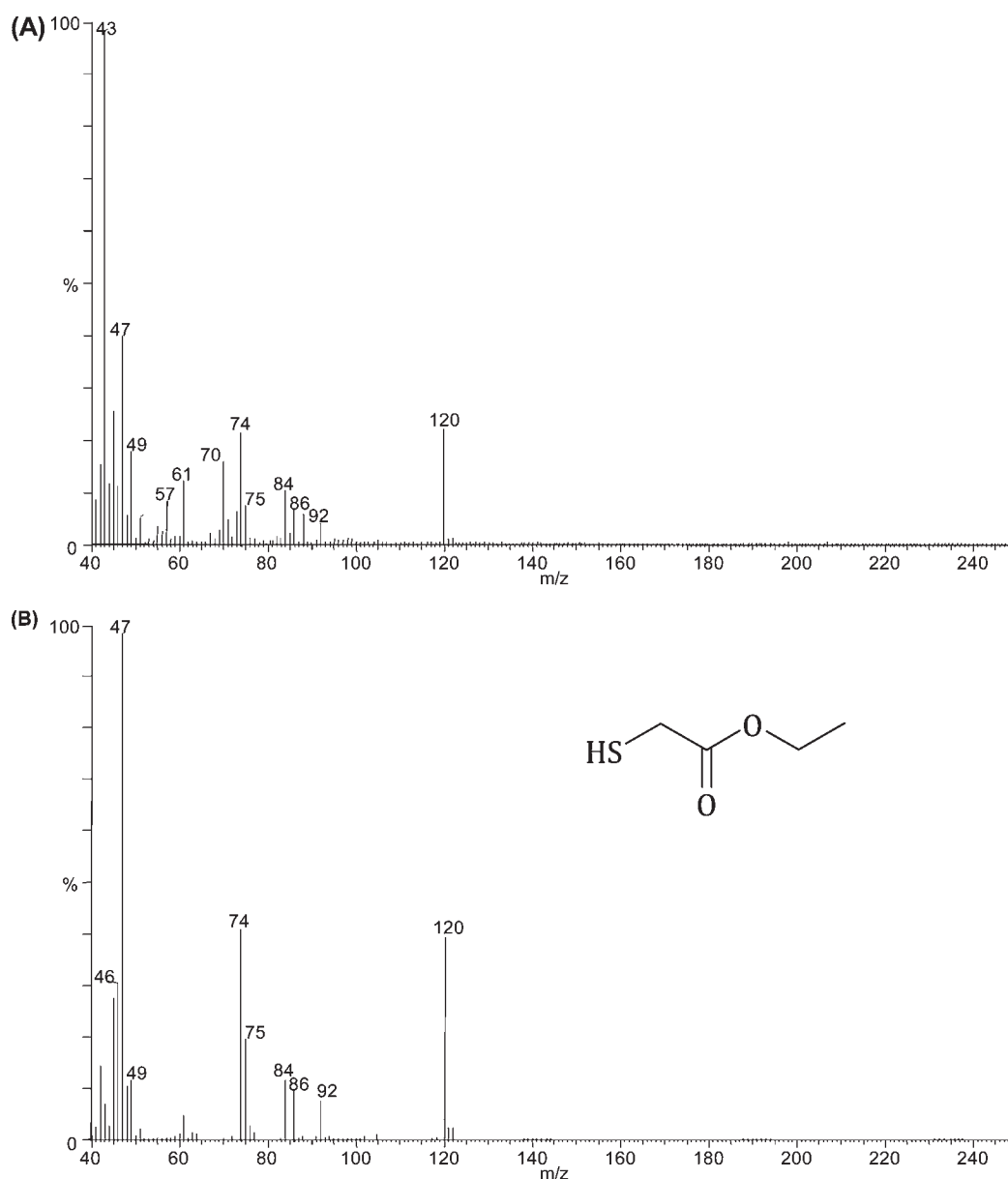
**Figure 1.** Mass spectra of ethyl 2-sulfanylacetate [OZ9, (A)] isolated from wine and pure ethyl 2-sulfanylacetate (B).

shown), so it was not possible to identify the compound(s) corresponding to the retention time of OZ9 ($LRI_{BP20}$ = 1411). This was probably due to the dilution related to HPLC fractionation. Consequently, we decided to characterize the compound(s) specifically responsible for OZ9 in crude wine extract. The fact that the off-odor of the in $F_{23}$ and $F_{24}$ fractions disappeared in the presence of a few milligrams of copper strongly indicated that the odor was produced by a compound with a thiol function group in its chemical structure. Consequently, the method described by Tominaga et al.[17] was used for selective extraction and GC−O and GC−MS analysis of volatile thiols in wine samples made from hard pressed juices obtained in contact with oxygen. GC−MS analysis gave a peak with the same linear retention index as that of OZ9. On the basis of mass spectrometry data obtained in EI mode (Figure 1, A) and a comparison of MS fragmentation patterns with mass spectra in the NIST library, the peak corresponding to the OZ9 was identified as **1**. Analysis of the mass spectra and

the retention time of the compound in comparison with those of the reference compound confirmed the identification of the volatile thiol (Figure 1, B).

