

Analysis, Occurrence, and Potential Sensory Significance of Five Polyfunctional Mercaptans in White Wines

LAURA MATEO-VIVARACHO, JULIÁN ZAPATA, JUAN CACHO, AND VICENTE FERREIRA*

Laboratory for Flavor Analysis and Enology, Analytical Chemistry, Faculty of Sciences, University of Zaragoza, 50009 Zaragoza, Spain

A previously developed analytical method has been improved, validated and adapted for the analysis of 2-furfurylthiol (FFT), 4-methyl-4-mercaptop-2-pentanone (MP), 3-mercaptophexyl acetate (MHA), 3-mercaptophexanol (MH) and benzylmercaptan (BM) in 136 white wines from different parts of the world. The overall uncertainty of the determinations was found to be around 20%, which was considered satisfactory given the low levels at which these compounds are found. The levels ranged from the method detection limits (0.5; 0.6; 2.0; 8.0 and 0.5 ng/L for FFT, MP, MHA, MH and BM, respectively) to 225; 87.9; 591; 7255 and 131 ng/L, which implies that nearly all of them can reach more than 100 Odor Units in some wines. The levels are significantly linked to both the grape variety (with the exception of FFT) and to the origin (in the case of Sauvignon Blanc samples), however, the range of variation within groups are so large that clear clusters could not be observed. Different sensory tests carried out on white wine models showed that all these compounds, even at low concentration, play an outstanding role on the aroma of wine, contributing to fruity, fresh and green notes. In some wines they are at concentrations high enough to act as genuine impact compounds.

KEYWORDS: Thiols; polyfunctional mercaptan; wine; Sauvignon blanc; white wine; variety; origin; impact compound; fruitiness

INTRODUCTION

There are several volatile or semivolatile polyfunctional mercaptans that can be responsible for positive and pleasant odor and flavor nuances of different foods such as meat (1), coffee (2), grapefruit (3), passion fruit (4), green tea (5), onions (6), or wine. Some of these polyfunctional mercaptans have the lowest olfactory detection thresholds known, which can be as low as 0.3 ng/L. Over the years, the presence of some of these compounds (2-furfurylthiol, FFT; 4-methyl-4-mercaptop-2-pentanone, MP; 3-mercaptophexanol, MH; 3-mercaptophexyl acetate, MHA; and benzylmercaptan, BM) in wine has been reported, but the role of these compounds in the sensory characteristics of wine is not yet well understood because of the difficulties involved in their analytical determination. These difficulties are related to the complexity of the matrix, the extremely low concentration levels that must be determined, and the well-known instability and elusiveness of these compounds.

Since these compounds were first discovered in wine, different works have reported their presence in wines elaborated with a limited number of cultivars. In 1995 and 1996 Darriet et al. (7) and Bouchilloux et al. (8) identified MP as a key varietal aroma compound in Sauvignon blanc wines, determining that those wines could contain between a few and 15 ng/L of this compound. Guth in 1997 (9) reported the content and sensory role of this compound in a sample from Scheurebe. By using a stable isotope dilution assay, this researcher reported that MP was present at

400 ng/L. In addition to MP, two other polyfunctional mercaptans (MH and MHA) were identified and quantified by Tomi-naga in different Sauvignon blanc wines from Bourdeaux and Sancerre and in red wines elaborated with Cabernet Franc and Merlot from Bourdeaux (10–13) and in other white varietals (14, 15). These researchers found levels ranging from <1 to 60 ng/L in the case of MP, whereas MH and MHA were found to be present at concentrations of up to several micrograms per liter and 500 ng/L, respectively. Other researchers have detected these compounds in the GC–olfactometric profile of several wines (16, 17) or even by sensory analysis using a trained panel (18, 19) in some cultivars, especially Sauvignon blanc.

The sensory relevance of MH and MHA has also been demonstrated in other papers. The outstanding role of MH in the aroma of rosé wines was demonstrated by Murat et al. (20), Ferreira et al. (21), and recently by Schneider et al. (22). In 2005 Fretz (23) also found that MH is an aroma impact compound of Petite Arvine wine. More recently, MH has been identified as a key compound in the aroma of botrytized Sauternes wines (24–27) being present at nearly constant levels as high as 5000 ng/L. Fedrizzi (28–30) published data about the concentrations of MH and MHA in different cultivars such as Sauvignon blanc, Traminer, Verdicchio, and Mueller Thurgau, and Campo et al. (16) demonstrated that the characteristic tropical fruit note of Verdejo wines is due to MHA. The importance of MH and MHA in Sauvignon blanc wines from different parts of the world has been recently reported by Lund et al. (31).

In the case of MP, data about its presence and potential importance in wines from other cultivars or origins is less clear.

*Author to whom correspondence should be addressed (phone 34 976762067; fax 34 976761292; e-mail vferre@unizar.es).

In a work about wines from Muscadet, Sauvignon blanc, and Bacchus cultivars Schneider et al. (32) found that this compound was in most cases below the method detection limits. In 2004, Escudero et al. (33) reported that very low amounts of MP (5 ng/L) were essential for the perception of freshness in a neutral white wine from Maccabeo, in which the characteristic scent of this compound could not be perceived. More recently, Pet'ka et al. (34) found that 2-methyl-3-furanthiol, MHA, and MP were among the most relevant odorants of white wines made with Devin grapes.

Another important compound, FFT, was found in wine in 1999 by Blanchard (35, 36), who attributed its presence in wine to oak wood. The relevance and range of occurrence of this compound in Bourdeaux red wines and Champagnes (1.0–40 ng/L) was shown by Tominaga and Bouchilloux (10, 37). Tominaga et al. (38, 39) identified and quantified for the first time BM, together with FFT, in old Champagnes and in some other white (Chardonnay, Sémillon, and Sauvignon blanc) and red (Merlot and Cabernet Sauvignon) wines, finding the highest concentrations (400 ng/L) in Champagnes aged for 15 years. Quantitative data about the presence of 2-methyl-3-furanthiol and FFT in some white (Sauvignon blanc and Chardonnay) and red wines (Merlot and Cabernet Sauvignon) and also in Champagnes have been reported by Tominaga and Dubourdieu (40).

All of these studies suggest that polyfunctional mercaptans play an outstanding role in the aroma of wines from very specific types, varietals, and regions, although the role of variety is not completely clear nor is the influence of geographical factors. In addition, in most of these studies just a limited number of samples from well-defined and specific wine types were analyzed, which does not allow having a clear picture about the real sensory role played by these compounds in normal (neutral) white table wines.

The question that this work will try to answer is which are the natural ranges of occurrence of these compounds and their potential sensory importance in a wide group of samples from different parts of the world, including both neutral samples and

samples known for the relevance of polyfunctional mercaptans in their aroma, especially Sauvignon blanc wines. To this aim, a previously developed method based on the in-cartridge pentafluorobenzyl-alkylation of polyfunctional mercaptans has been improved and adapted for the large-scale analysis (duplicated analysis of 136 samples, white and rosé wines) of these compounds.

MATERIALS AND METHODS

Reagents. *n*-Hexane for organic trace analysis (UniSolv) and dichloromethane (SupraSolv), methanol (SupraSolv), and ethanol, gradient grade for liquid chromatography (LiChrosolv), were from Merck (Darmstadt, Germany). Diethyl ether for instrumental analysis and mercaptoglycerol were from Fluka (Buchs, Switzerland). Anhydrous sodium sulfate was of analysis ACS-ISO quality from Panreac (Barcelona, Spain). Ethylenediaminetetraacetic acid disodium salt 2-hydrate (EDTA), L-cysteine hydrochloride hydrate 99%, 1,4-dithioerythritol, octafluoronaphthalene 96% (OFN), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were from Aldrich (Steinheim, Germany). *O*-Methylhydroxylamine hydrochloride purum >98% and 2,3,4,5,6-pentafluorobenzylbromide (PFBBr) were from Fluka. 4-Mercapto-4-methyl-2-pentanone (MP) 1% PG and 3-mercaptopohexyl acetate (MHA) were from Oxford Chemicals (Hartlepool, U.K.). 2-Furfurylthiol (FFT) and 3-mercaptopohexanol (MH) were from Lancaster (Strasbourg, France). Benzyl mercaptan (BM), 2-phenylethanethiol, and 4-methoxy- α -toluenethiol were from Fluka. Bond Elut-ENV resins, prepakced in a 50 mg cartridge (1 mL total volume) and semiautomated SPE Vac Elut 20 station, were from Varian (Walnut Creek, CA).

Wine Samples. The 130 white and rosé table wines plus 6 wines from Sauternes analyzed in the study are given in Table 1 (see also the Supporting Information). The alcoholic degree of all wines was between 12 and 14%. Wines from the Midi-Pyrenees region are controlled wines made with grapes from well-demarcated vineyards and made following nearly homogeneous wine practices.

