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Ability of Possible DMS Precursors To Release DMS during Wine Aging and in the Conditions of Heat-Alkaline Treatment

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The origin of dimethyl sulfide (DMS) produced during wine aging was examined through different assays. The production of DMS during the model aging of a wine and the concomitant decrease of residual potential DMS (PDMS), as DMS released by heat-alkaline treatment in 0.5 M sodium hydroxide at 100 °C for 1 h, were demonstrated. Then, dimethyl sulfoxide (DMSO), methionine sulfoxide (MSO), *S*-methylmethionine (SMM), and dimethylsulfonium propanoic acid (DMSPA), reported previously as possible DMS precursors, were investigated for their ability to be DMS precursors in wine in the conditions of this model aging and of the heat-alkaline treatment. The results showed that DMSO, MSO, and DMSPA could hardly be DMS precursors in the conditions used, whereas SMM appeared to be a good candidate. Finally, the use of [$^2H_6$]-DMSPA as an internal standard for PDMS determination was proposed, because it provided better reproducibility than [$^2H_6$]-DMS used as an external standard.

KEYWORDS: Aroma; wine; aging; dimethyl sulfide; DMS; precursors; DMSO; SMM

INTRODUCTION

Dimethyl sulfide (DMS) is a sulfur-containing volatile found in a wide range of foodstuffs of animal and plant origin (*1*−*9*) and of beverages such as rum (*10*) and beer (*11*). It was found in wines of most grape varieties, with sub-parts per billion to sub-parts per million levels (*12*) and often exceeds its perception threshold, particularly after aging (*13*−*17*). Its influence on aroma appears complex, because it can be perceived either positively or negatively depending on the DMS level and type of wine (*15*, *18*−*21*). The ability of yeast to release DMS during fermentation from various amino acids and derived compounds or from DMSO was demonstrated (*18*, *22*, *23*). However, DMS levels in freshly bottled wines are often low (*18*) and significantly increase during aging (*13*, *16*−*18*, *20*). Chemical pathways could be involved in this DMS production during bottle aging, although no precursor has been characterized in wine yet. DMS can be chemically produced from a variety of organosulfur precursors, in particular from methionine derivatives (*11*, *24*−*26*). In a previous paper, we demonstrated that free DMS in wines of different vintages and varieties was related to potential DMS (PDMS), as DMS released by heat-alkaline treatment from precursors present in grapes and wines (*20*). Dimethylsulfonium compounds that would easily release DMS in heat-alkaline conditions because of their chemical reactivity would hence be possible DMS precursors during wine aging. *S*-Methylmethionine (SMM or vitamin U), assumed to correspond to a storage and transport form of methionine (*27*, *28*), was reported as the main dimethylsulfonium compound in higher plants (*29*−*31*) and was established as a major DMS precursor in malt (*32*) and in beer through its thermal degradation during the brewing process (*11*). Heat-alkaline treatment, generating DMS from SMM, was used for the indirect determination of SMM in malt, beer, and vegetables (*4*, *33*, *34*). SMM has not been reported in grape or wine yet, but the same assay was proposed previously to assess PDMS in wine (*35*). On the other hand, only dimethyl sulfoxide (DMSO), found ranging from a trace to 1230 μg/L in Australian wines, has been proposed in previous work as a DMS precursor during wine aging, through a hypothetical reduction (*36*).

This paper aimed at testing the relevance of the heat-alkaline treatment to gauge PDMS in wine, i.e., DMS to be released during wine aging, and, on the other hand, at investigating DMSO and three methionine derived compounds (MSO, SMM, and DMSPA) as possible DMS precursors in wine. In addition, the conditions of PDMS determination in grapes and wines using heat-alkaline treatment were studied to improve the accuracy and precision of the method.

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MATERIALS AND METHODS

Analytical Reagents and Supplies. Toluene, acetic acid, K_2CO_3, and NaCl were purchased from Merck (Darmstadt, Germany). Tartaric acid (>99.5% pure) was from Fluka (Saint-Quentin Fallavier, France), and glucose, L-methionine (>98% pure), dimethyl sulfoxide (DMSO), l-methionine-sulfoxide (MSO), and L-methionine-*S*-methylsulfonium iodide (SMM iodide) were from Sigma (Saint-Quentin Fallavier, France). Sodium hydroxide (for analysis) and ethanol (absolute) were from Carlo Erba (Rodano, Italy). [$^2H_6$]-DMS (99.0% atom.), [$^2H_3$]-DMS (99.5% atom.), [$^2H_6$]-dimethyl sulfoxide ([$^2H_6$]-DMSO, 99.9% atom.), [$^2H_3$]-iodomethane (99.5% atom.), and deuterium oxide were from Aldrich (Saint-Quentin Fallavier, France). Dimethyl sulfide (99.0% pure) and acrylic acid (99.5% pure) were from Acros Organics (Noisy-le-Grand, France), and formic acid (>99% pure) was from Prolabo (France). Dichloromethane and methanol were from Riedel-de-Haën (Buchs, Switzerland). The carboxen poly(dimethylsiloxane) (CAR/PDMS, 75 μm) SPME fibers and the manual SPME holder used for DMS analysis were purchased from Supelco (Saint-Quentin Fallavier, France). The Chem Elut CE1010 cartridges used for DMSO extraction were supplied by Varian (Sunnyvale, CA). Water was purified with a Milli-Q system from Millipore S. A. (Saint-Quentin Fallavier, France).

Synthesis of the Deuterated Sulfoniums [$^2H_6$]-DMSPA Chloride and [$^2H_3$]-SMM Iodide. Deuterated dimethylsulfonium propanoic acid chloride ([$^2H_6$]-DMSPA chloride) was prepared as reported previously (*37*) from the reaction between [$^2H_6$]-DMS and acrylic acid in toluene at room temperature for 4 days and then precipitation of the sulfonium salt by bubbling HCl gas. The product was collected by filtration and washed with toluene (90% yield). The structure was confirmed by ^{1}H NMR analysis: [$(CD_3)_2S^+CH_2CH_2COOH$, Cl^-] $\delta(D_2O)$, 2.94 (t, H-2, 2H), 3.47 (t, H-3, 2H).