To the best of our knowledge, this was the first time that this off-odor compound had been identified in wines. It had previously been reported as an off-odor in a pharmaceutical packaging.[44] **1** was not detected in grape juice but its formation was noticed during alcoholic fermentation (data not shown). Closely related natural compounds had been previously identified in wine, e.g., ethyl 2-sulfanylproprionate[18,45] and ethyl 3-sulfanylproprionate.[18] Ethyl 3-sulfanylproprionate was also identified in grapes[46] and cheese,[47] whereas ethyl 2-sulfanylproprionate was identified in strawberries.[48]

**Olfactory Contribution of Ethyl 2-Sulfanylacetate in Wines.** The aromatic characteristics of the newly identified off-odor were described as follows by a sensory panel during the determination of its perception threshold in both water and model solution:

**Table 4. Quantitative Assays of Ethyl 2-Sulfanylacetate (ng/L) in Dry White and Rosé Wines from Various Appellations in France, Germany, Austria and New Zealand**

|  | origin (appellation) | vintage | variety | ethyl 2-sulfanylacetate |
|---|---|---|---|---|
| perception threshold |  |  |  | 200−400 |
| rejection threshold |  |  |  | 300−500 |
|  |  |  |  |  |
| white wines | Bordeaux (Graves) | 2009 | Sauvignon blanc | 225 ± 22 |
|  | Bordeaux (Graves) | 2008 | Sauvignon blanc | 759 ± 106 |
|  | Bordeaux (Pessac Léognan) | 2009 | Sauvignon blanc | 245 ± 30 |
|  | Bordeaux (Pessac Léognan) | 2008 | Sauvignon blanc | 364 ± 44 |
|  | Bordeaux (Entre Deux Mers) | 2009 | Sauvignon blanc | 797 ± 103 |
|  | Bordeaux (Entre Deux Mers) | 2009 | Sauvignon blanc | 670 ± 47 |
|  | Bordeaux (Entre Deux Mers) | 2008 | Sauvignon blanc | 745 ± 75 |
|  | Bordeaux (Entre Deux Mers) | 2008 | Sauvignon blanc | 1560 ± 13 |
|  | Bordeaux (Entre Deux Mers) | 2008 | Sauvignon blanc | 559 ± 79 |
|  | Bordeaux (Pauillac) | 2008 | Sauvignon blanc | 247 ± 29 |
|  | Bordeaux (Pauillac) | 2009 | Sauvignon blanc | 329 ± 40 |
|  | Loire (Sancerre) | 2009 | Sauvignon blanc | 169 ± 20 |
|  | Loire (Sancerre) | 2009 | Sauvignon blanc | 638 ± 58 |
|  | Loire (Sancerre) | 2009 | Sauvignon blanc | 348 ± 35 |
|  | Loire (Sancerre) | 2009 | Sauvignon blanc | 316 ± 22 |
|  | Loire (Sancerre) | 2009 | Sauvignon blanc | 407 ± 48 |
|  | Loire (Sancerre) | 2009 | Sauvignon blanc | 271 ± 32 |
|  | New Zealand (Marlborough) | 2008 | Sauvignon blanc | 262 ± 32 |
|  | Alsace | 2007 | Riesling | 1317 ± 158 |
|  | Austria (Wachau) | 2008 | Riesling | 548 ± 55 |
|  | Germany (Rheingau) | 2008 | Riesling | 214 ± 26 |
|  |  |  |  |  |
| rosés wines | Bordeaux (Graves) | 2009 | Merlot | 451 ± 54 |
|  | Provence (Bandol) | 2009 | Mourvedre, Grenache, Cinsault | 1105 ± 132 |
|  | Provence (Bandol) | 2009 | Mourvedre, Grenache, Cinsault | 933 ± 94 |
|  | Provence (Bandol) | 2009 | Mourvedre, Grenache, Cinsault | 1019 ± 119 |
|  | Provence (Côtes de Provence) | 2009 | Grenache, Cinsault, Syrah | 782 ± 94 |

irritating, baked beans, herbaceous, and *F. meleagris* bulbs. More-over, when the same test was carried out using young wines (vintage 2009) spiked with **1** in low concentrations (50−250 ng/L), assessors did not directly identify the off-odor, simply mention-ing a reduction in freshness and fruity nuances in the wine's aroma. When supplementation with **1** was increased (300−600 ng/L), it was perceived directly by the entire panel (data not shown) and caused a noticeable deterioration in varietal wine aroma.