Proposed Method. In a 24 mL screw-capped vial, spike 23 mL of wine with 0.2 g of EDTA (5 g/L) and 0.6 g of L-cysteine chloride (0.1 M) and keep it shaking for 2 min. After this, transfer a small volume of the wine to a 20 mL volumetric flask, spike it with 15 μ L of an ethanolic solution containing 1400 μ g/L of 2-phenylethanethiol as internal standard, shake to

Table 1. Identity, Origin, Variety, and Vintage of the Analyzed Wines

	wine	origin	cultivar	vintage
App. Sauternes Controlé, France (SAUT), <i>n</i> = 6	Chateau Raymond Lafon Chateau Violet-Lamothe Chateau Lafaurie-Peyraguey Castelnau de Suduiraut Chateau Laribotte Chateau Lamothe	Sauternes Contrôlée (France) Sauternes Contrôlée (France) Sauternes Contrôlée (France) Sauternes Contrôlée (France) Sauternes Contrôlée (France) Sauternes Contrôlée (France)	Sémillon, Sauvignon blanc Sémillon, Sauvignon blanc Sémillon, Sauvignon blanc Sémillon, Sauvignon blanc Sémillon, Sauvignon blanc Sémillon, Sauvignon blanc	2000 2002 2003 2003 2003 2004
Sauvignon blanc, Midi Pyrenees (SBPYR), <i>n</i> = 10	IFV Sud-Ouest v134 IFV Sud-Ouest v343 IFV Sud-Ouest v344 IFV Sud-Ouest v345 IFV Sud-Ouest v238 IFV Sud-Ouest v346 IFV Sud-Ouest v434 IFV Sud-Ouest v435 IFV Sud-Ouest v436 IFV Sud-Ouest v437	Midi Pyrenees (France) Midi Pyrenees (France)	Sauvignon blanc Sauvignon blanc Sauvignon blanc Sauvignon blanc Sauvignon blanc Sauvignon blanc Sauvignon blanc Sauvignon blanc Sauvignon blanc Sauvignon blanc	2005 2008 2008 2008 2007 2008 2009 2009 2009 2009
Sauvignon blanc, DO Rueda Spain (SBR), <i>n</i> = 10	Herederos del Marqués de Riscal José Pariente Doña Beatriz Villa Narcisa Palacio de Menade Cuatro Rayas Sanz Nieve Palacio de Bornos Palacio de Menade	DO Rueda (Spain) DO Rueda (Spain)	Sauvignon blanc Sauvignon blanc Sauvignon blanc Sauvignon blanc Sauvignon blanc Sauvignon blanc Sauvignon blanc Sauvignon blanc Sauvignon blanc Sauvignon blanc	2007 2007 2007 2007 2007 2008 2008 2008 2008 2008

Table 1. Continued

	wine	origin	cultivar	vintage
Sauvignon blanc Loira Valley (SBLO), n = 11				
	Vendangé à la Main	Sancerre (France)	Sauvignon blanc	2004
		Sancerre (France)	Sauvignon blanc	2005
		Sancerre (France)	Pouilly-Fumé	2006
	Saulétas	Sancerre (France)	Sauvignon blanc	2007
	Le Domaine du Moulin	Vin du Val de Loire (France)	Sauvignon blanc	2007
	Les Chailloux Chatelain	Sancerre, Loire (France)	Pouilly-Fumé	2007
	Les Boffants Charles Dupuy	Sancerre, Loire (France)	Sauvignon blanc	2007
	Le Calvaire Domaine Philippe Renaud	Sancerre, Loire (France)	Pouilly-Fumé	2007
	Domaine Les Renarderies	Sancerre, Loire (France)	Sauvignon blanc	2007
	Le Piere de al Chapelle	Sancerre, Loire (France)	Sauvignon blanc	2007
	Domaine Sébastien Riffault AKMENINE	Sancerre, Loire (France)	Sauvignon blanc	2008
Sauvignon blanc, New Zealand (SBNZ), n = 14				
	Staete Landt, estate grown	Marlborough (New Zealand)	Sauvignon blanc	2006
	Wairau River, family estate	Marlborough (New Zealand)	Sauvignon blanc	2005
	Woodthorpe Vineyard, Te Mata state	New Zealand	Sauvignon blanc	2007
	Cape Crest, Te Mata state	New Zealand	Sauvignon blanc	2006
	Cloudy Bay	Marlborough (New Zealand)	Sauvignon blanc	2008
	Wither Hills	Marlborough (New Zealand)	Sauvignon blanc	2008
	Schubert	Martinborough (New Zealand)	Sauvignon blanc	2008
	Goldridge	Marlborough (New Zealand)	Sauvignon blanc	2008
	Coopers Creek	Marlborough (New Zealand)	Sauvignon blanc	2008
	Dog Point	Marlborough (New Zealand)	Sauvignon blanc	2008
	Neudorf Nelson	New Zealand	Sauvignon blanc	2008
	Cape Cambell	Marlborough (New Zealand)	Sauvignon blanc	2008
	Cape Cambell	Marlborough (New Zealand)	Sauvignon blanc	2008
	Cape Cambell	Marlborough (New Zealand)	Sauvignon blanc	2008
Sauvignon blanc, Chile (SBCH), n = 12				
	EQ	Chile	Sauvignon blanc	2007
	Ninbus	Chile	Sauvignon blanc	2008
	Casas del Bogane	Chile	Sauvignon blanc	2008
	Floresta	Chile	Sauvignon blanc	2007
	Montes	Chile	Sauvignon blanc	2007
	Quintay	Chile	Sauvignon blanc	2008
	TH Undurraga	Chile	Sauvignon blanc	2007
	Veramonte	Chile	Sauvignon blanc	2008
	Castillo de Molina	Chile	Sauvignon blanc	2008
	Rua Especial	Chile	Sauvignon blanc	2008
	De Martino	Chile	Sauvignon blanc	2007
	JU Bouchou	Chile	Sauvignon blanc	2008
Verdejo, DO Rueda Spain (VER), n = 10				
	Herederos del Marqués de Riscal	DO Rueda (Spain)	Verdejo	2007
	José Pariente	DO Rueda (Spain)	Verdejo	2007
	Villa Narcisa	DO Rueda (Spain)	Verdejo	2007
	Blume	DO Rueda (Spain)	Verdejo	2007
	Doña Beatriz	DO Rueda (Spain)	Verdejo	2007
	Valpincia	DO Rueda (Spain)	Verdejo	2008
	Valtropín	DO Rueda (Spain)	Verdejo	2008
	Cuatro Rayas	DO Rueda (Spain)	Verdejo	2008
	Aura	DO Rueda (Spain)	Verdejo	2008
	Viña Albali	DO Rueda (Spain)	Verdejo	2008
Albariño, DO Rías Baixas Spain (ALB) n = 10				
	Pazo Piñeiro de Lusco	DO Rías Baixas (Spain)	Albariño	2004
	Zios de Lusco	DO Rías Baixas (Spain)	Albariño	2006
	Pazo Señorans	DO Rías Baixas (Spain)	Albariño	2007
	Albariño Mar de Frades	DO Rías Baixas (Spain)	Albariño	2007
	Albariño do Ferreiro	DO Rías Baixas (Spain)	Albariño	2007
	Viña Lareira	DO Rías Baixas (Spain)	Albariño	2008
	Martín Codax	DO Rías Baixas (Spain)	Albariño	2008
	Condes de Albarei	DO Rías Baixas (Spain)	Albariño	2008
	Vionta	DO Rías Baixas (Spain)	Albariño	2008
	LEiras	DO Rías Baixas (Spain)	Albariño	2008
Chardonnay, several DO, Spain (CHR), n = 9				
	Enate	DO Somontano (Spain)	Chardonnay	2008
	Idrias	DO Somontano (Spain)	Chardonnay	2008
	Fábrega	DO Somontano (Spain)	Chardonnay	2008
	Laus	DO Somontano (Spain)	Chardonnay	2008
	Aragus	DO Somontano (Spain)	Chardonnay	2008
	Raimat	DO Penedés (Spain)	Chardonnay	2008
	Consigna	Ciudad Real (Spain)	Chardonnay	2008
	Veranza	Huesca (Spain)	Chardonnay	2008
	Gran Feudo	DO Navarra (Spain)	Chardonnay	2008