Deuterated *S*-methylmethionine iodide ([$^2H_3$]-SMM iodide) was prepared as previously described (*38*) from the reaction between [$^2H_3$]-iodomethane and L-methionine in a mixture of formic acid and acetic acid (3:1, v/v). After 3 days at room temperature, the mixture was dried under vacuum and then the sulfonium salt was precipitated with methanol, filtered, dissolved in 50% aqueous ethanol, and precipitated again by adding pure ethanol, before final filtration (75% yield). The structure was confirmed by ^{1}H NMR analysis: [$(CD_3)(CH_3)S^+CH_2$-$CH_2CH(NH_2)(COOH)$, I^-], $\delta(D_2O)$ 2.34 (m, H-4, 2H), 2.90 (s, CH_{3-S}, 3H), 3.45 (m, H-3, 2H), 3.85 (m, H-2, 1H).

Grapes and Wines. Syrah and Grenache noir cultivars grapes and wines were analyzed. The grapes were grown in different locations of the Rhone Valley vineyard, France, selected by the experimental winery of the Inter-Rhone research station. The wines, of vintages 1992−2002, were elaborated in standard conditions by Inter-Rhone. Grapes were manually harvested, mechanically destemmed, crushed, and then put in 1 hL (100 L) stainless steel tanks. Musts were added with SO_2 at 5 g/hL and inoculated with 10 g/hL L2056 commercial yeast strain (Lallemand, France). Fermentation and maceration were carried out during 7 days at 25−30 °C. After pressing, malolactic fermentation was performed with the addition of Vitilactic bacteria (Martin vialatte, France). The wines were filtered before bottling, and bottle storage temperature was 13 °C. For grape juices analyses, the grapes were frozen at −20 °C immediately after harvest.

Synthetic Grape Juice and Model Base Wine. To obtain the synthetic grape juice, 220 g of glucose and 4 g of tartaric acid were added to 0.9 L of water. To obtain the model base wine, 3.5 g of tartaric acid were added to 120 mL of ethanol and 800 mL of water. The pH of both mixtures was adjusted to 3.5 with solid potassium carbonate, and their volume was then adjusted to 1 L.

Grape Juice Preparation. A total of 500 g of berries were destemmed, defrosted at 4 °C for one night, crushed in a mixer for 5 s at maximum power, and then filtered.

Analytical Determination of DMSO in Wines. A 10 mL aliquot of wine was added with 3 μg of [$^2H_6$]-DMSO (75 μL of a 40 μg/mL solution in ethanol) and concentrated under vacuum to remove ethanol. The sample was adsorbed at room temperature on a Chem Elut cartridge, which was then rinsed with 50 mL of CH_2Cl_2. The organic eluate was concentrated to dryness under vacuum, and the dry extract was dissolved in 200 μL of methanol for GC/MS analysis.

Calibration Curves. Serial dilutions of aliquots of a DMSO solution (40 μg/mL in ethanol) in 1 mL of methanol were made separately. Then, 4 μg of [$^2H]_6$-DMSO (100 μL of 40 μg/mL solution in ethanol) was added to each dilution as an internal standard. The calibration curves were obtained from these solutions by direct injection in GC/MS. Peak area ratios (peak area of the ion *m*/*z* 63/peak area of the ion *m*/*z* 66) were plotted against the concentration ratios (micrograms of DMSO/4 μg of [$^2H_6$]-DMSO) for the following DMSO concentrations: 0.05, 0.1, 0.2, 0.5, 1.0, 3.0, and 5.0 μg. The resultant curve was linear [response ratio = 1.1196 × concentration ratio; R^2 = 0.9998].

Analytical Determination of DMS and Potential DMS in Wines and Grapes. These analytical conditions were already described and discussed elsewhere (*20*).

Analysis of Free DMS by Solid-Phase Microextraction. A 15 mL aliquot of wine or grape juice was transferred at room temperature to a 22 mL vial equipped with a magnetic stir bar, and 1.75 g of NaCl was added. Then, 1.5 μg of [$^2H]_6$-DMS (15 μL of a 100 μg/mL solution in ethanol) was added, and the vial was sealed with a screw-top cap with Teflon-faced septum. The solution was equilibrated by magnetic stirring at 500 rpm for 5 min. The SPME needle (CAR/PDMS, 75 μm) was then inserted through the septum, and the fiber, previously conditioned at 280 °C for 5 min, was extended into the headspace and allowed to equilibrate for 30 min with stirring at 500 rpm, at room temperature. The fiber was then retracted, removed from the vial, and immediately desorbed into the injector of the GC, with an exposure time of 1 min to desorb the DMS analytes (see GC/MS conditions below).

Potential DMS Released by Heat-Alkaline Treatment (PDMS). The analysis of wines and grape juices was carried out using the same vials after the analysis of free DMS. Free DMS and [$^2H_6$]-DMS were removed from the sample by bubbling nitrogen at a 100 mL/min flow rate for 10 min, with magnetic stirring at 500 rpm. PDMS was then released by performing a thermal treatment in alkaline conditions, as follows: about 300 mg of sodium hydroxide (pelletized) was added to the 15 mL sample to obtain a 0.5 N solution. The vial was sealed with a new screw-top cap with Teflon-faced septum, heated at 80 °C for 1 h, and then allowed to cool. A total of 1.5 μg of [$^2H_6$]-DMS (15 μL of a 100 μg/mL solution in ethanol) was introduced into the sample through the septum and equilibrated, and the DMS released by the reaction was quantitatively determined as described above for free DMS.

Calibration Curves. A model base wine and a synthetic grape juice were used to obtain the calibration curves for DMS quantification in wines and grape juices, respectively. Serial dilutions in 15 mL of the model base wine or synthetic grape juice of aliquots of an ethanol solution of DMS (100 μg/mL) were made separately in the 22 mL septum-sealed glass vials used for SPME. Then, 1.5 μg of [$^2H_6$]-DMS (15 μL of a 100 μg/mL solution in ethanol) was added to each dilution as an internal standard. The calibration curves were obtained from these solutions by SPME analysis (see above) coupled to GC/MS. Peak area ratios (peak area of the ion *m*/*z* 62/peak area of the ion *m*/*z* 68) were plotted against the concentration ratios (micrograms of DMS/1.5 μg of [$^2H]_6$-DMS) for the following DMS concentrations: 0.15, 0.75, 1.5, 3.0, and 4.5 μg. The resultant curve was linear [response ratio = 1.1673 × concentration ratio; R^2 = 0.9986].

