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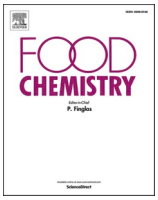

A method for the quantitative and reversible trapping of sulfidic gases from headspaces and its application to the study of wine reductive off-odors

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ABSTRACT

Some relevant food systems release tiny amounts of sulfidic gases, whose measurement is difficult because of their inherent instability. The present paper demonstrates that Cu(I) solutions trap quantitatively and stabilize sulfidic gases. Once trapped, the gases remain stable for weeks at 4 °C and at least 8 days at 75 °C. Trapped gases can be quantitatively released with tris(2-carboxyethyl) phosphine (TCEP) and brine dilution and then determined by GC. Trapping solutions, placed in 20-mL opened vials housed in 100 mL hermetically-sealed flasks containing wine in anoxia, have been used to monitor the release of sulfidic gases by wines, revealing that at 50 °C, up to 400 μg/L of H_2S and 58 μg/L of MeSH can be released in 68 days, and 3–5 times more at 75 °C in 28 days. The possibility to differentiate between released and accumulated amounts provides key clues to understanding the fate of sulfidic gases in wine and other food systems.

1. Introduction

Reductive problems are linked to the development of sulfur off-odors during the anoxic storage of wines. These odors are caused by hydrogen sulfide (H_2S), methanethiol (MeSH) and, eventually, other sulfhydryls and their derivatives (Siebert, Solomon, Pollnitz, & Jeffery, 2010). Reductive problems affect a significant number of wines (Goode & Harrop, 2008) implying strong economical and brand-image losses. Incidentally, the problem is becoming more frequent as present winemaking strategies limits contact with air during the whole winemaking process (Bekker, Day, Holt, Wilkes, & Smith, 2016) and more hermetic canning systems are proposed (AWRI, 2021).

The molecules causing the problems are mainly formed during fermentation. Yeasts naturally produce little to moderate amounts of H_2S and MeSH, but in certain circumstances, not completely understood, produce relatively high levels of these molecules. While a fraction is lost by evaporation, a significant amount can remain in wine under stable and odorless metal complexes (Franco-Luesma & Ferreira, 2014), disulfides, polysulfides (Bekker, Kreitman, Jeffery, & Danilewicz, 2018) and, eventually, polythionates (Müller & Rauhut, 2018; Müller, Rauhut, & Tarasov, 2022). These molecules can be formed by metal catalyzed chemical oxidation (Kreitman, Danilewicz, Jeffery, & Elias, 2016b, 2017), or by enzymatic processes (Dekker, Fedrizzi, van Leeuwen, Roman, Nardin, & Larcher, 2022; Pilkington et al., 2019), and form a complex pool of precursors of H_2S and MeSH. Little amounts of sulfidic gases will be slowly re-formed from the different precursors as soon as the wine is stored without any contact with air (Franco-Luesma & Ferreira, 2016a, 2016b). The chemical processes responsible for such re-formation are not completely understood. On the one hand, several slow but spontaneous chemical reactions in which electrons are released, such as those involving condensation of polyphenols and SO_2-related reactions, take place during anoxic storage (Ontanon, Sanchez, Saez, Mattivi, Ferreira, & Arapitsas, 2020). On the other hand, a number of reactions, such as sulfitolysis, thiosulfatolysis and thiolysis could play relevant roles (Kreitman, Elias, Jeffery, & Sacks, 2019; Müller & Rauhut, 2018; Müller et al., 2022) in the reduction of the pool of precursors and

Abbreviations: BCDA, bathocuproine disulfonic acid; BR, brine-releasable; EDTA, Ethylenediaminetetraacetic acid; EMS, Ethyl methyl sulphide; HS-GC-SCD, Head space Gas Chromatography with sulfur chemiluminescent detection; I.D, Internal diameter; MeSH, methanethiol; MMI, Multi mode injector; RA, Accelerated reductive aging; TCEP, tris(2-carboxyethyl) phosphine; VSCs, Volatile sulfur compounds.

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the release of H_2S and MeSH.

Because of its complexity, the direct chemical analysis of the pool of precursors is extremely difficult. Advanced HPLC-MS based analytical methods for the quantitative analysis of polysulfides from cysteine and glutathione have been developed (Jastrzembski, Allison, Friedberg, & Sacks, 2017; Kreitman et al., 2017; van Leeuwen, Nardin, Barker, Fedrizzi, Nicolini, & Larcher, 2020) and have been successfully applied to demonstrate the implication of these molecules in H_2S release (Bekker et al., 2018), to trace their origin in fermentation (Dekker, Fedrizzi, van Leeuwen, Nardin, Dell'Anna, & Larcher, 2022), to assess the role of yeast (Dekker, Fedrizzi, van Leeuwen, Roman, et al., 2022) and to verify that their levels are negatively affected by the addition of some antioxidants before fermentation (Nardin et al., 2020). However, those HPLC-MS methods can quantify, so far, just a fraction of the total pool of precursors of H_2S and MeSH. In particular, polysulfides bound to proteins, polythionates (Müller et al., 2022) and species forming complexes with copper (Kreitman et al., 2016b) are not, at present, quantifiable through those methods.