Table 1. Continued

	wine	origin	cultivar	vintage
Maccabeo, several DO Spain (MAC), <i>n</i> = 9	Monte Ducay	DO Cariñena (Spain)	Maccabeo	2007
	Monopole	DO Rioja (Spain)	Viura (Maccabeo)	2008
	Faustino VII	DO Rioja (Spain)	Viura (Maccabeo)	2008
	Paternina	DO Rioja (Spain)	Viura (Maccabeo)	2008
	Diacoño	DO Navarra (Spain)	Viura (Maccabeo)	2008
	Pleno	DO Navarra (Spain)	Viura (Maccabeo)	2008
	Viña Miedes	DO Calatayud (Spain)	Maccabeo	2008
	Navitum	DO Calatayud (Spain)	Maccabeo	2008
	Borsao	DO Campo de Borja (Spain)	Maccabeo	2008
Maccabeo, Xarel-lo, Parellada, DO Penedés Spain (CAT), <i>n</i> = 5	Vall de Juy	DO Penedés (Spain)	Maccabeo, Xarel-lo, Parellada	2007
	Ermita D'Espiells	DO Penedés (Spain)	Maccabeo, Xarel-lo, Parellada	2007
	Ermita d'Espiells	DO Penedés (Spain)	Maccabeo, Xarel-lo, Parellada	2008
	Conde de Caralt	DO Penedés (Spain)	Maccabeo, Xarel-lo, Parellada	2008
	René Barbier	Catalunya (Spain)	Maccabeo, Xarel-lo, Parellada	2008
Grenache rosé wine, several DO Spain (RGR), <i>n</i> = 9	Ralon Rosado	DO Navarra (Spain)	Garnacha	2007
	Gran Feudo Rosado	DO Navarra (Spain)	Garnacha	2007
	Valdemadera Rosado	DO Cariñena (Spain)	Garnacha	2007
	Viña Miedes Rosado	DO Calatayud (Spain)	Garnacha	2007
	Reino de los Mallos	Ribera del Gállego (Spain)	Garnacha	2008
	Señorio de Sarria	DO Navarra (Spain)	Garnacha	2008
	Beamonte	DO Navarra (Spain)	Garnacha	2008
	Baltasar Gracián	DO Calatayud (Spain)	Garnacha	2008
	Homenaje	DO Navarra (Spain)	Garnacha	2008
rosé wines from different origins (R), <i>n</i> = 21	House Wine Rosé	Vin de Pays D'Oc (France)		2006
	Le Froglet Rosé	Vin de Pays D'Oc (France)		2006
		Vin de Pays D'Oc (France)		2006
		Bordeaux (France)		2006
	Les Ruettes	Sancerre (France)		2006
	Domaine Grand Milord, Rosé Organic	Vin de Pays du Gard (France)		2006
	Domaine de Verlaque Rosé	Côtes de Provence (France)		2006
	Las Falleras	DO Utiel-Requena (Spain)		2006
	Ralon Rosado	DO Navarra (Spain)		2007
	Manón Pinot Grigio Sicilia-Ramato	Blush (Italy)		2006
	Woodhaven	California (USA)	Shiraz rosé	2006
	White Zinfandel Rosé	California Iodi (USA)		2006
	Kaituna Hills, East Coast	New Zealand	Merlot-Cabernet Sauvignon	2006
	Casa Leona	Rapel (Chile)		2006
	Los Nucos	Valle Central (Chile)	Shiraz-Cabernet rosé	2007
	IFV Sud-Ouest	Midi Pyrenees (France)	Négrette	2008
	IFV Sud-Ouest	Midi Pyrenees (France)	Négrette	2008
	IFV Sud-Ouest	Midi Pyrenees (France)	Négrette	2008
	IFV Sud-Ouest	Midi Pyrenees (France)	Négrette	2009
	IFV Sud-Ouest	Midi Pyrenees (France)	Négrette	2009
	IFV Sud-Ouest	Midi Pyrenees (France)	Négrette	2009

ensure a complete dissolution, and make up to volume with the wine. This volume is transferred to a 24 mL screw-capped vial to which 0.2 g of *O*-methylhydroxylamine was added, the mixture stirred for 15 s, and the vial purged gently with pure nitrogen, sealed, and incubated in a water bath at 55 °C for 45 min. Six milliliters of this incubated sample are then loaded onto a 50 mg BondElut-ENV SPE cartridge (previously conditioned with 1 mL of dichloromethane, 1 mL of methanol, and 1 mL of water). Some wine major volatiles are removed by rinsing with 4 mL of a 40% methanol/water solution 0.2 M in phosphate buffer at pH 7.7 and, after this, with 1 mL of water. A second internal standard is added to the cartridge; 20 μ L of an ethanolic solution containing 150 μ g/L of 4-methoxy- α -toluenethiol and 200 μ L of water are mixed and loaded onto the cartridge. Mercaptans retained in the cartridge are directly derivatized by passing first 1 mL of an aqueous solution of DBU (6.7%) and later 50 μ L of a 2000 mg/L solution of PFBr in hexane, and letting the cartridge imbibe with the reagent for 20 min at room temperature (25 °C). Excess of reagent is removed by adding 100 μ L of a 2000 mg/L solution of mercaptoglycerol in 6.7% DBU aqueous solution, and letting the cartridge react again for 20 min at room temperature. The cartridge is then rinsed with 4 mL of a 40% methanol/water solution 0.2 M in H₃PO₄ and with

1 mL of water. Derivatized analytes are finally eluted with 600 μ L of a solvent mixture (hexane 25% in diethyl ether), and then 10 μ L of the chromatographic internal standard solution (octafluoronaphthalene 22.5 μ g/L in hexane) is added to the extract. The eluate is finally washed with five 1 mL volumes of brine (200 g/L NaCl water solution), transferred to a 2 mL vial, and spiked with a small amount of anhydrous sodium sulfate. Four microliters of this sample is directly injected in cold splitless mode into the GC-negative chemical ionization (NCI) MS system.

GC-NCI-MS Analysis. The apparatus is a Shimadzu QP-2010Plus gas chromatograph with a quadrupole mass spectrometric detection system. Four microliters of extract is injected in a split/splitless liner packed with silanized glass wool in a Shimadzu PTV injector. The initial temperature of the injector is 65 °C, and after 25 s, it is heated at 16 °C/s to 260 °C, remaining at this temperature until the end of the analysis. The carrier gas is He, flowing through the column during the splitless time (4.15 min) at 2.69 mL/min and then, when the split valve is opened, the flow is fixed at 1.44 mL/min. The column is a Factor Four capillary column DB-5 from J&W Scientific, 20 m \times 0.18 mm i.d., with 0.18 μ m film thickness. The column initial temperature is 40 °C for 4.15 min, heated to 140 at 25 °C/min, then to 180 at 15 °C/min, then to 210 at 30 °C/min, and finally to 280 at 250 °C/min, remaining at that

Table 2. Basic Method Calibration Data^a

analyte	IS	av slope	RSD (%)	av R
FFT	4Met α Tol	0.00205	16.1	0.9996
MP	OFN	0.00000357	11.3	0.9982
MHA	OFN	0.00000359	9.9	0.9990
MH	4Met α Tol	0.000513	28.7	0.9962
BM	4Met α Tol	0.00423	6.9	0.9996

^a Internal standards used for each analyte. Average slopes found in seven independent standard addition experiments with their relative standard deviation. Correlation coefficients (R) are also the mean of the seven values.

temperature for 10 min. The ion source is operated in NCI mode using methane at 3 bar as reagent gas. The temperature of the ion source was 220 °C, and the interface was kept at 270 °C. The analytes and internal standards ions are acquired in the single ion monitoring (SIM) mode from minute 5.5 to minute 17 at 0.18 s/point: OFN is quantified with *m/z* 272; FFT, retention time, 11.18 min, is quantified with *m/z* 274 (113 as qualifier ion); MP, 11.88 min, is quantified with *m/z* 160 (194); MHA, 12.25 min, with *m/z* 175 (194); MH, 12.03 min, with *m/z* 133 (194); and BM, 12.13 min, with *m/z* 284 (162). Finally, the quantification of the internal standards was carried out with *m/z* 135 (194) and 314 for 2-phenylethanethiol (12.48 min) and 4-methoxy- α -toluenethiol (12.835 min), respectively. To obtain the concentration data, the corresponding analyte peak relative areas were simply divided by the average slopes calculated in **Table 2**.

Method Validation. Internal Standards, Linearity, and Matrix Effects. Seven calibration curves were built in different wines: 5 white wines (1 Sauvignon blanc from Loira, 1 from Chile, 1 from Verdejo, 1 from Chardonnay, 1 from Maccabeo), 1 Sauternes wine, and 1 rosé wine; furthermore, an eighth calibration curve was prepared by adding the analytes directly to the cartridge (without wine). Seven calibration points were used in all cases, and each calibration point was analyzed in duplicate. Concentrations for each compound in calibration curves were in the following ranges: FFT from 3.96 to 253 ng/L; MP from 6.25 to 400 ng/L; MHA from 12.4 to 795 ng/L; MH from 121 to 7796 ng/L; and BM from 3.97 to 254 ng/L. Per each analyte and wine, three standard addition lines (one per potential internal standard, 2-phenylethanethiol, 4-methoxy- α -toluenethiol, and OFN) were built. The internal standards for each analyte were finally selected as those providing the least dissimilar slopes between different wines.