Gas Chromatography Coupled with Mass Spectrometry. GC/MS analyses were carried out using a Hewlett−Packard gas chromatograph 5890 series II fitted with a 30 m fused silica column (0.25 mm inside diameter and 0.5 μm film thickness), coated with DB-WAX (J&W Scientific).

For DMS analysis, the injector (splitless) temperature was held at 300 °C, constantly. Transfer of the sample to the GC/MS column was accomplished by keeping the SPME fiber for 1 min in the heated chromatograph injector. The oven temperature program was 30 °C (for 3 min), then increased at 1 °C/min to 40 °C, and then increased at 10 °C/min to 250 °C.

For DMSO analysis, the injector (splitless) temperature was increased from 20 to 250 °C at 180 °C/min. The oven temperature program was 60 °C (for 3 min) and then increased at 3 °C/min to 245 °C. The column was connected to the injector with a 1 m deactivated fused silica precolumn (J&W Scientific; 0.53 mm inside diameter). The carrier gas was helium 6.0 (Linde gaz, Marseille, France), with a flow rate of 1.3

mL/min. The GC instrument was coupled to a Hewlett−Packard 5989 A mass selective detector and a Hewlett−Packard B.05.02 MS Chemstation. The transfer line was heated at 250 °C. The electron impact (EI) energy was 70 eV, the MS source and quadrupole temperatures were set at 250 and 120 °C, respectively. The following ions in the selective ion monitoring (SIM) mode were used: *m*/*z* 62 (999) and *m*/*z* 68 (999) as quantifiers for DMS and [$^2H_6$]-DMS, respectively, and *m*/*z* 47 (875) and *m*/*z* 50 (1150) as qualifiers, respectively, on the other hand, *m*/*z* 63 (999) and *m*/*z* 66 (999) as quantifiers for DMSO and [$^2H_6$]-DMSO, respectively, and *m*/*z* 78 (727) and *m*/*z* 84 (799) as qualifiers, respectively.

Study of DMS Release by Model Aging Experiments. *Model Aging of a Wine.* A total of 33 15 mL aliquots of a 2001 Syrah wine were placed separately into flame-sealed glass tubes. The wine-filled tubes were submitted to model aging by controlled heating at 45 °C for 24 days. All along the controlled heating, aliquots were sampled, with a total of 10 samplings, and stored at 4 °C. All aliquots were then submitted to analysis of free and potential DMS, with each sampling consisting of three tubes for triplicate analysis.

Model Aging of Wines and Model Wines Spiked with DMS Precursors. Three separate portions of a Syrah wine (vintage 2002) and three separate portions of a model wine were separately spiked with three hypothetical DMS precursors. Each 150 mL portion was spiked with only one DMS precursor, at a level close to 2 mg/L of DMS equivalents. One portion was added with 1400 μg of SMM iodide (100 μL of a 14.06 mg/mL solution in water); one portion was added with 400 μg of [$^2H_6$]-DMSO (100 μL of a 4.06 mg/mL solution in ethanol); and one portion was added with 793 μg of MSO (100 μL of a 7.93 mg/mL solution in water). In addition, one 150 mL portion of a Grenache wine (vintage 2002) was also spiked with SMM iodide, at the same level. All portions were separately conditioned in 15 mL flame-sealed glass tubes, submitted to model aging by controlled heating at 45 °C for 10 weeks, and then submitted to analysis of free and potential DMS. In the samples added with [$^2H_6$]-DMSO, the DMS and PDMS levels assessed were those of both [$^2H_6$]-DMS and natural DMS, determined by the addition of known amounts of these compounds to the samples.

Use of the Deuterated Sulfoniums Salts [$^2H_6$]-DMSPA Chloride and [$^2H_3$]-SMM Iodide as Internal Standards in the PDMS Assay. The three pure commercial DMS, [$^2H_3$]-DMS, and [$^2H_6$]-DMS, each one in ethanol solution, were analyzed separately by GC/MS with an extended mass range to obtain the mass spectra of the pure compounds.

Three 15 mL model wine aliquots were spiked separately with one of the following compounds: 4.0 μg of [$^2H_6$]-DMS (50 μL of a 80 μg/mL solution in ethanol), 14.8 μg of [$^2H_6$]-DMSPA chloride (70 μL of a 211 μg/mL solution in water), or 20 μg of [$^2H_3$]-SMM iodide (50 μL of a 400 μg/mL solution in water). The three samples (assays encoded A, B, and C, respectively) were submitted to heat-alkaline treatment (0.5 M NaOH, 100 °C for 1 h). The DMS released was analyzed by GC/MS as described above but without standard addition, to determine its labeling only.

Two 3 mL aliquots of a 2001 Syrah wine were separately concentrated to dryness under vacuum. One residue was dissolved in 3 mL of water, and the second was dissolved in 3 mL of deuterium oxide (assays encoded D and E, respectively). Both samples were submitted to heat-alkaline treatment (0.5 N NaOH, 100 °C for 1 h) and then to DMS analysis as described above but without standard addition, to determine the labeling of the produced DMS.

Comparison between PDMS Analysis Using [$^2H_6$]-DMS as an External Standard and [$^2H_6$]-DMSPA Chloride as an Internal Standard. PDMS of a Syrah wine (2001 vintage) was determined using [$^2H_6$]-DMSPA chloride as an internal standard, by modifying the procedure described above as following: after nitrogen bubbling, the 15 mL sample was added with 3.9 μg of [$^2H_6$]-DMSPA chloride (18 μL of a 211 μg/mL solution in water), corresponding to 1.5 μg of [$^2H_6$]-DMS equivalents. Heat-alkaline treatment (0.5 M NaOH, 100 °C for 1 h) and DMS quantification were performed as described above but without [$^2H_6$]-DMS addition. PDMS of the same wine was also determined using the conventional procedure described above ([$^2H_6$]-DMS as an external standard). Each analysis was performed in triplicate.