Moreover, the few analytical tests developed to date to assess potential levels of H_2S and MeSH in wine do not provide reliable estimates of the magnitude of the pool of precursors capable of producing H_2S and MeSH. In the test proposed by Franco-Luesma and Ferreira, the wines are incubated in strict anoxia for 2 weeks at 50 °C (Franco-Luesma & Ferreira, 2016b). Free and brine-releasable (BR) levels of H_2S and MeSH accumulated in the wine after such incubation were correlated with the levels of free and BR forms accumulated by the wines after 1 year of anoxic aging in the bottle at room temperature (Franco-Luesma & Ferreira, 2016a). While this suggests that the levels of free forms of H_2S and MeSH measured after accelerated reductive aging can predict the relative tendencies of a set of wines to accumulate free H_2S and MeSH during standard aging, and hence, to develop reductive off-odors, the other results of the test are not adequate to assess the magnitude of the pool of H_2S and MeSH precursors. In particular, levels of BR forms measured after accelerated reductive aging may not be directly related to the actual magnitude of the pool of precursors as initially thought (Ferreira, Franco-Luesma, Vela, López, & Hernández-Orte, 2018), since neither the proportions of the pools of precursors that are finally transformed into H_2S and MeSH during anoxic incubation are known, nor are the proportions of these two re-formed molecules that reacted with other wine components during the incubation time. The test proposed by Kreitman et al (Kreitman et al., 2017) is based on the combined addition of a strong reducing agent and a Cu(I)-complexing agent, so that it directly targets the complete pool of precursors. However, the proportion of precursors that such a method can reveal in real wine is not really known, since validation has been carried out only with model wines. In fact, levels of "total" H_2S in wines reported using this method (Chen, Jastrzembski, & Sacks, 2017; Kreitman et al., 2017) are relatively low (from 3 μg/L to 77 μg/L), which may suggest that the release is incomplete.

In this context, it seems necessary to have at hand a method able to trap H_2S and sulfhydryls as far as they are released, so that they are quickly removed from the media. This would avoid any further reaction of these molecules with other wine components, would also facilitate a shift in chemical equilibria towards the formation of the sulfidic gases and, if trapped H_2S and sulfhydryls can be efficiently quantified, would provide more accurate estimates of the magnitude and chemical nature of the pool of precursors. Because of this, the main goal of the present research was to develop and validate a method for the quantitative and reversible trapping of H_2S and sulfhydryls released to headspaces. The method was also applied to assess the amounts of sulfidic gases released by wines during anoxic storage. The effects of temperature and of the combined addition of TCEP and BCDA were also briefly assessed.

2. Material and methods

2.1. Solvents and chemical standards

Absolute ethanol was purchased from Merck (Darmstadt, Germany). Ultrapure water was obtained from a Milli-Q system from Millipore (Merck, Germany); sodium hydroxide (NaOH), sodium chloride (NaCl), tartaric acid −2,3-dihydroxybutanedioic acid ($C_4H_6O_6$), ascorbic acid -(2R)-2-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxy-2H-furan-5-one ($C_6H_8O_6$), silver nitrate ($AgNO_3$) and zinc diacetate dihydrate ($ZnCH_3CO_2{*}2H_2O$) were purchased from PanReac AppliChem (Barcelona, Spain). Copper(I) chloride (CuCl), disodium sulfide (Na_2S), sodium methanethiolate (CH_3SNa) and ethyl methyl sulfide (EMS) -methylsulfanylethane (C_3H_8S) were obtained from Sigma-Aldrich (Germany). The flaks containing Na_2S, CH_3SNa and CuCl were vacuum packed immediately after use. Na_2S was stored at 4 °C, and CH_3SNa and CuCl were stored in a desiccator. Copper sulfate pentahydrate ($CuSO_4{*}5H_2O$) was purchased from LabKem (Spain). For calibration purposes, 1 g/L stock alkaline aqueous solutions of Na_2S and CH_3SNa were freshly prepared in an anoxic chamber P[box] (Jacomex, France) with Argon atmosphere. Stock, intermediate and working solutions of EMS were prepared in ethanol in amber vials with Mini-inert valves (Supelco, Ca, USA) and were stored at −20 °C. Brine contained 350 g/L of NaCl and 0.5 g/L of ascorbic acid in Milli-Q water. Model wine was a pure water solution containing 5 g/L of tartaric acid, 12 % v/v ethanol and pH 3.4 adjusted with diluted NaOH (0.1 M). The optimal trapping solution contained 100 mg/L of CuCl and was 15 mM in HCl. This solution was daily prepared by dilution of a stock trapping solution containing 10 g/L CuCl in 1.5 M HCl, prepared and stored under Argon. All these solutions were manipulated inside the anoxic chamber.

2.2. Analysis of free and BR-volatile sulfur compounds (VSCs) by HS-GC-SCD

Free and BR forms of H_2S and MeSH were analysed following the procedure proposed by Ontanon et al (Ontanon, Vela, Hernandez-Orte, & Ferreira, 2019). Except the GC analysis, all experimental steps took place inside the anoxic chamber. For the determination of free VSCs, 12 mL of wine and 40 μL of the internal standard solution (10 mg/L EMS in ethanol) were transferred to a 20 mL vial, which was closed, taken out of the glove box and placed in the sampler tray. After 15 min of incubation at 30 °C, 1 mL of the headspace was taken with a syringe thermostated at 40 °C and injected at 30 μL/s with a 1:2 split ratio in the GC injector. For the determination of the BR fraction of VSCs, 1.2 mL of wine, 10.8 mL of brine and 40 μL of the internal standard solution (2 mg/L EMS in ethanol) were transferred to the 20 mL vial. The vial was incubated for 25 min at 70 °C, the syringe was at 80 °C, and the injection of 1 mL of headspace was carried out at 1000 μL/s using a 1:15 split ratio.