Method Recovery and Limits of Detection. During the analysis of real samples, wines spiked with the analytes (4 ng/L FFT; 75 ng/L MP; 150 ng/L MHA; 1500 ng/L MH; and 45 ng/L BM) were included in the batches to control the system. These samples were used, by comparison with their respective unspiked samples, to determine the overall system recovery.

Method limits of detection and quantification were determined by the analysis of real samples, which were defined as the concentration of analyte in wine which would give a signal 3 or 10 times higher than the noise, respectively.

Sensory Role of Polyfunctional Mercaptans. A model fruity white wine was prepared. Such model was made from a dearomatized neutral white wine sample made with Maccabeo. Dearomatization took place by adding 4 g of Amberlite XAD-2 resins (Rhom and Hass, Philadelphia, PA) to 1 L of wine for 24 h with gentle agitation). Dearomatized wine was then partially rearomatized by adding ethyl esters, fusel alcohol acetates, terpenols, cinnamates, γ -lactones, vanillins, and ethyl cinnamates at the concentrations usually found in neutral wines (33, 41). The model was spiked with different amounts of FFT (1.9, 3.5, 5.3, 21.1, and 79.2 ng/L), MP (1.0, 1.7, 3.4, and 10.0 ng/L), MHA (6.4, 11.4, 25.0, 50.0, and 125 ng/L), MH (20.5, 82.1, 148, 246, 850, and 1497 ng/L), and BM (0.8, 1.4, 3.0, 8.1, and 22.4 ng/L). The sensory effect of these additions was evaluated by a panel of 12 trained judges; the panelists compared the spiked sample with the glass containing the unspiked reference, first in triangular tests to assess the existence of differences and, later, in the cases in which significant differences were found, by freely describing the aroma differences found in the spiked sample. The descriptors and their citation frequency were recorded to provide a description of the sample aroma.

RESULTS AND DISCUSSION

Method Setup. We have previously developed a method for the analysis of polyfunctional mercaptans in wines (42). However, the

large-scale application of the method had failed several times due to different reasons, such as persistent problems with blanks, the instability of spiking solutions, and the deterioration of chromatographic performance, particularly evident in the case of MH.

Blank problems seem to have three major causes. The most important is related to the handling of the concentrated standard samples used for standardization and calibration. The laboratory surroundings become easily contaminated with small but perceptible levels of these compounds that blur the small signals found in most of the samples. These problems could not be completely avoided even after taking elemental precautions such as working with the standards in a different laboratory and keeping all of the equipment perfectly clean. It became evident that no one pure standard or concentrated solution ($C > 1000$ mg/L) could be handled in the 2 weeks before the analysis of the samples, which implied that only diluted solutions ($C < 10$ mg/L) were used for method control and that a complete standardization was carried out only once all of the samples have been analyzed. A second source of contaminations arose from the deuterated analogues. Small levels of the unlabeled molecules in the solutions caused serious interferences in the samples containing the lowest levels of the analytes. Similarly, the use of diluted (μ g/L) spiking solutions containing mixtures of mercaptans as internal standards also seemed to contribute to the presence of spurious peaks and to the general increase of the signal noise. Because of this, it was decided to reduce the number of internal standards used in the procedure and to avoid the use of the deuterated molecules. These decisions brought about a clear improvement on the blank samples, and it was only after that, that good and unequivocal signals from the analytes in some of the samples could be obtained. Only two surrogate standards (2-phenylethanethiol and 4-methoxy- α -toluenethiol, 4Met α Tol), added in different steps of the procedure, and the chromatographic standard (octafluoronaphthalene, OFN) were finally kept in the procedure. As the ability of these standards to correct for matrix effects is considerably lower than that of the deuterated analogues, different essays were developed to evaluate the intensity of such matrix effects.

As for the instability of the spiking solutions, it is well-known that all mercaptan solutions are unstable, and the contact with oxygen must be kept minimal. However, we noted in a previous work (49) that the presence in the calibration solution of 2-methyl-3-furanthiol, a particularly unstable molecule, can induce the oxidation of the other analytes present in that solution, even working under reasonable protective measures. Because of this, 2-methyl-3-furanthiol was excluded from the standard solution, and this compound has not been used at all during this work.

Finally, for an accurate quantification of MH the chromatographic system must be in optimal conditions because its derivative is strongly adsorbed in a dirty chromatographic inlet. Such adsorption causes first tailing and then a shift in retention time and can eventually lead to complete peak extinction. To overcome this problem, the injected sample volume was limited to 4 μ L, and whenever a slight peak distortion was noted, both the injector liner and the chromatographic precolumn were replaced. In practice, this means that the aforementioned maintenance operations should be done every 15–20 injections.

Method Validation. Eight linear calibration graphs were built by carrying out standard addition experiments on seven different wines and a synthetic sample. For each analyte, the internal standard producing straight lines least dissimilar among wines was selected as the most adequate. A summary of the results is given in **Table 2**. As can be seen, linearity is in general satisfactory in all cases, with average correlation coefficients of > 0.996 in all cases. Linear ranges include the normal range of occurrence of these compounds in wine. The calibration graphs built on

different matrices (normalized by their corresponding internal standard) were slightly different but, as can be seen, the standard

Table 3. Recovery Assay^a

sample	FFT	MP	MHA	MH	BM
SBNZ2 recovery	26.8 (31.5) 118	88 (174) 115	443 (618) 117	7255 (9295) 136	6.78 (60.3) 119
CAT1 recovery	3.51 (7.27) 94	1.18 (83.7) 110	<2 (145) 97	78.0 (1413) 89	3.82 (59.2) 123
SBFR5 recovery	7.43 (11.6) 104	5.46 (65.5) 80	118 (289) 114	3002 (4907) 127	8.56 (50.0) 92
SBFR2 recovery	102 (108) 148	2.78 (65.8) 84	56.7 (172) 77	60.7 (1981) 128	2.32 (43.3) 91
SBFR3 recovery	127 (131) 108	<0.6 (95.2) 127	36.8 (185) 99	<8 (1440) 96	12.1 (59.8) 106
RGR3 recovery	3.05 (6.61) 89	2.22 (94.5) 123	425 (621) 131	463 (1693) 82	6.90 (57.7) 113
RNE2 recovery	56.3 (60.7) 111	2.75 (65) 83	124 (236) 75	25.8 (1616) 106	3.76 (48.3) 99
av recovery	110.3	103.1	101.4	109.1	106.1
mean standard error	7.3	7.7	7.9	8.0	4.8
standard deviation	19	20	21	21	13

^a Results of the analysis of seven spiked (concentration results in parentheses) and unspiked wine samples (concentration results in bold). Concentrations are given in ng/L and recovery is expressed as %. All results are the average of two replicated analyses. Amounts spiked in all cases were 4 ng/L FFT, 75 ng/L MP, 150 ng/L MHA, 1500 ng/L MH, and 45 ng/L BM.

deviations of the slopes were not very high except for MH, for which quantification was more problematic due to the broad and tailing peak obtained and for which some of the samples contained very different levels. The average slopes were used for the estimation of the levels of these compounds in the different samples analyzed. The efficiency of this approach can be measured by means of the recovery data shown in **Table 3**. Recovery has been calculated as $(C_{\text{spiked}} - C_{\text{unspiked}})/C_{\text{added}}$; as can be seen in the table, the mean recoveries do not differ significantly from 100% in any case, which indicates that, on average, the method is giving reliable estimates for all analytes.

The overall method uncertainty can be also estimated from the deviation standards of the recoveries found in the last row of the table. As can be seen, overall uncertainty ranges from 13 to 21%. Such uncertainty values were considered to be satisfactory for the low concentrations at which these compounds are found.

Quantitative Results. Results of the analysis of the 5 analytes in 136 wines from different countries and types are summarized in **Table 4**. The table is divided into three sections: the first one gives results from the 57 wines from Sauvignon blanc; the second, from 73 white or rosé wines made from different grape varieties; and the third includes results from 6 wines from Sauternes taken just as reference.