Table 1. Free DMS and PDMS[a] Levels at Different Times of the Model Aging[b] of a Wine[c]

model aging time[b]	DMS[d]	PDMS[a,d]	ratio [(free DMS)/(free DMS and PDMS)][e]
0	36.9 (0.9)	96.0 (5.9)	27.8
2	53.6 (0.4)	66.8 (2.0)	44.5
4	85.5 (2.1)	72.0 (2.0)	55.3
6	94.0 (8.8)	56.3 (1.6)	62.1
8	107.5 (5.2)	53.2 (0.9)	66.9
13	123.7 (2.5)	43.6 (1.7)	73.9
17	138.6 (3.3)	32.2 (1.3)	81.1
19	203.2 (6.3)	41.3 (0.2)	83.1
21	187.7 (8.9)	36.0 (2.1)	83.9
24	213.9 (11.5)	34.6 (0.9)	86.1

[a] PDMS refers to DMS released by heat-alkaline treatment (0.5 N NaOH, heating at 80 °C for 1 h). [b] Model aging by heating at 45 °C, time in days. [c] This Syrah wine was chosen for its high PDMS level, determined in a previous study (code S01d in ref *20*). [d] Mean levels and standard deviation ($n = 3$) of free DMS and PDMS, in μg/L. [e] Mean of the ratio in percent ($n = 3$).

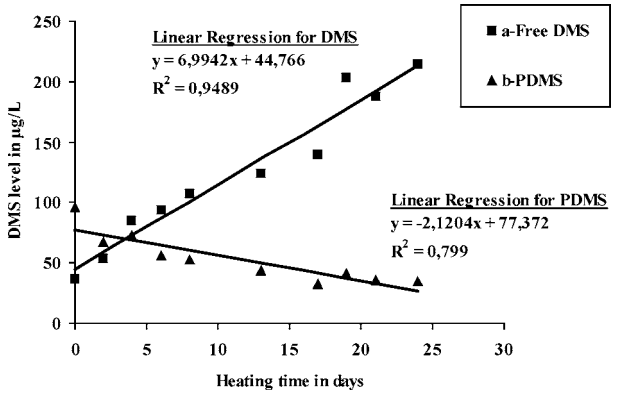

Figure 1. Linear regressions between the heating time of the model aging of a Syrah wine and (a) free DMS (■) and (b) PDMS determined in 0.5 M NaOH, at 80 °C for 1 h (▲) (data from **Table 1**).

RESULTS AND DISCUSSION

Model Aging of a Wine. Previous work showed an increase in the DMS level with wine storage time and temperature (*13*, *14*, *16*, *36*). Some authors, measuring DMS levels in different wines but not in the same wine at different storage times, reported an upward trend of free DMS level with vintage (*14*, *17*), whereas others did not observe any trend (*15*, *36*). Our previous results obtained on 22 Grenache and Syrah wines of different vintages and vineyards showed that DMS was released from PDMS during wine aging and that wine age was correlated to the proportion of the free DMS level relative to the sum (free DMS and PDMS levels) but not to the free DMS level (*20*). Indeed, the samples analyzed were wines of different vintages but not the same wine sampled at different aging times, and the range of the PDMS observed in different wines for the same vintage was wide (8.7−97 ppb). Thus, in this study, the DMS and PDMS levels were determined during the model aging of only one wine. A 2001 Syrah wine was chosen, because it presented the highest PDMS level in the Syrah and Grenache wines previously analyzed (*20*). This wine was submitted to a controlled heating, at 45 °C for 24 days, which was previously used as a model aging for acid-catalyzed reactions occurring during wine aging (*39*). As shown in **Table 1** and **Figure 1**, between the 1st and the 24th day, the free DMS level increased continuously from 36.9 to 213.9 μg/L. Controlled heating hence appeared to be of interest for the study of DMS release kinetics. The PDMS levels simultaneously decreased from 96.0 to 34.6

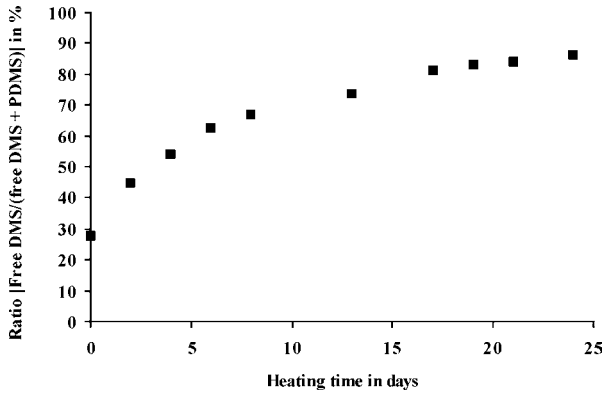

Figure 2. Ratio [free DMS/(free DMS and PDMS)] in percent as a function of the heating time of the model aging of a Syrah wine. PDMS was determined in 0.5 M NaOH, at 80 °C for 1 h (data from **Table 1**).

μg/L (**Table 1** and **Figure 1**), and the proportion of free DMS relative to the sum (free DMS and PDMS) increased from 28 to 86% (**Figure 2**). This increase was consistent with our previous results obtained on wines of different varieties and vintages (*20*), showing that this ratio increased with wine aging. However, the curved outline obtained in our present results, from the same wine sampled at different aging times, differed from the linear model ($R^2 = 0.85$) that we proposed previously for wines of different varieties and vintages. As shown by the different absolute values of the slopes of the DMS increase and PDMS decrease in **Figure 1**, the assay initially proposed (*20*, *35*) to gauge the levels of DMS released during wine storage gave lower levels than those really released. The discrepancy between these levels could be attributed to the heat-alkaline treatment, not suitable to liberate DMS from some DMS precursors in wine. Thus, the ability of some sulfur compounds, suggested as wine DMS precursors in the literature, to release DMS in the conditions of wine aging, as well as in the conditions of the heat-alkaline treatment, was investigated in the following.

DMSO and MSO as DMS Precursors. The ability of yeast to release DMS during fermentation from DMSO was demonstrated in beer (*40*) as in wine (*18*). De Mora et al. (*36*) measured DMSO levels in wine up to 1230 μg/L and led bottle aging experiments suggesting that this compound could be reduced into DMS during aging. Because of its chemical structure, methionine sulfoxide (MSO), a natural oxidized form of methionine (*41*), may similarly be reduced into DMS through DMSO release. DMSO and MSO as DMS precursors in wine were thus investigated. First, the DMSO levels were determined in seven wines and compared to the DMS levels previously determined (*20*). Second, DMS formation was studied during the model aging of wines spiked with DMSO and MSO.