Analyses were carried out using an Agilent 7890B gas chromatograph with a selective detector SCD 8355. The capillary column was a SPB-1 SULFUR (30 m × 0.32 mm I.D. × 4 um film thickness) from Supelco, (Bellefonte PA, USA) preceded by a precolumn, 3 m × 0.32 mm I.D. of deactivated fused silica (polar deactivation). The precolumn crosses inside the Cryogenic Trapping System (CTS 2, Gerstel). The injection was made into a MMI injector equipped either with a 1 mm I.D. ultra-inert liner from Agilent for the analysis of free forms, or with a 4 mm I.D. ultra-inert liner for the analysis of BR forms. The autosampler was a Combi-PAL from CTCAnalytics (Zwingen, Switzerland) with a static headspace unit. After the injection, the syringe was purged with nitrogen for 5 min. For the analysis of free forms, the chromatographic oven was held at 35 °C for 3.8 min then heated to 160 °C at 10 °C/min and held at this temperature for 0.5 min. The cryogenic unit was kept at − 150 °C for 0.8 min and then raised at 20 °C/s up to 300 °C. Helium was used as carrier gas at 2 mL/min during the first 0.8 min and then at 1.4 mL/min. Chromatographic conditions in the analysis of BR forms were similar, but the initial oven temperature was kept for 3 min, Helium was

set the first 0.8 min at 0.9 mL/min and the cryogenic unit was not used. Detector conditions: the base temperature of the detector was 280 °C and that of the burner was 800 °C; air flow was set to 50 mL/min as oxidizer gas and H_2 flow was set to 38 mL/min in the upper flow and to 7 mL/min in the lower flow.

2.3. Metal cations as potential trapping systems

Unless otherwise stated, all the experiments involving sample preparation described in this and in the next sections, were carried out inside the anoxic chamber. In a preliminary set of experiments, aqueous solutions containing 200 μg/L of H_2S and 20 μg/L of MeSH, 20 μg/L of EMS, the internal standard, and 0.5 mg/L of ascorbic acid, were spiked with the metal cations to make their molar levels 10 times [cases of Cu (II) and Zn(II)] or 20 times [Cu(I) and Ag(I)] in excess with respect to the H_2S content, so that the ratios -SH/metal cation are very similar. The headspaces of the solutions were monitored using the method for quantifying free forms of VSCs before and after spiking with the metals.

For the study of the kinetics of the trapping of sulfidic gases by aqueous solutions of metal cations, a double vial system was used. The external vial was a closed screw capped 10 mL headspace vial with 4 mL of model wine (5 g/L tartaric acid, 12% ethanol, pH 3.4) containing 200 μg/L of H_2S and 20 μg/L of MeSH, further spiked with 15 μL of the internal standard solution (10 mg/L EMS in ethanol). The internal vial was a 2 mL opened glass vial containing either 1.5 mL of the trapping solution or, in the case of controls, 1.5 mL of purified water. Trapping solutions contained in all cases 167.4 μM of the cation, which is a 10-molar excess with respect to the total SH groups present in the model wine. Contact between the model wine and the trapping solution was exclusively through the headspace. Eighteen different complete sets of double vials were prepared for each cation studied, nine with the trapping solution and nine controls. The vials were incubated at room temperature and their internal headspaces were analysed at different times, between 0 and 7 h following the procedure used for the analysis of free forms. Each vial was analysed just once and was discarded after the analysis. The experiment was duplicated.

2.4. Recovering VSCs from metal-trapped solutions

This study was carried out with aqueous solutions containing 125 μM of one of the four metal cations. Four mL of these solutions were further spiked with a concentrated solution containing H_2S and MeSH, so that the final concentrations were 220 and 10 μg/L, respectively. Then, 1.2 mL aliquots of the mixture were transferred to a 20 mL headspace vial containing 10.8 mL of brine or water, and were, eventually, further spiked with either TCEP (1 mL of aqueous TCEP at 30 g/L, making it 250 mg/L), BCDA (1 mL of aqueous BCDA at 66 g/L, making it 550 mg/L) or EDTA (0.6 mL of aqueous EDTA, making it 20 mg/L), as detailed in Table 1. The mixtures were finally spiked with the internal standard and analysed following the procedure for BR-forms. Two replicated vials were prepared for each experiment and each vial was analysed in duplicate. In the case of Cu(I), additional levels of TCEP (2500 mg/L) and of dilution with brine (1:24 and 1:100) were assayed. Furthermore, recovery was also checked with the optimal trapping solutions containing 1015 μM of Cu(I) (100 mg/L of CuCl), 0.015 M HCl and three different levels of sulfidic gases: level 1: 250 and 30 μg/L; level 2: 1250 and 150 μg/L; level 3: 6250 and 750 μg/L of H_2S and MeSH, respectively. In all these cases, 0.5 mL of the solution was mixed with 11.5 of brine (1:24 dilution) and spiked with 30 mg of TCEP (2.5 g/L).

2.5. Analytical characterization of the stability, release and further HS-GC-SCD determination of sulfidic gases trapped in CuCl solutions

These experiments were carried out with the optimal trapping solutions which were 10 mL aqueous solutions containing 100 mg/L of CuCl and 0.015 M HCl. VSCs contained in these trapping solutions were determined by transferring 0.5 mL of the trapping solution to a 20 mL vial containing 11.5 mL of brine, spiking with TCEP (30 mg, to make it 2.5 g/L) and IS (40 μL of 2 mg/L EMS) and analysing the headspace following the GC-SCD procedure for BR forms described in section 2.2.