As can be seen, ranges of occurrence are widest in the case of wines from Sauvignon blanc because, leaving aside MHA and MH, some samples made with those grapes were found to contain the compounds below the method detection limits, whereas some other samples made with this varietal reached the maximum levels found in the whole data set. In geometric terms, this means that the quotient between the maxima and the minima can be up to nearly 3 orders of magnitude in the case of MH and nearly 2 in the case of MHA. As odor thresholds are really low, these differences

Table 4. Summary of the Results and Basic Descriptive Statistics Obtained in the Analysis of Five Polyfunctional Mercaptans in a Large Sample of Wines^a

	analyte: threshold: detection limit:	FFT	MP	MHA	MH	BM
Sauvignon blanc						
Sauvignon blanc all of them, <i>n</i> = 57	range max/min SD RSD (%) mean median range OAV	<0.5–225 450 38.9 223 17.4 3.9 <1.25–562	<0.6–87.9 146 16.9 166 10.2 4.6 <1–146	6.7–591 88.2 117 120 97.1 53.7 1.67–148	25.8–7256 281 1566 88.2 178 1775 1368 0.43–121	<0.5–131 262 27.0 178 15.2 5.1 <1.77–437
Other Cultivars						
non Sauvignon blanc different cultivars and origins, <i>n</i> = 73	range max/min SD RSD (%) mean median range OAV	1.4–56.3 40.2 9.9 130 7.6 4.0 3.5–141	<0.6–22.6 37.7 3.4 154 2.3 1.1 <1–37.7	<2–425 212 57.3 126 45.4 26.3 <0.5–106	10–2349 235 546 134 406 197 0.17–39.1	<0.5–35.8 71.6 115 62.14 4.8 3.5 <1.67–119
Sauternes						
Sémillon, Sauvignon blanc DO Sauternes (France) (SAUT), <i>n</i> = 6	range max/min SD RSD (%) mean median range OAV	10.0–20.6 2.1 3.8 23.9 15.9 15.2 25–51.5	<0.6–2.7 4.5 1.1 102 1.08 0.47 <1–4.5	7.16–10.8 1.51 1.22 13.4 9.10 9.10 1.79–2.7	3911–5224 1.34 523 11.6 4499 4470 65.2–87.1	15.8–53.9 3.42 13.1 41.0 31.9 29.4 52.6–180

^a All quantitative data are expressed in ng/L.

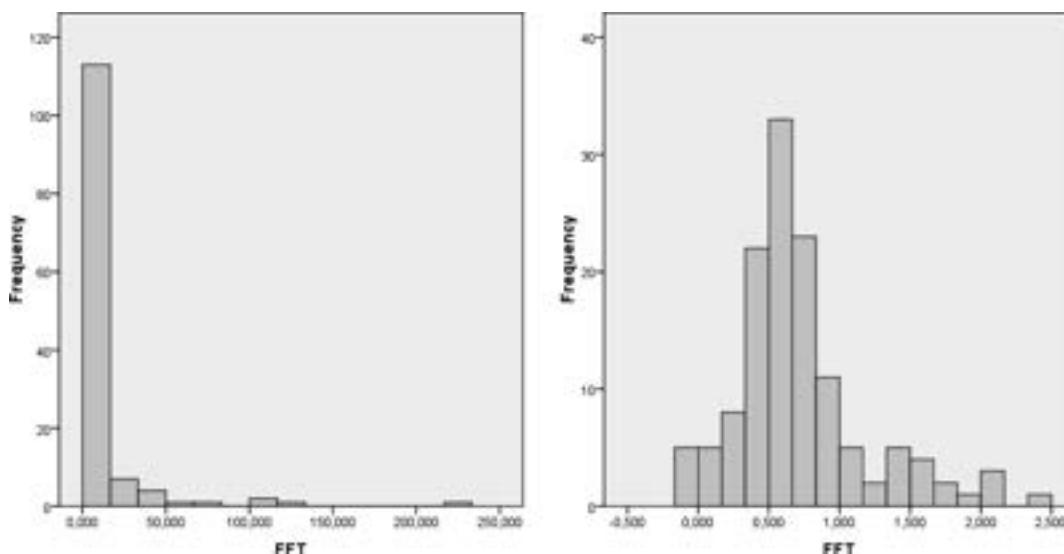


Figure 1. Histogram representing the distribution of FFT in the whole data set of samples in normal (left) or in logarithmic scales (right).

are enormous in terms of odor activity values (OVAs), which range from around 1 to 562, 437, or 148 in the cases of FFT, BM, and MHA, respectively, and from <1 to 148 and 121 in the cases of MP and MH, respectively. As will be later discussed, these differences have deep sensory consequences and reveal the existence of a broad range of aroma types among wines made with Sauvignon blanc. Such differences are the consequence of a combination of factors exerting a strong influence on the levels of these compounds, such as the strain of yeast (43–45), climatological and edafological factors, and, particularly, all of the technological decisions related to the management of oxygen and to the redox state of the wine (46–48). In any case, even if the maximum levels of these compounds are found in wines made with Sauvignon blanc, it cannot be said that containing high levels of these compounds is a common attribute of all wines from this variety. It should be noted that these ranges of variation are the most important found in wine for aroma molecules with positive characteristics, being even larger than those observed for some molecules responsible for off-odors, such as 4-ethylphenol (49).

In the case of the other varietals, ranges are narrower because the maximum registered levels are between 3 and 4 times smaller than those found in Sauvignon blanc wines, but still the lengths of the ranges of occurrence are very high and will have a deep sensory impact. This is in clear contrast to the ranges of occurrence found in wines from Sauternes, which have a quite characteristic profile of polyfunctional mercaptans, as is shown in the table and in accordance with other papers (24–27). As expected from such a wide range of occurrence, the distributions of these compounds among the sets or the different subsets of samples do not follow normal (Gaussian) distribution patterns. In fact, in most cases the histograms reveal the existence of much skewed distributions with the largest part of samples containing low levels of the compound. This observation can be clearly seen in **Figure 1**, which contains the histogram with the distribution of FFT in the whole data set in both normal and log-normal scales. As can be seen, the distributions follow a rather log-normal pattern, which means that geometric rather than arithmetic means are best representatives of the centroids of the distributions and also suggest that statistical tests must be carried out on data after a logarithmic transformation.

The first statistical study carried out intended to evaluate whether the levels of these compounds are linked to the variety of grape. As shown in **Table 5**, the ANOVA study reveals that

Table 5. One-Way ANOVA Study To Check the Effect of Grape Variety on the Mean Levels of Polyfunctional Mercaptans in White Table Wines (Input Data Were Log-Transformed)

	FFT sig 0.645 F = 0.706	MP sig 0.000 F = 4.62	MHA sig 0.000 F = 6.56	MH sig 0.000 F = 48.2	BM sig 0.007 F = 3.18
SB, n = 57	5.35 a	4.29 c	52.9 c	1166 c	4.59 b
VER, n = 10	5.33 a	0.72 a	80.4 c	1547 c	5.88 b
ALB, n = 10	7.72 a	1.87 ab	19.5 b	280 b	2.91 ab
CHR, n = 9	4.93 a	3.32 bc	25.5 b	33.1 a	1.01 a
MAC, n = 9	4.64 a	1.27 ab	19.0 ab	25.0 a	1.00 a
CAT, n = 5	4.28 a	1.65 abc	8.26 ab	30.6 a	1.41 ab
RGR, n = 9	2.95 a	2.27 bc	67.4 c	286 b	3.16 ab

variety exerts a highly significant influence in all cases, except FFT. However, the huge variability found within each group limits the number of significant differences in the different pairwise comparisons, and in fact the different multidimensional representations of those samples (principal component analysis and cluster analysis) revealed that samples from a single variety are relatively scattered, not showing clear groupings or clusters (data not shown). On the other hand, the quotients between the highest and the lowest means range from around 6 or 10 (BM and MHA) to around 60 for MH, differences that are large enough to have a high sensory relevance. These results confirm that there is a varietal influence on the levels of these compounds but that such influence is not strong enough to determine the existence of clearly defined compositional profiles.