Analysis of DMSO in Wines. Dimethyl sulfoxide, as a highly polar and hydrophilic compound, is not easily quantified in aqueous media. Usual methods are either indirect determination, through GC analysis of DMS formed by chemical reduction of DMSO, or direct determination, through solvent extraction, with or without the previous concentration under vacuum (*40*, *42*, *43*). These indirect methods are not convenient for complex aqueous media such as wine and not suitable for the parts per billion levels found in wine. The method that we developed for this study used DMSO extraction with the diatomaceous earth-filled Chem Elut cartridges (Varian). The DMSO recovery yield, assessed with an external standard, was 30% only, but it was close to the recovery yield using chloroform extraction from wine, lower than 38% (*36*). To circumvent the problems of the extraction step, a stable isotope dilution assay was used, with commercial [$^2H_6$]-DMSO as labeled internal standard, as reported by Dumoulin (*42*) for analyzing DMSO at parts per million levels in malt, wort, and beer. GC/EIMS in the selected ion monitoring mode allowed suitable selective and sensitive detection of DMSO and [$^2H_6$]-DMSO. The detection limit was 5.0 μg/L in the wines with an estimated signal/noise ratio of 3:1. The repeatability, estimated by the coefficient of variation for six replicates of one sample, was 6%.

The seven wines analyzed were four Syrah and three Grenache wines, 0.5−9.5 years aged (vintages 1992−2002), with PDMS range from 11.3 to 47.4 μg/L (**Table 3**) as reported previously (*20*). Their DMSO levels ranged from 9.9 to 41.9 μg/L and were lower than the PDMS levels in six of the seven wines. Thus, except in the S92a wine (11.3 μg/L of PDMS versus 41.9 μg/L of DMSO), these DMSO levels could be partially responsible for the PDMS levels observed in these wines but could not explain these levels alone.

Model Aging of Wines Added with DMSO and MSO. Because of their chemical reactivity, DMSO would hardly release DMS in the heat-alkaline treatment and MSO would only release DMSO. This could explain the discrepancy discussed above between DMS and PDMS during the model aging of wine, if these compounds were DMS precursors in the conditions of wine aging. This hypothesis was tested by submitting to model aging a natural and a model wine spiked with DMSO and MSO, at a level close to 2 mg/L of DMS theoretical equivalents. The deuterated analogue of DMSO, [$^2H_6$]-DMSO, was used for spiking, because it was commercially available. A Syrah wine with low DMS and PDMS contents [lower than 3.7 and 20.9 μg/L, respectively (*20*)] was selected. The same model aging as above (heating at 45 °C) was extended to 10 weeks to reach the end of the DMS production. Free DMS and PDMS levels measured in these assays are displayed in **Table 2**. Free DMS and PDMS levels in the control samples, i.e., the wines without spiking and after the model aging, were consistent with their levels in the initial samples. In the Syrah wine and the model wine spiked with [$^2H_6$]-DMSO and MSO, only very low amounts of [$^2H_6$]-DMS and DMS were observed, neither after model aging nor by heat-alkaline treatment (PDMS determination).

Thus, DMSO at the parts per billion levels measured in this study or at the parts per million levels measured by others (*36*) could hardly lead to significant DMS amounts in the model-aging conditions described above. It can be concluded from these results that DMSO or MSO could only be minor DMS sources during wine aging compared to DMS precursors assessed by the PDMS assay. However, because it was previously shown that yeast can reduce DMSO to DMS, its relevance to release DMS during aging on lees or in wines spoiled with *Brettanomyces dekkera* yeasts cannot be excluded and should be investigated.

***S*-Methylmethionine (SMM) as a DMS Precursor in Wine.** *Model Aging of Wines Added with SMM.* The ability of yeast to release DMS during fermentation from various amino acids and derived compounds was demonstrated (*22*, *23*). The formation of DMS during wine aging was reported by several work (*16*, *17*, *18*, *36*), but the possible involvement of methionine derivatives was not investigated. In beer, *S*-methylmethionine (SMM) was identified as the main DMS precursor (*11*, *44*), through its thermal degradation occurring during the brewing process at temperatures higher than 60 °C (*34*). SMM was not yet reported in wine, and the winemaking process is carried out at lower temperatures (<35 °C). However, it was recently shown that this degradation occurred in cheese model

Table 2. Free DMS and PDMS[a] Levels in Two Wines[b] and in a Model Wine Added with DMS Precursors[c] and Submitted to Model Aging[d]

medium[b]	compound added[c]	level of added compound[e]	treatment applied[d]	free DMS level after treatment[f]	PDMS[a,f] level after treatment	conversion of compound added in free DMS (%)[g]
Syrah wine			storage at 4 °C	3.7 (0.4)	20.9 (0.7)	
			model aging	27.2 (0.8)	6.8 (0.2)	
	[$^2H_6$]-DMSO[h]	2710.0 (2189.1 equiv)	model aging	26.0 (DMS)	19.1 (DMS)	
				9.9 ([$^2H_6$]-DMS)[h]	2.1 ([$^2H_6$]-DMS][h]	0.45
	SMM iodide	9373.3 (1996.4)	model aging	1529.8 (21.3)	70.0 (0.1)	77
	MSO	5286.7 (1984.1 equiv)	model aging	30.0 (1.1)	11.0 (2.0	0.14
Grenache wine			storage at 4 °C	4.4 (0.3)	14.1 (0.3)	
			model aging	23.1 (0.5)	7.4 (0.6)	
	SMM iodide	9373.3 (1996.4 equiv)	model aging	1501.1 (1.7)	119.6 (0.7	75
model wine			model aging	<LOD	<LOD	
	[$^2H_6$]-DMSO[h]	2710.0 (2189.1 equiv)	model aging	<LOD	<LOD	<LOD
	SMM iodide	9373.3 (1996.4 equiv)	control	<LOD	699.9	<LOD
	SMM iodide	9373.3 (1996.4 equiv)	model aging	1562.0 (47.5)	112.6 (5.5)	78
	MSO	5286.7 (1984.1 equiv)	model aging	<LOD	<LOD	<LOD