The repeatability of the release of the trapped material and its

Table 1
Recoveries of H_2S and MeSH trapped in different metal cation solutions (125 μM unless otherwise stated) after different treatments to induce complex cleavage. If nothing else is specified, dilution with brine was 1:10 and TCEP, EDTA or BCDA added were 250, 550 or 20 mg/L, respectively. Uncertainties are standard deviations of two independent experiments. Different superscript letters indicate the existence of significant differences (*t* test).

Metal cation	Treatment	H_2S recovered	MeSH recovered
Zn^{2+}	Dilution with brine	97 ± 4.1% ^{a}	Not retained
	Dilution with brine after 24 h	102 ± 3.2% ^{a}	Not retained
	TCEP addition	105 ± 6.6% ^{a}	Not retained
	TCEP addition after 24 h	95 ± 4.2% ^{a}	Not retained
	TCEP addition + dilution with brine	91 ± 3.7% ^{a}	Not retained
	TCEP addition + dilution with brine after 24 h	108 ± 6.8% ^{a}	Not retained
	EDTA addition + dilution with brine	103 ± 2.3% ^{a}	Not retained
Ag^{+}	Dilution with brine	79 ± 5.9% ^{a}	88 ± 9.4% ^{a}
	TCEP addition + dilution with brine	96 ± 4.4% ^{a}	102 ± 7.9% ^{a}
Cu^{2+}	Dilution with brine	18 ± 4.0% ^{b}	80 ± 4.6% ^{bcd}
	TCEP addition	0.6 ± 3.4% ^{a}	98 ± 6.3% ^{c}
	TCEP addition after 24 h	0.5 ± 0.9% ^{a}	67 ± 7.2% ^{b}
	TCEP addition + dilution with brine	22 ± 4.4% ^{b}	101 ± 6.0% ^{c}
	TCEP addition + dilution with brine after 24 h	19 ± 2.9% ^{b}	69 ± 8.4% ^{b}
	TCEP addition + dilution with brine (low VSC level)	70 ± 5.4% ^{c}	82 ± 4.5% ^{bc}
	TCEP addition + dilution with brine after 24 h (low VSC level)	4 ± 3.6% ^{ab}	6 ± 6.2% ^{a}
Cu^{+}	Dilution with brine	11 ± 5.8% ^{ab}	80 ± 7.3% ^{bc}
	TCEP addition	0.1 ± 2.9% ^{a}	75 ± 3.4% ^{bc}
	TCEP addition after 24 h	0.3 ± 2.2% ^{a}	69 ± 4.6% ^{bc}
	TCEP addition + dilution with brine	85 ± 5.1% ^{de}	95 ± 3.2% ^{de}
	TCEP addition + dilution with brine after 24 h	82 ± 5.2% ^{de}	96 ± 5.7% ^{de}
	TCEP addition + dilution with brine (1:24)	66 ± 6.7 % ^{cd}	74 ± 6.5% ^{bc}
	TCEP addition (+2.5 g/L) + dilution with brine (1:24)	94 ± 5.9% ^{ef}	95 ± 4.8% ^{de}
	TCEP addition (+2.5 g/L) + dilution with brine (1:24) after 24 h	100 ± 5.2% ^{ef}	97 ± 5.5% ^{de}
	TCEP addition (+2.5 g/L) + dilution with brine (1:24) after 48 h	106 ± 6.0 % ^{fg}	96 ± 3.5%
	TCEP addition + dilution with brine (1:100)	40 ± 8.9% ^{bc}	42 ± 5.2% ^{a}
	TCEP addition (+2.5 g/L) + dilution with brine (1:100)	68 ± 7.6 % ^{cd}	68 ± 2.3% ^{b}
	TCEP + BCDA + dilution with brine (1:24) after 48 h	80 ± 9.3% ^{de}	78 ± 2.2% ^{c}
	TCEP (+2.5 g/L) + BCDA + dilution with brine (1:24) after 48 h	105 ± 5.3 % ^{fg}	82 ± 5.1% ^{bcd}
Cu^{+} (1015 μM)	250 μg/L H_2S, 30 μg/L MeSH + TCEP (+2.5 g/L) + brine (1:24 dilution)	99.2 ± 3.2% ^{ef}	97.3 ± 5.0% ^{de}
	1250 μg/L H_2S, 150 μg/L MeSH + TCEP (+2.5 g/L) + brine (1:24 dilution)	102.3 ± 4.3 % ^{fg}	99.5 ± 3.8% ^{e}
	6250 μg/L H_2S, 750 μg/L MeSH + TCEP (+2.5 g/L) + brine (1:24 dilution)	96.9 ± 4.6% ^{ef}	101 ± 2.9% ^{e}