As expected, means from Sauvignon blanc (geometric means) are in all cases relatively high but, surprisingly, in no single case were they significantly the highest among all of the varietals, and in the cases of MHA and BM the highest mean levels were in fact found in Verdejo wines. This is just a consequence of the existence of a relatively large proportion of wines from Sauvignon blanc with low levels of these compounds, as was aforementioned. It should be remembered that in all cases the highest levels of mercaptans are found in specific groups of wines from Sauvignon blanc as will be shown later. In the case of MP, the highest levels are found in Sauvignon blanc, but unexpectedly, also wines from Chardonnay contain a small but significantly higher level than other varietals, whereas wines from the Spanish varietal Verdejo present by far the lowest levels. In the case of MHA, contrarily, wines from Verdejo had the highest levels in accordance with previous studies (16), whereas wines from Maccabeo and Catalonian

Table 6. One-Way ANOVA Study To Check the Influence of the Factor Origin between Wines Made with Sauvignon blanc (Input Data Were Log-Transformed)

	FFT sig 0.007 <i>F</i> = 3.99	MP sig 0.000 <i>F</i> = 7.92	MHA sig 0.000 <i>F</i> = 6.61	MH sig 0.000 <i>F</i> = 7.26	BM sig 0.000 <i>F</i> = 14.0
SBPYR, <i>n</i> = 11	12.4 b	2.28 ab	100 c	325 a	5.30 b
SBR, <i>n</i> = 9	4.59 ab	1.44 a	57.7 bc	1452 bc	21.9 c
SBLOI, <i>n</i> = 11	8.05 b	3.66 ab	19.7 a	913 b	4.95 b
SBNZ, <i>n</i> = 14	6.02 b	15.1 c	106 c	2308 c	7.26 b
SBCH, <i>n</i> = 12	1.80 a	4.77 b	31.6 ab	1588 bc	0.60 a

varieties contained the lowest amounts of this compound. In the case of MH, wines from Sauvignon blanc contain higher mean levels, followed by wines from Verdejo, Albariño, and rosés elaborated with Grenache, whereas the lowest levels were found in this case in wines made from Chardonnay, Maccabeo, and other Catalonian varieties. More or less the same pattern is found for BM, although in this case the maximum was found in wines from Verdejo.

The influence of the geographic origin of the grapes on the wine contents on polyfunctional mercaptans was studied by comparing wines from Sauvignon blanc from different regions of the world. The ANOVA study shown in **Table 6** demonstrates that origin exerts a significant influence on the levels of all these compounds. Ranges of variations within each group are again rather high, as can be seen in the box plots given in **Figure 2**, which limits the number of pairwise significant differences, although in this case the multidimensional study of the data set revealed the existence of some clusters of wines from a given region (data not shown). Differences between the maxima and minima group means range in this case from 5 (MHA) to 30 (BM), ranges again large enough to have a deep sensory influence. As shown in **Table 6**, the levels of FFT are maximal in the two groups of wines from France and minimal in wines from Chile, which should be primarily related to the specific winemaking practices of each region but also to the climate differences between the regions (36, 50). In the case of MP, the levels found in wines from New Zealand are by far the highest and, in fact, it can be said that the highest level of this compound is a characteristic of wines from this country. On the contrary, Sauvignon blanc wines from Rueda (Spain) and from Midi Pyrenees in France have the lowest levels of this compound, whereas wines from the Loire Valley and from Chile have intermediate values. The fact that wines from Verdejo are grown also in Rueda and that they were found to contain minimum levels of this compound (**Table 5**) may suggest that geographical factors affecting the level of this compound have similar effects on different grape varieties. In the case of MHA, the maximum levels were found in wines from New Zealand and from Midi Pyrenees and the minimum in wines from the Loire Valley, this last observation in accordance with data reported by Lund et al. (31). As for MH, data suggest that this compound is essential in the characteristics of Sauvignon blanc from New Zealand, Chile, and Rueda, the levels of which are > 7 times higher than those from the rest of the wines. Finally, in the case of BM, wines from Rueda contain the highest levels, whereas wines from Chile contain by far the lowest levels. It should be noted that high levels of BM were also found in wines from Verdejo (**Table 5**).

It is not easy to establish a detailed comparison of data reported here with those reported previously, but in general it can be said that data are in general agreement with some exceptions. In the case of MP the values here reported are in accordance with those given in refs 7, 13, 15, 32, and 51, but are well below the 400 ng/L found in Scheurebe in 1997 (9); in the specific case of Maccabeo, our present data are below the 5 ng/L figure reported in 2004 (33). In the case of MHA and MH our data agree with data published in refs 15, 21, 29, 30, and 51–53 but are slightly

below those recently reported by Lund et al. (31). Such differences may be in part due to the use of normal statistics used by these authors that, with such highly skewed distributions, tend to overestimate mean values.

Sensory Role. As was previously mentioned, the large ranges of occurrence of polyfunctional mercaptans in white wines, together with their low odor thresholds, suggest that these compounds play a relevant role in the aroma properties of nearly every type of white wine and that they could even be responsible for relevant sensory differences between wine types, origins, and varieties. To have a preliminary estimation of the contribution of these compounds to the aroma of wine, a series of sensory tests have been conducted. In the tests, model white wines (prepared by partial rearomatization of a dearomatized wine, as described in a previous section) containing different levels of one of the polyfunctional mercaptans were sensory compared with similar reference models not containing the mercaptan. The results of the tests are summarized in the box plots shown in **Figure 2**. Dashed lines indicate the different concentrations tested, and the most frequently cited sensory descriptors (cited by at least 4 of the 12 panelists) have been printed close to the lines.

In the case of FFT, the smallest concentration tested was 1.9 ng/L, and the sensory effects caused by these additions were highly significant (difference with *P* < 0.001) and were described by the judges as an increase of wine fruitiness and of pineapple character. Notably, only at the 5.3 ng/L level and above were the sensory nuances toasty and coffee detected. As indicated in the box plot, these results suggest that this compound is an active contributor to wine sensory properties in at least 90% of the wines and that only in some Sauvignon blanc from Chile does it play a negligible role. The plot also suggests that in most Sauvignon blanc wines from Loire (typically called “Fumé blanc”), in most wines from Albariño, and in some wines from New Zealand and from the French Pyrenees, the compound is at a concentration high enough to act as a genuine impact compound communicating to wine its specific odor nuances.

In the case of MP, the first concentration tried was 1.0 ng/L, which again was significantly identified by the judges (*P* < 0.001), who noted an increase of the fruity and green character of the wine. The same sensory effect was noted at 1.7 and 3.4 ng/L, and only at 10.0 ng/L did the judges clearly note the typical box tree note of this compound. These results suggest that this compound plays a relevant role in the perception of fruitiness in most wines and that only in most wines from Verdejo and in some other sporadic samples will its contribution be negligible. Similarly, only in Sauvignon blanc wines from New Zealand and in some other isolated samples did this compound act as genuine impact compound.

In the case of MHA, the first concentration assayed was 6.4 ng/L, which was again easily identified by the judges, who reported a clear increase of wine fruitiness and freshness. At concentrations above 25 ng/L, the tropical fruit character was noted, and only at concentrations above 50 ng/L was the odor nuance clearly identified as tropical fruit and box tree. This implies that in most wines this compound is a relevant contributor to the wine fruity

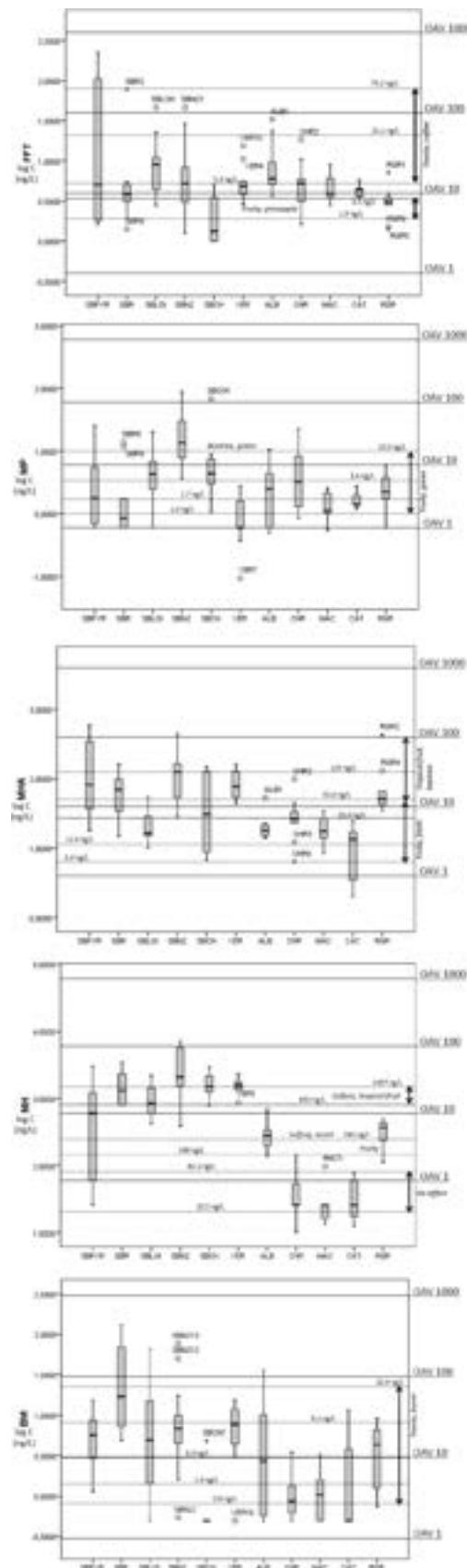


Figure 2. Box plots for each of the 5 polyfunctional mercaptans showing the basic distribution in the 11 specific wine groups considered in the study (wines from Sauternes and some other isolated wine samples were not considered for best visualization). Samples marked with a circle are outliers, whereas samples marked with an asterisk are extreme samples.

and fresh character and only in some wines made with Maccabeo, Xarel-lo, and Parellada does its contribution seem to be negligible. Besides, this compound will communicate to many wines its characteristic tropical fruit (passion fruit) character, and to some others it will even impart box tree notes.