[a] PDMS refers to DMS released by heat-alkaline treatment (0.5 N NaOH, 80 °C for 1 h). [b] The Syrah and Grenache wines were chosen from a previous study and encoded accordingly (*20*). [c] SMM iodide, *S*-methylmethionine iodide; MSO, methionine sulfoxide; [$^2H_6$]-DMSO, deuterated dimethyl sulfoxide. [d] Model aging by controlled heating, at 45 °C for 10 weeks. [e] Level (μg/L) of added compound and DMS theoretically released (equivalence in parentheses). [f] Mean levels and standard deviation of free DMS and PDMS, in μg/L; <LOD: level lower than the limit of detection (2.0 μg/L). [g] Calculated as the ratio (%) of the free DMS level after model aging to the DMS level theoretically released from the DMS precursor initially added to the sample. [h] When [$^2H_6$]-DMSO was added, DMS and PDMS levels measured were both natural and deuterated DMS was determined by the addition of known amounts of these compounds to the samples.

Table 3. Free DMS, PDMS[a], and DMSO Levels in Syrah and Grenache Noir Wines[b] of Various Vine Locations and Vintages

wine	vintage	code[b]	DMS[c]	PDMS[a,c]	DMSO[c]
Syrah	1992	S92a	46.0 (0.8)	11.3	41.9 (1.2)
Syrah	1998	S98c	44.5 (1.0)	47.4	17.1 (0.7)
Syrah	2000	S00c	11.0 (0.2)	33.4	9.9 (1.1)
Syrah	2002	S02e	3.7 (0.4)	20.9	14.0 (0.8)
Grenache	1997	G97j	13.4 (0.5)	14.6	11.9 (1.5)
Grenache	1998	G98k	15.6 (0.1)	19.5	10.9 (0.7)
Grenache	2000	G00k	10.0 (0.5)	26.0	11.2 (1.5)

[a] PDMS refers to DMS released by heat-alkaline treatment (0.5 N NaOH, 80 °C for 1 h). [b] The Syrah and Grenache wines were chosen from a previous study and encoded accordingly (*20*). [c] Mean levels and standard deviation in parentheses ($n = 3$) of DMS and DMSO, in μg/L.

Table 4. PDMS[a] of Wines at Two Heating Temperatures and Different NaOH Molarities

		80 °C[b]	100 °C		
sample	code[c]	0.5 M NaOH	0.5 M NaOH	1.0 M NaOH	1.5 M NaOH
Grenache wine	G01p	27.2	70.3	55.5	
Syrah wine	S01d	96.0	262.3	251.6	238.1
Grenache wine	G01q	8.6	20.1		
Grenache wine	G01n	22.8	52.3		
Syrah wine	S01e	60.5	181.9		
Syrah wine	S01f	31.5	84.9		
Syrah wine	S01g	53.9	145.7		
model wine and SMM iodide		35%[d]	100%		

[a] DMS released by heat-alkaline treatment; free DMS was removed by N2 stripping before heat-alkaline treatment. [b] DMS released in μg/L of wine, after a reaction for 1 h at the temperature and NaOH molarity mentioned. [c] Wines encoded as mentioned in a previous paper (*20*). [d] Conversion (%) of SMM into DMS, data from Table 2.

medium at 25 °C (*45*). Thus, this study aimed at investigating the ability of SMM to be a DMS precursor in wine, by submitting to model aging a natural and a model wine spiked with SMM iodide. The model-aging conditions and the Syrah base wine were the same as for the assay with DMSO and MSO (see above). Free DMS and PDMS levels measured in these assays are displayed in **Table 2**. DMS was formed from SMM iodide in high yields in both natural and model wines (77 and 78%, respectively). In addition, in a Grenache noir wine spiked with SMM iodide and submitted to model aging in the same conditions, a similar yield (75%) was also observed (**Table 2**), confirming that SMM degradation would not depend on the wine matrix. It can be concluded that, if SMM is present in wine, it would produce DMS during aging.

Moreover, it is noted that the PDMS level, before model aging of the model wine spiked with SMM iodide (control sample in **Table 2**), represented only 35% of the theoretical DMS (699.9 compared to 1996.4 μg/L of DMS equivalents). As a consequence, if SMM is indeed a wine DMS precursor, it would be underestimated by the PDMS assay in the conditions used, which would explain the discrepancy between free DMS and PDMS observed above (**Figure 1**).

Influence of the Heat-Alkaline Treatment Conditions on PDMS Determination. From the results discussed above, the PDMS assay proposed by Swan (*35*) may underestimate the levels of DMS precursors, which led us to investigate the influence of heat-alkaline conditions on PDMS determination in wines. Moreover, because the levels of free and potential DMS in wine were related to the PDMS level in grape juices (*20*), the conditions for PDMS determination in grape juices were also studied. The PDMS levels at the heating temperature initially used (80 °C) were compared to those obtained at 100 °C, which was the maximum temperature suitable in our conditions of analysis. In addition, the influence of the sodium hydroxide molarity used in this assay was evaluated.

Wines. Temperature had a great influence on PDMS in wines, because in the seven wines studied above (three Grenache and four Syrah wines), DMS release was about 3 times higher at 100 °C than at 80 °C (**Table 4**). Furthermore, at 100 °C, PDMS tended to decrease with higher sodium hydroxide molarity. As displayed in **Figure 3**, a good linear regression was observed between the PDMS levels of wines at 100 °C and those at 80 °C. Thus, the PDMS levels measured during the model aging of a wine were recalculated using this relationship (see above and **Figure 1**), which led to a PDMS decrease consistent with the DMS increase (**Figure 4**). This suggested that all of the

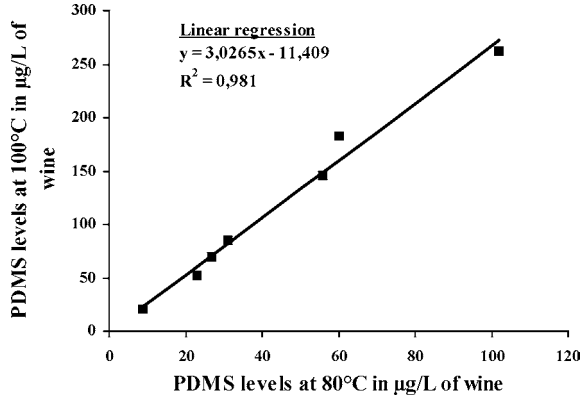

Figure 3. Linear regression between the PDMS levels (means of triplicate analyses) of seven wines determined using the heat-alkaline treatment (1 h with 0.5 M NaOH), at 80 and 100 °C (data from **Table 4**).