subsequent HS-GC-SCD determination was evaluated by studying the 191 standard deviations obtained in the duplicate analysis of the 191 real trapping solutions produced in the experiment described in 2.7. The limits of detection were calculated from the trapping solutions containing the smallest levels of sulfidic gases as the concentration that gave a signal 3 times higher than the noise. For the study of the stability of trapped solutions, two series of fourteen 10-mL vials containing 10 mL of the trapping solutions spiked either with 320 μg/L of H_2S and 50 μg/L of MeSH (low level) or with 1.6 and 0.20 mg/L (high level), were prepared. Two similar series of ten 10-mL vials containing equivalent amounts of H_2S and MeSH were used as controls. The vials were stored under strict anoxia in an incubator at 75 °C. Every day or every other day for a period of 8 days, a pair of vials per level were taken and analysed. Similar experiments were carried out at room temperature (25 °C) and at 4 °C at a single concentration level (850 μg/L of H_2S and 95 μg/L of MeSH). At room temperature, vials were analysed 2 times per week for a period of 3 weeks, and at 4 °C, they were analysed weekly for 7 weeks. These experiments were carried out in duplicate. Linearity, stability and recovery of the release of trapped material, were further investigated by preparing a set of five trios of vials containing trapping solutions spiked at five different levels of H_2S (0, 0.84, 2.45, 4.67 and 7.0 mg/L) and MeSH (0, 0.20, 0.42, 0.63 and 0.84 mg/L). The vials were stored for 4 days at 50 °C and were then analysed. The areas obtained were compared with those obtained in the duplicate analysis of freshly prepared aqueous solutions without copper and containing equivalent amounts of H_2S and MeSH.

2.6. Design and validation of a device for the continuous trapping of sulfidic gases emanated from wine

A series of preliminary tests were carried out to verify the airtightness of different closure systems and glassware, which was assessed by using a PSt3 or PSt6 O2 sensor from Nomasense (Nomacorc, France). The first functional "trapping device" was made with a 25 mL Erlenmeyer type flask equipped with a 19/26 ground glass stopper, inside which was housed a small test tube (0.8 cm diameter × 5.5 cm length) containing 1.5 mL of the trapping solution.

The finally proposed trapping device is schematized in the supplementary material (Figure S1) and consists of a 100 mL flat bottom round glass flask fitted with a 29/32 glass stopper and of a 20-mL standard headspace glass vial containing 10 mL of the trapping solution. This vial is housed inside the main flask, which also contains the wine (80 mL). The wine then surrounds, without actually submerging, the 20 mL-vial. This allows wine and trapping solution to only come into contact through the gas phase. A metallic clamp is further used to keep the cap closed throughout the incubation. All the sample preparation takes place within the anoxic chamber. The trapping devices are then taken out of the anoxic chamber and incubated at the specified temperature. After incubation, the devices are cooled down and re-introduced in the chamber. Then, 0.5 mL aliquots of the trapping solution were transferred, as described in 2.5., to a 20 mL vial containing 11.5 mL of brine. The mixture was further spiked with TCEP (30 mg, to make it 2.5 g/L) and IS (40 μL of 2 mg/L EMS). The vial was closed, taken out of the glove chamber and left in the GC autosampler tray, where it was analysed following the GC-SCD procedure for BR forms to determine the concentrations of H_2S and MeSH contained in the 0.5 mL of trapping solution. These amounts, multiplied by 20 and divided by 80, correspond to the total concentration of H_2S and MeSH released by the wine in the incubation time.

The repeatability of the trapping devices was evaluated by studying the 82 standard deviations obtained in the analysis of 55 pairs and 27 triplets of independent replicates of trapping solutions produced in the experiment described in 2.7. The efficiency of the trapping devices was assessed in three experiments, one with model wine, one with red wine and a third with white wine. In each experiment, 2 × 6 trapping devices containing 80 mL of wine or synthetic wine and increasing concentrations of H_2S and MeSH, were prepared. Levels of H_2S and MeSH used were 50 and 5 μg/L, 100 and 10 μg/L, 150 and 15 μg/L, 200 and 20 μg/L, 400 and 30 μg/L and 600 and 40 μg/L of H_2S and MeSH, respectively. Volumes of synthetic wine containing the corresponding amounts of H_2S and MeSH, were introduced in the trapping devices, were incubated at 50 °C for 24 h and then, the trapping solutions were taken and analysed. In the case of real wines, twelve 80-mL volumes were incubated in the trapping devices at 50° for 7 days to make them become reductive. After this, the flasks were cooled down, re-introduced in the anoxic chamber, the original trapping solutions were discarded and replaced by new ones, and the 80 mL of wine was then spiked with H_2S and MeSH to provide the concentrations previously described. Then, the trapping devices were incubated at 50° for 24 h, after which, the trapping solutions were taken and analysed.

The use of magnetic agitation to expand the working range of the traps was also considered. For this, different magnetic nuclei (two types of standard PTFE-covered and one glass-made) were considered and added to the trapping solution. The trapping devices were then set on an agitator plate installed within the incubator. A comparison of the areas obtained with and without agitation during the incubation of model wines containing 200, 500 and 750 μg/L of H_2S was made in triplicate.

2.7. The spontaneous release of H_2S and MeSH from wines stored in anoxia

Seven different wines, as described in Table 2, were used in this experiment. For each wine, two sets of trapping devices were prepared in duplicate (in triplicate in two of the wines), one was incubated at 50 °C and the other at 75 °C. In samples at 50 °C, the trapping solutions were replaced at least once per week, with up to three changes in the first weeks. In samples stored at 75 °C, the trapping solutions were replaced daily or every three days. The amounts of H_2S and MeSH contained in the trapping solutions were analysed, as indicated in 2.5, by transferring 0.5 mL of the trapping solution to a 20 mL vial containing 11.5 mL of brine, spiking with TCEP (30 mg, to make it 2.5 g/L) and IS (40 μL of 2 mg/L EMS) and analysing the headspace following the procedure for BR forms.