The perception thresholds of **1** in water and model solution were 70 and 200 ng/L, respectively, and values ranged from 267 to 400 ng/L in two different dry white wines (2009 vintages). In addition, a paired comparison test was also performed to determine the off-odor's rejection threshold in order to under-stand its direct impact on wine aroma. For each concentration, the proportion of assessors who chose the sample without **1** is shown in Figure 2, illustrating the determination of its rejection threshold in two different Sauvignon Blanc wines, selected for their low **1** content. Lines (0.81*, 0.90***) correspond to the minimum number of assessors at the 5% and 1% level of significance in a paired test, respectively. The concentrations at which **1** was identified as an off-odor in dry white wines ranged from under 300 to 500 ng/L, indicating a strong dependence on the wine matrix.[49]

**Ethyl 2-Sulfanylacetate Content in Wines.** **1** was quantified in various white Sauvignon Blanc and Riesling wines from several French, Austrian, New Zealand, and German appellations, as well as several rosé wines made from Cabernet Sauvignon, Merlot, Mourvèdre, Grenache, Cinsault, and Syrah. All of the young white and rosé wines (2008 and 2009 vintages) analyzed con-tained **1** (Table 4). The average concentrations of **1** in the wines analyzed varied from 169 to 1560 ng/L. The highest values were found in Sauvignon Blanc wines from the Entre Deux Mers appellation in the Bordeaux region, while the majority of Pessac Léognan and Sancerre wines contained values close to its rejection threshold. Additionally, higher concentrations of **1** were also detected in Riesling wine samples from France (Alsace) and Austria (Wachau) than in those from Germany (Rheingau). Moreover, this compound was also present in rosé wines at levels comparable or higher to those measured in dry white wines.

**Impact of Juice Pressing Conditions on Ethyl 2-Sulfany-lacetate Concentrations in Wines.** General procedures for white wine production involve careful protection of must from oxidation. Our experiment revealed that winemaking methods were a significant factor in the formation of **1**. The use of antioxidant juice protection technology (inert) resulted in wines with lower concentrations of **1** (Figure 3). On the contrary, the
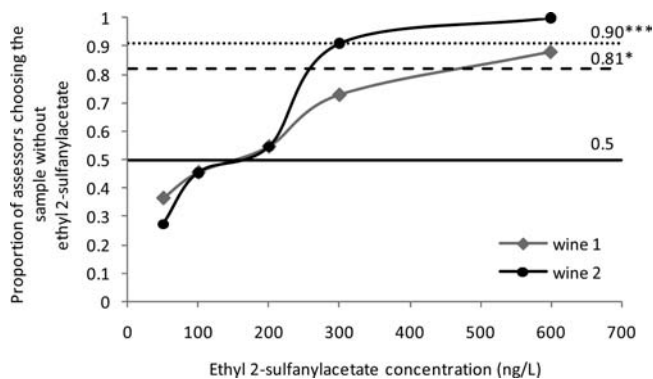
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**Figure 2.** Cumulative proportion of assessors choosing each white wine sample (wine 1, wine 2) without ethyl 2-sulfanylacetate addition, at each concentration used. The line at 0.5 represents the results obtained by random reponse, and the lines at 0.90 and 0.81 indicate the 5% (*) and 1% (***) significance criterion, respectively, for the determination of rejection threshold using the binomial distribution for a paired test ($N = 11$).
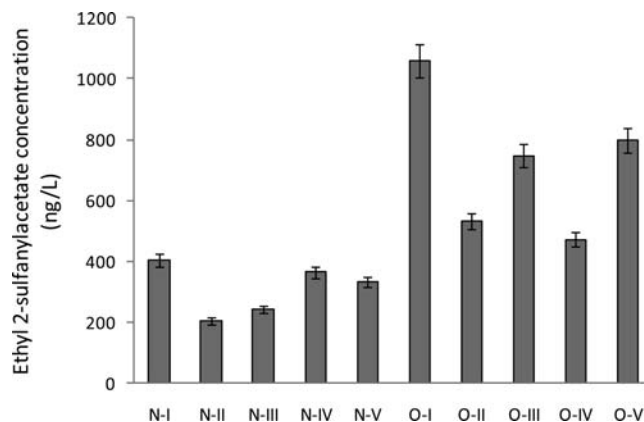