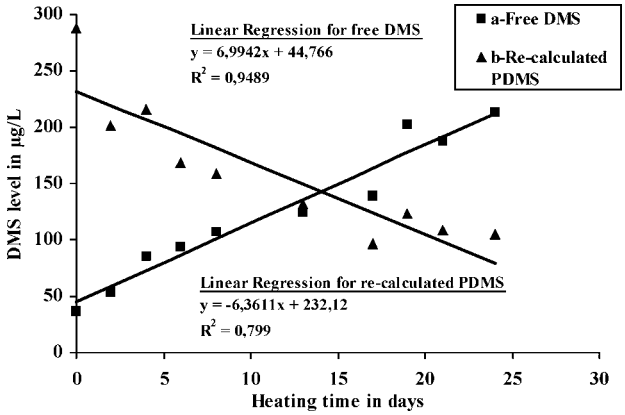

Figure 4. Linear regressions between the heating time of the model aging of a Syrah wine and (a) free DMS (■) and (b) PDMS calculated using the linear relationship given in **Figure 3**, i.e., from PDMS determined in the conditions (0.5 M NaOH, at 80 °C for 1 h) extrapolated to the conditions (0.5 M NaOH, at 100 °C for 1 h) (▲) (data from **Table 1**).

DMS precursors in wine were entirely degraded to DMS by the PDMS assay carried out in the new conditions (0.5 N NaOH, 100 °C for 1 h).

Moreover, the underestimation factor of the PDMS determination in the previous conditions relative to the new ones (approximately $^1/_3$) was consistent with the SMM conversion calculated using the previous PDMS conditions (35%, control sample in **Tables 2** and **4**). An additional assay confirmed that PDMS of a model wine spiked with SMM iodide, using the new conditions, led to the total conversion of SMM into DMS (**Table 4**). Hence, if SMM is a DMS precursor in wine, the PDMS assay with 0.5 M NaOH at 100 °C for 1 h would be enabled to gauge it.

Finally, when the free DMS levels in the natural wines after model aging are compared to the PDMS levels of the controlled wines stored at 4 °C (**Table 2**), it is noted that the conditions of this model aging did not release the total re-estimated PDMS. This was consistent again with the SMM conversion into DMS during the wine model aging (77% in **Table 2**).

Grape Juices. As observed above for wines, much more DMS was released in grape juices using 100 °C heating temperature than 80 °C (**Table 5**). Moreover, the PDMS levels determined at 100 °C tended to increase with sodium hydroxide molarity, up to a maximum level obtained with 1.5 M. Thus, performing the heat-alkaline conditions in 1.5 M sodium hydroxide at 100 °C would provide better assessment of PDMS in grape juices. As displayed by **Figure 5**, a good linear regression was observed

Table 5. PDMS[a] of Grape Juices at Two Heating Temperatures and Different NaOH Molarities

		80 °C[b]	100 °C			
grape juice variety	code[c]	0.5 M NaOH	0.5 M NaOH	1.0 M NaOH	1.5 M NaOH	2.0 M NaOH
Grenache	G01n	74.0	137.5	185.3	185.6	
Syrah	S01f	214.7	444.5	513.0	544.6	525.1
Syrah	S01e	187.0		448.9	509.1	507.3
Grenache	G02p	24.9			57.2	
Grenache	G01p	61.5			156.2	
Syrah		199.9			528.3	

[a] DMS released by heat-alkaline treatment; free DMS was removed by N2 stripping before heat-alkaline treatment. [b] DMS released in µg/L of grape juice, after a reaction for 1 h at the temperature and NaOH molarity mentioned. [c] Grapes encoded as mentioned in a previous paper (*20*).

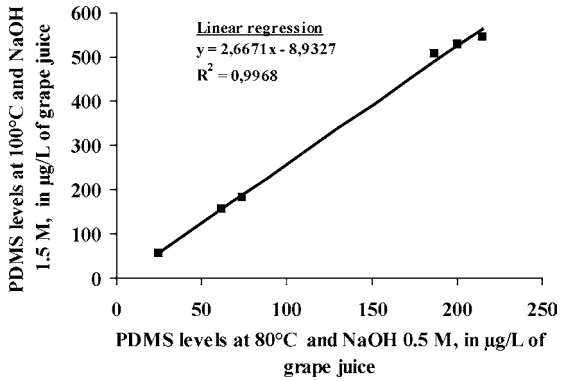

Figure 5. Linear regression between the PDMS levels (means of triplicate analyses) of six grape juices determined using heat-alkaline treatment (1 h with 1.5 M NaOH), at 80 and 100 °C.

between the PDMS levels of grape juices determined using these new conditions and the previous conditions.

Use of the Deuterated Sulfonium Salts [$^2H_6$]-DMSPA Chloride and [$^2H_3$]-SMM Iodide, as Internal Standards in the PDMS Assay. Because DMS loss during the heat-alkaline treatment for PDMS estimation was possible and not controlled, the use of [$^2H_6$]-DMS as an external standard, by addition to the sample after the treatment, could lead to false results. Thus, an internal standard, able to release labeled DMS in the PDMS assay, should be preferred. From the results discussed above, SMM appeared chemically close to DMS precursors in wine and thus would be a good candidate. On the other hand, dimethyl sulfonium propanoic acid (DMSPA), biologically derived from methionine (*24*), was previously reported as a DMS precursor in some plants (*46*−*48*) and is assumed to be a significant precursor of DMS in seawater and atmosphere, via its enzymatic hydrolysis by marine microorganisms and algae (*49*, *50*). A method using alkali treatment to gauge DMS released from this compound has been developed, and [$^2H_6$]-DMSPA chloride was used as an internal standard for the determination of DMSPA in seawater (*25*, *37*).