2.8. Wine characterization

Free and BR contents of VSCs of the seven wines were determined as indicated in 2.2. Additionally, the seven wines were subjected to standard accelerated anoxic aging at 50 °C following the methodology developed by Franco-Luesma and Ferreira (Franco-Luesma & Ferreira, 2016b) but using 14 days as standard incubation time. For this, the wine bottles were introduced into the anoxic chamber, where they were opened and distributed in three 60-mL aliquots in three 60-mL screw capped glass tubes (Wit Deluxe, Denmark). The tubes were tightly closed and double vacuum bagged, including a layer of powder containing an O_2 scavenger (AnaeroGen™ from Thermo Scientific Waltham, Massachusetts, United States) between both bags. Bagged samples were incubated at 50 °C for 2 weeks, after which were cooled down, introduced in the anoxic chamber and analysed for free and BR forms of VSCs as indicated in 2.2.

2.9. Release of H_2S and MeSH after the direct addition of TCEP

A white wine, different to those used previously, displaying a strong reductive off-odour was used in the first part of this experiment. Sixteen 80 mL aliquots of wine were spiked in duplicate with four different levels of TCEP (0, 12, 60 and 200 mg) and were kept at room temperature in complete anoxia. Samples were taken and analysed at days 0, 1, 2, 3 and 6th. Parameters controlled were the redox potential and the free and BR levels of H_2S. Redox potential was measured according to Vela et al. (Vela et al., 2018) with a commercial electrode consisting of a Pt electrode, an Ag-AgCl(s) reference electrode and a HI 98191 ORPmeter

Table 2
The amounts of H_2S and MeSH accumulated or released by the seven different wines used in the main experiment. All data are given in μg/L. Free and BR refer to the free and BR levels of the original wines, RA-free and RA-BR, to the corresponding levels after 2 weeks of anoxic incubation at 50 °C. The two last columns are data extracted from the plots in Fig. 3. Uncertainties are standard deviations of two independent replicates analyzed each twice.

		H_2S						MeSH					
Wine code	Wine type, grape cultivar, vintage, geographical origin	Free	BR	RA-free	RA-BR	Released after 21 d at 50 °C	Released after 7 d at 75 °C	Free	BR	AR-free	AR-BR	Released after 21 d at 50 °C	Released after 7 d at 75 °C
TT17	Red, tempranillo, 2017, La Rioja	0.14 ± 0.01	23.2 ± 9.7	0.76 ± 0.51	63.04 ± 2.5	42.1 ± 3.2	443 ± 34	0.61 ± 0.09	3.94 ± 0.39	5.2 ± 0.03	9.37 ± 0.39	11.65 ± 0.71	65.7 ± 4.4
TT20	Red, tempranillo, 2020, Toro	3.3 ± 0.22	21.39 ± 0.47	14.6 ± 2.4	44.17 ± 1.7	94.6 ± 7.2	402 ± 31	0.79 ± 0.1	3.84 ± 0.88	2.76 ± 0	5.28 ± 0.75	11.25 ± 0.68	29.1 ± 1.9
TG20	Red, garnacha, 2020, Cariñena	0.17 ± 0.02	83.26 ± 6.6	2.48 ± 0.51	116.16 ± 6.1	119.6 ± 9.2	540 ± 42	0.36 ± 0.06	2.71 ± 1.35	2.41 ± 0.12	5.41 ± 0.09	12.04 ± 0.73	47.4 ± 3.2
TG16	Red, garnacha, 2016, Cariñena	0.29 ± 0.01	28.63 ± 5.68	4.3 ± 1.6	38.24 ± 7.1	54.7 ± 4.2	373 ± 29	0.58 ± 0.2	2.49 ± 0.65	2.9 ± 0.37	6.38 ± 0.79	10.61 ± 0.64	48.2 ± 3.2
RG20	Rosé, garnacha, 2020, Cariñena	0.2 ± 0.02	18.96 ± 5.39	19.14 ± 0.23	38.46 ± 0.93	108.0 ± 8.3	510 ± 39	0.56 ± 0.11	3.97 ± 3.9	5.36 ± 0.24	6.67 ± 0.66	4.96 ± 0.30	25.29 ± 0.15
WCR20	White, chardonnay, 2020, Somontano*	3.85 ± 0.64	147.25 ± 0.55	16.47 ± 0.72	93.62 ± 0.43	117.4 ± 9.0	341 ± 26	1.19 ± 0.47	3.4 ± 1.0	6.4 ± 0.02	8.93 ± 0.31	10.34 ± 0.63	16.3 ± 1.1
TTR20	Red, tempranillo, 2020, La Rioja*	3.22 ± 0.27	32.8 ± 2.5	17.06 ± 0.68	40.05 ± 1.31	154 ± 12	1261 ± 97	1.87 ± 0.01	3.94 ± 0.63	3.55 ± 0.05	7.74 ± 0.27	17.8 ± 1.1	52.0 ± 3.5

*These two wines were experimental wines to which elemental sulfur was added before fermentation to induce the formation of reductive off-odors.

(Hanna Instruments, Woonsocket, RI, U.S.A.). All measurements were carried out inside the anoxic chamber. Measurements were taken by immersing the electrode into 5 mL-samples and allowing for 35 min of equilibration.