**Figure 3.** Quantitative assay of ethyl 2-sulfanylacetate in different young white wines (2009 vintage) elaborated from hard pressed juices extracted under nitrogen gas atmosphere (N) or with conventional vinification process, in contact with oxygen (O).
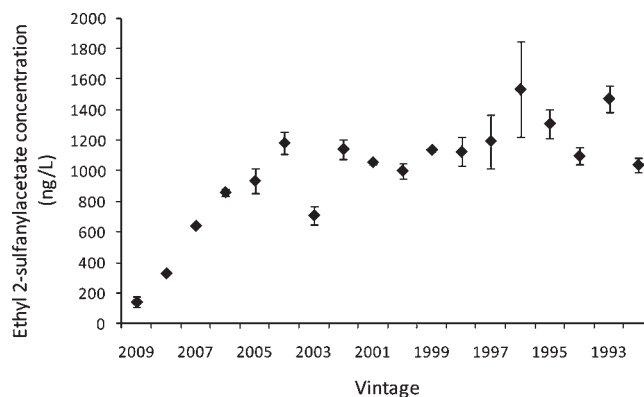


**Figure 4.** Ethyl 2-sulfanylacetate concentrations in Sauvignon blanc and Semillon blend wines from the same winery (Bordeaux appellation, Pauillac, France) in relation with age. All quantification assays were performed at April 2009.

presence of oxygen during the pressing (oxidation) significantly favored the development of this off-odor (**1**) (Figure 3).

The metabolic pathways leading to the formation of **1** in wine have not yet been elucidated and are likely to be relatively complex. However, it may be supposed that they are similar to those described for other thiol compounds in wine and cheese (ethyl 2-sulfanylpropionate and ethyl 3-sulfanylpropionate). In all situations, **1** is probably formed after esterification of the corresponding low molecular weight organic acid (thioglycolic acid) with ethanol during alcoholic fermentation. As suggested by Sourabié et al.[50] for ethyl 2-sulfanylproprionate and ethyl 3-sulfanylproprionate, the formation of the precursor acid (thioglycolic acid) may be related to an Ehrlich degradation reaction following the catabolism of a sulfur amino acid, i.e., cysteine, by *S. cerevisiae* yeast. **1** has also been reported in red wines,[51] which are obviously obtained from red varieties not with the same winemaking process.

To date, a great deal of attention has been paid to elucidating the impact of oxygen availability during alcoholic fermentation on concentrations of attractive flavor compounds, such as medium-chain fatty acid esters,[32,52] but fewer studies have focused on short-chain fatty acid esters.[53] It is known that the amount of molecular oxygen dissolved in must affects the metabolism of yeast cells.[54] Reduced oxygen availability has been reported to enhance the production of attractive flavor compounds, such as medium chain fatty acid esters, during alcoholic fermentation.[32] In contrast, Moio et al.[32] reported that, in some cases, a vinification process involving the presence of free oxygen increased the production of short-chain fatty acids (e.g., ethyl 3-methylbutanoate). Thus, in view of these results, we hypothesize that available dissolved oxygen in the must modulates yeasts metabolic activity, promoting **1** formation from its low molecular weight precursor. This off-odor's formation mechanism, as well as the parameters that control it, need to be elucidated in order to prevent spoilage of wine aroma.

**Impact of Bottle Aging on Ethyl 2-Sulfanylacetate.** The development of **1**, an off-odor volatile thiol, during bottle aging was studied by simultaneously analyzing 18 different vintages of one type of Bordeaux white wine. The **1** content in the wine increased during the bottle aging time (Figure 4). This result agreed with the findings presented by Tominaga et al.[18] concerning the proportional increase in ethyl 3-sulfanylproprionate

in Champagne wines after 13—15 years' of bottle aging. The formation of esters continues throughout the aging process, perhaps due to the presence of the corresponding organic acid in wine, together with large quantities of ethanol. The total ester concentration is governed by the wine's composition and age. The formation mechanisms of these compounds have not yet been determined.

In conclusion, in this study, we analyzed an off-odor due to a thiol in white wines and identified it as ethyl 2-sulfanylacetate. Additional experiments to evaluate the sensory properties of this volatile compound revealed that it had a very low perception threshold and a moderate rejection threshold in various white wines, indicating that the contribution of **1** to off-odors in wine was highly dependent on the type of wine. **1** was quantified in a range of white and rosé wines of different origins, revealing concentrations from below the aroma perception threshold to several times that value. Oxygen exposure during juice preparation appeared to play a role, as juices pressed in an inert atmosphere had lower **1** levels in the finished wine than those exposed to oxygen. Different vintages of one Bordeaux wine were analyzed for **1** to assess the impact of bottle aging. All the different vintages of the wines contained **1**, with higher concentrations in older vintages. Further studies will also be required to investigate its formation pathway in wine.

**Corresponding Author**
*Telephone: +33-557575860. Fax: +33-557575813. E-mail: philippe.darriet@oenologie.u-bordeaux2.fr.

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