Thus, we tested [$^2H_6$]-DMSPA chloride and [$^2H_3$]-SMM iodide as internal standards for the measurement of PDMS in wine (assays B and C, respectively, in **Table 6**). The labeling of the DMS produced by heat-alkaline treatment from these deuterated precursors was determined by comparing the mass spectra of the DMS released to those of authentic isotopomers. In assay A (**Table 6**), the stability of the [$^2H_6$]-DMS labeling during the heat-alkaline treatment was checked. The relative abundances of characteristic ions of assay A product, compared to those of initial [$^2H_6$]-DMS, showed that the labeling was not

Table 6. Relative Abundances of Nine Reference Ions in the MS Analysis of DMS Obtained from Five PDMS Assays

assay[a]		*m/z* 62	*m/z* 63	*m/z* 64	*m/z* 65	*m/z* 66	*m/z* 67	*m/z* 68	*m/z* 47	*m/z* 50
A	model wine spiked with $[^2H_6]$-DMS	0	0	5	0	35	6	100	0	117
B	model wine spiked with $[^2H_6]$-DMSPA	2	0	2	5	33	12	100	2	114
C	model wine spiked with $[^2H_3]$-SMM	100	4	4	0	0	0	0	91	0
D	Syrah wine dry extract with heat-alkaline treatment in H_2O	100	5	4	0	0	0	0	86	0
E	Syrah wine dry extract with heat-alkaline treatment in D_2O	5	0	3	3	31	13	100	6	113

[a] The five assays were submitted to heat-alkaline treatment (0.5 M NaOH, 100 °C for 1 h) before GC–MS analysis. See the Materials and Methods for details.

Table 7. PDMS, as DMS Released by Heat-Alkaline Treatment, of a Wine Using Different Standards

standard[a]	PDMS[b]	percent standard deviation ($n = 3$)
$[^2H_6]$-DMS	260.3	6.5
$[^2H_6]$-DMSPA	269.4	2.7

[a] Standard was added to the reaction vial respectively before ($[^2H_6]$-DMSPA) and after ($[^2H_6]$-DMS) the heat-alkaline treatment (*20*). [b] Mean levels ($n = 3$) of DMS released by heat-alkaline treatment (0.5 M NaOH, 100 °C for 1 h) in μg/L.

significantly affected by the heat-alkaline treatment. The results of assay B (**Table 6**) showed that $[^2H_6]$-DMSPA chloride produced only $[^2H_6]$-DMS, consistently with what was reported previously (*37*). On the contrary, the result of assay C (**Table 6**) showed that $[^2H_3]$-SMM iodide produced unlabeled DMS exclusively, showing that the deuterated methyl group had been back-exchanged in the reaction conditions. Because assay A showed that DMS labeling was stable, $[^2H_3]$-SMM should totally back exchange its protons with the reaction medium, before the thermal degradation leading to DMS. The deuterium exchange could be explained by the rapid formation of sulfur ylides under the basic conditions used, before Hoffmann β elimination or nucleophilic substitution by hydroxide ion of $[^2H_3]$-SMM. The ability of sulfonium groups to stabilize adjacent carbanions is well-known, with the properties of the sulfur center resulting in enhanced acidity of hydrogen atoms in α positions to the sulfur. The abstraction of these hydrogens in α positions to the sulfoniums has been previously observed in basic conditions, similar to those used in our experiments (*51*). On the contrary, $[^2H_6]$-DMSPA should much more rapidly release $[^2H_6]$-DMS, stable in the basic conditions, because the alkene formed through the retro-Michael addition (acrylic acid) was stabilized by resonance with the carboxylic group. As a consequence of the instability of SMM deuterium labeling during the heat-alkaline treatment, $[^2H_6]$-DMSPA chloride could only be used as an internal standard in the PDMS assay. However, a stable ^{13}C-labeled SMM could be employed, which would significantly increase the cost of the analysis.

Furthermore, the PDMS assay in a wine using $[^2H_6]$-DMS as an external standard that we reported previously (*20*) was compared to the same analysis using $[^2H_6]$-DMSPA chloride as an internal standard (**Table 7**). No significant difference was observed between the levels measured (one-way × three repetitions ANOVA, $p = 0.05$), showing that the conversion of $[^2H_6]$-DMSPA into $[^2H_6]$-DMS was total and that no loss of $[^2H_6]$-DMS occurred in both assays.

However, the standard deviation was lower when $[^2H_6]$-DMSPA chloride was used, which could be explained by a more accurate standard addition considering the very high volatility of DMS compared to the stable crystals of $[^2H_6]$-DMSPA chloride. Hence, $[^2H_6]$-DMSPA chloride could be a convenient internal standard for PDMS determination.

Finally, the observation of the different chemical reactivities of SMM and DMSPA suggested investigation of the natural DMS precursors in wine. Thus, in assays D and E (**Table 6**), the ability of the wine DMS precursors to exchange protons during the heat-alkaline treatment was tested. When the aqueous matrix was changed for a deuterium oxide medium (assay E compared to D), only $[^2H_6]$-DMS was produced by heat-alkaline treatment, showing that the natural DMS precursor in wine did exchange their protons in α positions to the sulfur atom. Thus, the chemical reactivity of this or these unknown precursor(s) was similar to that of SMM but different from that of DMSPA, excluding the latter compound as a wine DMS precursor.

From the different results discussed above, the chemical reactivity of DMS precursors in wine was shown to be similar to that of SMM, through their conversions into DMS during the model aging of a wine, their conversions into DMS in different heat-alkaline conditions, and their exchange of protons in the α positions to the sulfur atom in the heat-alkaline assay. Hence, SMM appeared to be a good candidate for the DMS precursor during wine aging, but further work is needed to identify this precursor in wine or must. Then, the costly synthesis of the ^{13}C-labeled SMM would allow us to improve the assessment of PDMS levels in wine and must, using the conditions developed in this work.

ABBREVIATIONS USED

DMS, dimethyl sulfide; DMSO, dimethyl sulfoxide; SMM, *S*-methylmethionine; MSO, methionine sulfoxide; DMSPA, dimethyl-sulfonium propanoic acid; PDMS, potential dimethyl sulfide, as DMS released by heat alkaline treatment; GC/MS, gas chromatography/mass spectrometry; SPME, solid-phase microextraction.

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