In the second part of the experiment, two of the wines studied in 2.7 (WCR20 and TTR20) were treated with TCEP and BCDA (1 mM each) as described by Kreitman et al (Kreitman et al., 2017) and were then incubated in the trapping device proposed in the present paper at room

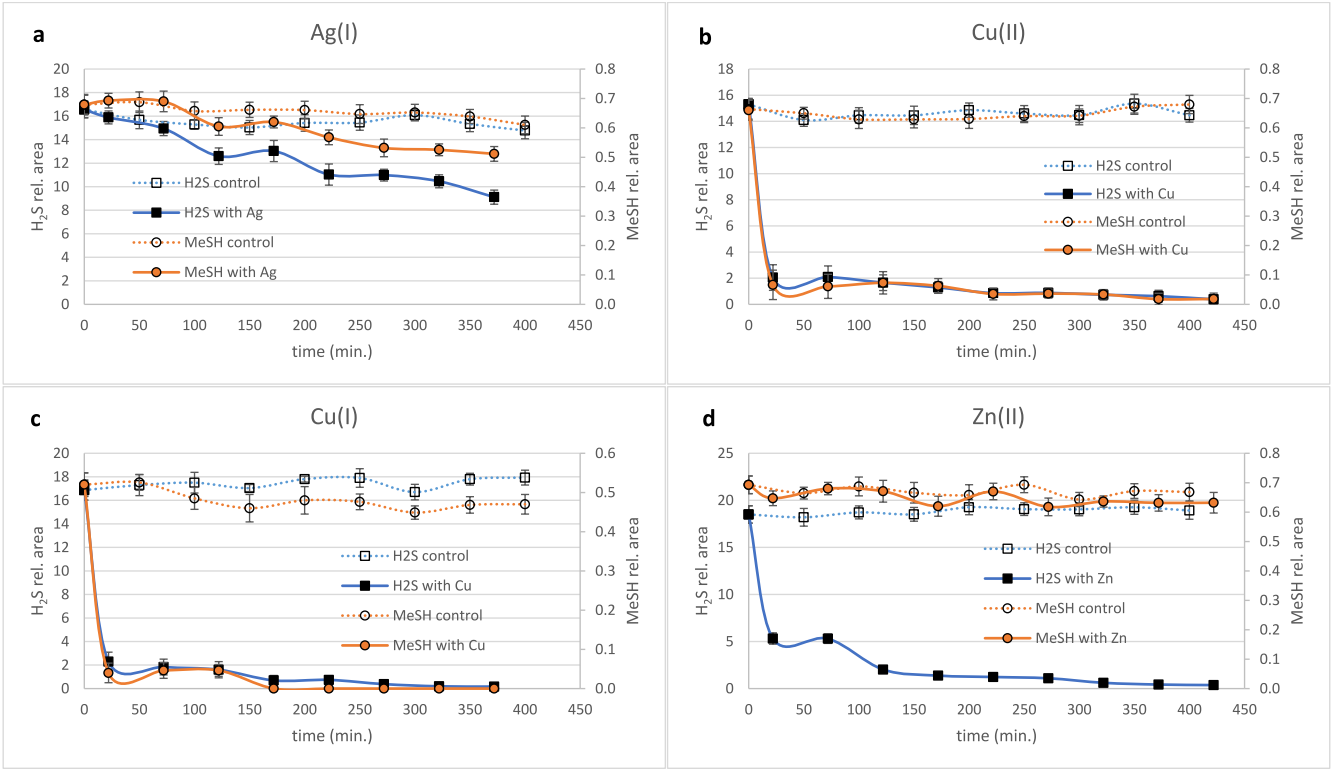

Fig. 1. The ability of solutions from different metal cations to remove H_2S and MeSH from the headspace of a hydro alcoholic solution containing 200 μg/L of H_2S and 20 μg/L of MeSH. The data are the relative areas of H_2S and MeSH (vs EMS) measured in the headspaces of the analyte solutions, in contact via gas-phase with the different trapping solutions. Error bars are standard deviations of two independent replicates.

temperature. The trapping solutions were replaced and analysed daily or every three days for three weeks. In a final experiment, a third different white wine with a strong reductive off-odour and a model wine containing 400 μg/L of H_2S, both spiked or not with 50 mg/L of SO_2, were treated with TCEP and BCDA, incubated in the trapping device along 19 days, and the trapping solutions replaced and analysed as in the previous experiment. All these experiments were carried out in duplicate.

3. Results

The first objective of this work is to develop a reversible trapping system capable of removing and stabilizing sulfidic gases as they are released into the gas phase. For this, different metal cations have been considered.

3.1. Optimization of the trapping system

3.1.1. Study of different metal cations as potential trapping systems

Cu(I), Cu(II), Ag(I) and Zn(II) were initially selected as potential constituents of the trapping systems. In a first experiment, the effects of the direct addition of little amounts of these cations to aqueous solutions containing H_2S and MeSH was observed. Results revealed (Supp. Mat. Fig. S2) that the four cations completely eliminate H_2S from the headspace, and that all but Zn removed also completely MeSH, in agreement with the corresponding solubility products (Martell and Smith, 1982).

In a second experiment, the headspaces of solutions containing H_2S and MeSH were exposed to solutions with the metal cations and the compositions of the headspaces were monitored over time. Results can be seen in Fig. 1 and reveal that only Cu cations quickly and effectively removed the two VSCs from the headspace. The process is very fast and in around 20 min more than 80%-90% of both VSCs were eliminated. Cu (I) was particularly effective so that levels of H_2S in the headspace remaining after 7 h were below 1% of the original value. In the case of Cu(II), levels of H_2S in the headspace were never below 4% of the initial value. It can be observed that Ag(I) was particularly ineffective, which should be attributed to the rapid formation of a conspicuous Ag_2S surface layer, which makes the trapping process to be controlled by slow diffusion kinetics. As expected, Zn was quite efficient at trapping H_2S (less than 3% remaining) and had no effect on MeSH vapors.