The case of MH is more complex. Below or close to the reported odor threshold (21 or 82 ng/L), the sensory effect of the odorant was negligible, and at 148 ng/L it was clearly detectable ($P < 0.001$), contributing to the general fruity note of wine. At concentrations above 246 ng/L, the contribution was less pleasant, imparting to wine a sulfury and burnt-like note, and only at 1497 ng/L level was the descriptor tropical fruit clearly identified in addition to the sulfury note. Therefore, this molecule seems to be the least important of the five mercaptans considered in the study, and in fact it seems to be an irrelevant aroma molecule in nearly all wines made with Chardonnay, Maccabeo, Xarel-lo, and Parellada. In most rosés from Grenache and in accordance with a previous study (21) and even in most wines from Albariño, the effect of 3-mercaptopropanol is a complex aroma described as fruity, sulfury, or even burnt. This compound seems to be a relevant odorant only in Sauvignon blanc wines, especially from New Zealand.

In the case of BM the addition of very low levels (0.7 and 1.4 ng/L) caused strong changes in the aroma of wines, delivering the toasted, burnt, and empyreumatic notes characteristics of the compound, in accordance with previous reports (38, 39). This compound seems to be an active aroma compound in all of the studied wines, except perhaps in some wines made with Maccabeo, Xarel-lo, and Parellada.

Finally, it should be noted that the sensory tests carried out have not considered the potential interactions between these compounds, which surely will change and complicate the results. Similarly, the sensory tests have been carried out on a single white wine model. However, data reported here conclusively demonstrate that polyfunctional mercaptans are very important odor molecules in nearly any kind of white wine, contributing mostly to the fruit, green, and fresh notes typical of white wines, and, in some particular cases, communicating to the wine their specific toasted, coffee, box tree, or tropical fruit odor nuances.

Supporting Information Available: Additional figures and tables. This material is available free of charge via the Internet at <http://pubs.acs.org>.

LITERATURE CITED

- (1) Kerscher, R.; Grosch, W. Quantification of 2-methyl-3-furanthiol, 2-furfurylthiol, 3-mercaptopropanone, and 2-mercaptopropanone in heated meat. *J. Agric. Food Chem.* **1998**, *46*, 1954–1958.
- (2) Elmore, J. S.; Nursten, H. E. The effect of 2-furanmethanethiol upon the flavor of instant coffee. *Int. J. Food Sci. Technol.* **1993**, *28*, 531–536.
- (3) Buettner, A.; Schieberle, P. Characterization of the most odor-active volatiles in fresh, hand-squeezed juice of grapefruit (*Citrus paradisi* Macfayden). *J. Agric. Food Chem.* **1999**, *47*, 5189–5193.
- (4) Engel, K. H.; Tressl, R. Identification of new sulfur-containing volatiles in yellow passion fruits (*Passiflora edulis* f. *flavicarpa*). *J. Agric. Food Chem.* **1991**, *39*, 2249–2252.
- (5) Kumazawa, K.; Kubota, K.; Masuda, H. Influence of manufacturing conditions and crop season on the formation of 4-mercaptopropanone in Japanese green tea (sen-cha). *J. Agric. Food Chem.* **2005**, *53*, 5390–5396.
- (6) Granvogl, M.; Christlbauer, M.; Schieberle, P. Quantitation of the intense aroma compound 3-mercaptopropanone in raw and processed onions (*Allium cepa*) of different origins and in other *Allium* varieties using a stable isotope dilution assay. *J. Agric. Food Chem.* **2004**, *52*, 2797–2802.
- (7) Darriet, P.; Tominaga, T.; Lavigne, V.; Boidron, J.-N.; Dubourdieu, D. Identification of a powerful aromatic component of *Vitis vinifera*

L. var. Sauvignon wines: 4-mercaptopentan-2-one. *Flavour Fragrance J.* **1995**, *10*, 385–392.

(8) Bouchilloux, P.; Darriet, P.; Dubourdieu, D. Mise au point d'une méthode de dosage de la 4-mercaptopentan-2-one dans les vins de Sauvignon. *J. Int. Sci. Vigne Vin.* **1996**, *30*, 23–29.

(9) Guth, H. Quantification and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3027–3032.

(10) Bouchilloux, P.; Darriet, P.; Henry, R. Identification of volatile and powerful odorous thiols in Bordeaux red wine varieties. *J. Agric. Food Chem.* **1998**, *46*, 3095–3099.

(11) Tominaga, T.; Darriet, P.; Dubourdieu, D. Identification de l'acétate de 3-mercaptopropanol, composé à forte odeur de buis, intervenant dans l'arôme des vins de Sauvignon [Identification of 3-mercaptopropanol acetate in Sauvignon wine, a powerful aromatic compound exhibiting box-tree odor]. *Vitis* **1996**, *35*, 207–210.

(12) Tominaga, T.; des Gachons, C. P.; Dubourdieu, D. A new type of flavor precursors in *Vitis vinifera* L. cv. Sauvignon blanc: S-cysteine conjugates. *J. Agric. Food Chem.* **1998**, *46*, 5215–5219.

(13) Tominaga, T.; Furrer, A.; Henry, R.; Dubourdieu, D. Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon blanc wines. *Flavour Fragrance J.* **1998**, *13*, 159–162.

(14) Lopez, R.; Ortin, N.; Perez-Trujillo, J. P.; Cacho, J.; Ferreira, V. Impact odorants of different young white wines from the Canary Islands. *J. Agric. Food Chem.* **2003**, *51*, 3419–3425.

(15) Tominaga, T.; Baltenweck-Guyot, R.; Des Gachons, C. P.; Dubourdieu, D. Contribution of volatile thiols to the aromas of white wines made from several *Vitis vinifera* grape varieties. *Am. J. Enol. Vitic.* **2000**, *51*, 178–181.

(16) Campo, E.; Do, B. V.; Ferreira, V.; Valentin, D. Aroma properties of young Spanish monovarietal white wines: a study using sorting task, list of terms and frequency of citation. *Aust. J. Grape Wine Res.* **2008**, *14*, 104–115.

(17) Campo, E.; Ferreira, V.; Escudero, A.; Cacho, J. Prediction of the wine sensory properties related to grape variety from dynamic-headspace gas chromatography–olfactometry data. *J. Agric. Food Chem.* **2005**, *53*, 5682–5690.

(18) Parr, W. V.; Green, J. A.; White, K. G. Wine judging, context and New Zealand Sauvignon Blanc. *Eur. Rev. Appl. Psychol.* **2006**, *56*, 231–238.

(19) Parr, W. V.; Green, J. A.; White, K. G.; Sherlock, R. R. The distinctive flavour of New Zealand Sauvignon blanc: sensory characterisation by wine professionals. *Food Qual. Pref.* **2007**, *18*, 849–861.

(20) Murat, M.-L.; Tominaga, T.; Dubourdieu, D. Mise en évidence de composés clefs dans l'arôme des vins rosés et clairets de Bordeaux [Impact of some components on Bordeaux roses and clairets aroma]. *J. Int. Sci. Vigne Vin.* **2001**, *35*, 99–105.

(21) Ferreira, V.; Ortin, N.; Escudero, A.; Lopez, R.; Cacho, J. Chemical characterization of the aroma of Grenache rose wines: aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. *J. Agric. Food Chem.* **2002**, *50*, 4048–4054.

(22) Masson, G.; Schneider, R. Key compounds of provence rose wine flavor. *Am. J. Enol. Vitic.* **2009**, *60*, 116–122.

(23) Fretz, C. B.; Luisier, J. L.; Tominaga, T.; Amado, R. 3-Mercaptopropanol: an aroma impact compound of Petite Arvine wine. *Am. J. Enol. Vitic.* **2005**, *56*, 407–410.

(24) Bailly, S.; Jerkovic, V.; Marchand-Bryaert, J.; Collin, S. Aroma extraction dilution analysis of Sauternes wines. Key role of polyfunctional thiols. *J. Agric. Food Chem.* **2006**, *54*, 7227–7234.

(25) Campo, E.; Cacho, J.; Ferreira, V. The chemical characterization of the aroma of dessert and sparkling white wines (Pedro Ximenez, Fino, Sauternes, and Cava) by gas chromatography–olfactometry and chemical quantitative analysis. *J. Agric. Food Chem.* **2008**, *56*, 2477–2484.

(26) Sarrazin, E.; Shinkaruk, S.; Tominaga, T.; Bennetau, B.; Frerot, E.; Dubourdieu, D. Odorous impact of volatile thiols on the aroma of young botrytized sweet wines: identification and quantification of new sulfanyl alcohols. *J. Agric. Food Chem.* **2007**, *55*, 1437–1444.

(27) Thibon, C.; Dubourdieu, D.; Darriet, P.; Tominaga, T. Impact of noble rot on the aroma precursor of 3-sulfanylhexanol content in *Vitis vinifera* L. cv Sauvignon blanc and Semillon grape juice. *Food Chem.* **2009**, *114*, 1359–1364.

(28) Fedrizzi, B.; Magno, F.; Moser, S.; Nicolini, G.; Versini, G. Concurrent quantification of light and heavy sulphur volatiles in wine by headspace solid-phase microextraction coupled with gas chromatography/mass spectrometry. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 707–714.

(29) Fedrizzi, B.; Versini, G.; Lavagnini, I.; Badocco, D.; Nicolini, G.; Magno, F. Hyphenated gas chromatography–mass spectrometry analysis of 3-mercaptopropanol and 3-mercaptopropanyl acetate in wine – comparison with results of other sampling procedures via a robust regression. *Anal. Chim. Acta* **2008**, *621*, 38–43.

(30) Fedrizzi, B.; Versini, G.; Lavagnini, I.; Nicolini, G.; Magno, F. Gas chromatography–mass spectrometry determination of 3-mercaptopropanol and 3-mercaptopropanyl acetate in wine. A comparison of headspace solid phase microextraction and solid phase extraction methods. *Anal. Chim. Acta* **2007**, *596*, 291–297.

(31) Lund, C. M.; Thompson, M. K.; Benkwitz, F.; Wohler, M. W.; Triggs, C. M.; Gardner, R.; Heymann, H.; Nicolau, L. New Zealand Sauvignon blanc distinct flavor characteristics: sensory, chemical, and consumer aspects. *Am. J. Enol. Vitic.* **2009**, *60*, 1–12.

(32) Schneider, R.; Kotseridis, Y.; Ray, J. L.; Augier, C.; Baumes, R. Quantitative determination of sulfur-containing wine odorants at sub parts per billion levels. 2. Development and application of a stable isotope dilution assay. *J. Agric. Food Chem.* **2003**, *51*, 3243–3248.

(33) Escudero, A.; Gogorza, B.; Melus, M. A.; Ortin, N.; Cacho, J.; Ferreira, V. Characterization of the aroma of a wine from Macabeo. Key role played by compounds with low odor activity values. *J. Agric. Food Chem.* **2004**, *52*, 3516–3524.

(34) Pet'ka, J.; Ferreira, V.; Gonzalez-Vinas, M. A.; Cacho, J. Sensory and chemical characterization of the aroma of a white wine made with Devin grapes. *J. Agric. Food Chem.* **2006**, *54*, 909–915.

(35) Blanchard, L. Recherche sur la contribution de certains thiols volatils à l'arôme des vins rouges étude. *Etude de leur Genèse et de leur Stabilité*; Université de Bordeaux 2: Bordeaux, France, 2000.

(36) Blanchard, L.; Tominaga, T.; Dubourdieu, D. Formation of furfurylthiol exhibiting a strong coffee aroma during oak barrel fermentation from furfural released by toasted staves. *J. Agric. Food Chem.* **2001**, *49*, 4833–4835.

(37) Tominaga, T.; Blanchard, L.; Darriet, P.; Dubourdieu, D. A powerful aromatic volatile thiol, 2-furanmethanethiol, exhibiting roast coffee aroma in wines made from several *Vitis vinifera* grape varieties. *J. Agric. Food Chem.* **2000**, *48*, 1799–1802.

(38) Tominaga, T.; Guimbertau, G.; Dubourdieu, D. Role of certain volatile thiols in the bouquet of aged Champagne wines. *J. Agric. Food Chem.* **2003**, *51*, 1016–1020.

(39) Tominaga, T.; Guimbertau, G.; Dubourdieu, D. Contribution of benzenemethanethiol to smoky aroma of certain *Vitis vinifera* L. wines. *J. Agric. Food Chem.* **2003**, *51*, 1373–1376.

(40) Tominaga, T.; Dubourdieu, D. A novel method for quantification of 2-methyl-3-furanthiol and 2-furanmethanethiol in wines made from *Vitis vinifera* grape varieties. *J. Agric. Food Chem.* **2006**, *54*, 29–33.

(41) Mateo-Vivaracho, L.; Cacho, J.; Ferreira, V. Improved solid-phase extraction procedure for the isolation and in-sorbent pentafluorobenzyl alkylation of polyfunctional mercaptans. Optimized procedure and analytical applications. *J. Chromatogr., A* **2008**, *1185*, 9–18.

(42) Loscos, N.; Hernandez-Orte, P.; Cacho, J.; Ferreira, V. Release and formation of varietal aroma compounds during alcoholic fermentation from nonfloral grape odorless flavor precursors fractions. *J. Agric. Food Chem.* **2007**, *55*, 6674–6684.

(43) Howell, K. S.; Swiegers, J. H.; Elsey, G. M.; Siebert, T. E.; Bartowsky, E. J.; Fleet, G. H.; Pretorius, I. S.; de Barros Lopes, M. A. Variation in 4-mercaptopentan-2-one release by *Saccharomyces cerevisiae* commercial wine strains. *FEMS Microbiol. Lett.* **2004**, *240*, 125–129.

(44) Murat, M. L.; Masneuf, I.; Darriet, P.; Lavigne, V.; Tominaga, T.; Dubourdieu, D. Effect of *Saccharomyces cerevisiae* yeast strains on the liberation of volatile thiols in Sauvignon blanc wine. *Am. J. Enol. Vitic.* **2001**, *52*, 136–139.

(45) Swiegers, J. H.; Kievit, R. L.; Siebert, T.; Lattey, K. A.; Bramley, B. R.; Francis, I. L.; King, E. S.; Pretorius, I. S. The influence of yeast

on the aroma of Sauvignon blanc wine. *Food Microbiol.* **2009**, *26*, 204–211.

(46) Lopez, R.; Lapena, A. C.; Cacho, J.; Ferreira, V. Quantitative determination of wine highly volatile sulfur compounds by using automated headspace solid-phase microextraction and gas chromatography–pulsed flame photometric detection – critical study and optimization of a new procedure. *J. Chromatogr., A* **2007**, *1143*, 8–15.

(47) Munch, P.; Schieberle, P. Quantitative studies on the formation of key odorants in thermally treated yeast extracts using stable isotope dilution assays. *J. Agric. Food Chem.* **1998**, *46*, 4695–4701.

(48) Ugliano, M.; Kwiatkowski, M. J.; Travis, B.; Francis, I. L.; Waters, E. J.; Herderich, M. J.; Pretorius, I. S. Post-bottling management of oxygen to reduce off-flavour formation and optimize wine style. *Wine Ind. J* **2009**, *24*, 24–28.

(49) Suarez, R.; Suarez-Lepe, J. A.; Morata, A.; Calderon, F. The production of ethylphenols in wine by yeasts of the genera *Brettanomyces* and *Dekkera*: a review. *Food Chem.* **2007**, *102*, 10–21.

(50) Tominaga, T.; Masneuf, I.; Dubourdieu, D. Powerful aromatic volatile thiols in wines made from several *Vitis vinifera* grape varieties and their releasing mechanism. In *Nutraceutical Beverages: Chemistry, Nutrition, and Health Effects*; 2004; Vol. 871, pp 314–337.

(51) Rodriguez-Bencomo, J. J.; Schneider, R.; Lepoutre, J. P.; Rigou, P. Improved method to quantitatively determine powerful odorant volatile thiols in wine by headspace solid-phase microextraction after derivatization. *J. Chromatogr., A* **2009**, *1216*, 5640–5646.

(52) Murat, M. L.; Tominaga, T.; Saucier, C.; Glories, Y.; Dubourdieu, D. Effect of anthocyanins on stability of a key odorous compound, 3-mercaptophenol, in Bordeaux rose wines. *Am. J. Enol. Vitic.* **2003**, *54*, 135–138.

(53) Tominaga, T.; Murat, M. L.; Dubourdieu, D. Development of a method for analyzing the volatile thiols involved in the characteristic aroma of wines made from *Vitis vinifera* L. cv. Sauvignon blanc. *J. Agric. Food Chem.* **1998**, *46*, 1044–1048.

Received for review March 22, 2010. Revised manuscript received August 1, 2010. Accepted August 1, 2010. This work has been funded by the Spanish government (Project 2007-65139). L.M. has received a grant from the Spanish FPU program. J.Z. has received a grant from the Universidad de Zaragoza–Banco Santander Central Hispano.