3.1.2. Recovering VSCs from metal-trapped solutions

The abilities of different chemicals to cleave the corresponding complexes, and in some cases, also to reduce any oxidized species formed, were studied. Chemicals tested were NaCl, since Cl^- anions form complexes with Ag(I), Cu(I) and Zn(II); the strong reducing agent TCEP; BCDA, which forms complexes with Cu(I); and EDTA, which forms complexes with divalent anions. Results of these experiments are summarized in Table 1 and confirm that the facility to recover H_2S from the complexed cations follows the order $Zn^{2+} > Ag^+ > Cu^+ > Cu^{2+}$.

In the case of Zn^{2+}, dilution with brine, TCEP addition or EDTA addition are sufficient for quantitative and consistent recoveries. For Ag^+, the recovery with dilution with brine is high, but the addition of TCEP is required to achieve quantitative results. In the case of Cu(II) solutions, the H_2S recoveries were never quantitative and strongly depended on the H_2S concentration and the timing of the addition of reagents after mixing the cation with the sulfidic components, in accordance with previous observations (Franco-Luesma & Ferreira, 2014). As can be seen, when the mixture Cu(II) with H_2S and MeSH is left to age 24 h, the recoveries of H_2S drop to a residual value of just 4% (not significantly different from 0%), confirming that the complex mixture of disulfides, Cu(I) and H_2S, evolves into polymeric structures (Kreitman, Danilewicz, Jeffery, & Elias, 2016a). Results in the table reveals that those structures cannot be cleaved by the combination of TCEP and dilution with brine.

The best results were obtained with Cu(I), as can be seen in the table. Results show that a high level of TCEP, together with a 1:24 dilution with brine provides very high and time-consistent recoveries. It can also be seen in the table that even with solutions with high concentrations of CuCl (1015 mM), the recoveries remain quantitative. These high levels of Cu(I) were further used in the optimal trapping systems.

3.1.3. Analytical characterization of the stability, release and further HS-GC-SCD determination of sulfidic gases trapped in CuCl solutions

The repeatability was determined by the study of the 191 standard deviations obtained in the duplicate analysis of the 191 trapping solutions produced in the experiment presented in section 3.3. The representations of repeatabilities, as standard deviations, vs. concentration reveal a significant correlation (P less than 10^{-17} and less than 10^{-33} for H_2S and MeSH, respectively; Fig. S3 in the Supplementary Material), while RSDs remained constant and independent of concentration, so that it can be considered that the method has constant RSDs. The average figures for such RSDs were obtained by pooling all the measurements and was 15.5% for H_2S and 12.1% for MeSH. These values are typical of analyses in which there are complexes forming colloidal-type structures, such as those obtained in the analysis of brine-releasable forms of H_2S and MeSH (Ontanon et al., 2019). Overall, the uncertainty of a mean obtained by duplicate analysis of a trapping solution is 11% for H_2S and 8.6 for MeSH. The limits of detection were estimated from those traps containing smallest amounts of H_2S and MeSH, and were found to be 0.18 μg/L and 1 μg/L, respectively.

As shown in Fig. 2a and 2b, trapped sulfidic gases are stable at 75 °C and can be quantitatively recovered after storages as long as 8 days at this temperature. The linearity, stability and recovery of the trapping system were further demonstrated by the analysis of CuCl solutions with known amounts of sulfidic gases after incubation in anoxia at 50 °C for 4 days. As can be seen in Fig. 3c and 3d, not only linearity was satisfactory, but the regression lines were just slightly smaller than those obtained in the analysis of fresh calibrated solutions of H_2S and MeSH without copper. Overall, results suggest that more than 95% of the H_2S and MeSH trapped in the Cu(I) solution can be recovered after 4 days of storage at 50 °C.

Other studies (Supp. Mat. Fig S4) demonstrated that the stability of the trapping solutions keeps at room and fridge temperatures for at least 3 or 7 weeks, respectively, as soon as they are stored in anoxia and in tightly closed vials. Also, that once the complexes are cleaved in the 20 mL headspace vials, the signals remain stable for at least 12 h, so samples can be left unattended in the autosampler tray for analysis (Supp. Mat. Fig S5).

3.2. Design and validation of a device for the continuous trapping of sulfidic gases emanated from wine stored in anoxic conditions

First designs containing the trapping solutions within narrow test tubes (0.8 cm internal diameter) demonstrated a poor performance. The problem was caused by the formation of a conspicuous surface layer of metallic aspect caused by the too-fast precipitation of Cu_2S. This was avoided by increasing the internal diameter of the vial containing the trapping solution. The final trapping device consists of a 100 mL flat bottom round glass flask with ground glass stopper, housing inside a standard-20 mL SPME glass vial containing 10 mL of the trapping solution, as schematized in Figure S1 in the supplementary material. No signs of the formation in the surface of Cu_2S precipitates were observed during the validation of this device with synthetic solutions containing less than 200 μg/L of H_2S at 50 °C or with less than 150 μg/L at 75 °C. Those concentrations represent the maximum levels of H_2S in the headspace that the Cu(I) solutions can efficiently trap in a very short period of time (less than 2 h), but do not represent the upper limits of the traps.

The repeatability of the complete system was assessed by the study of the 82 standard deviations obtained in the replicated samples (n = 2 or 3) coming from the wines studied in 3.3. Those standard deviations (Supp. Mat. Fig. S6) increased significantly with C (correlation

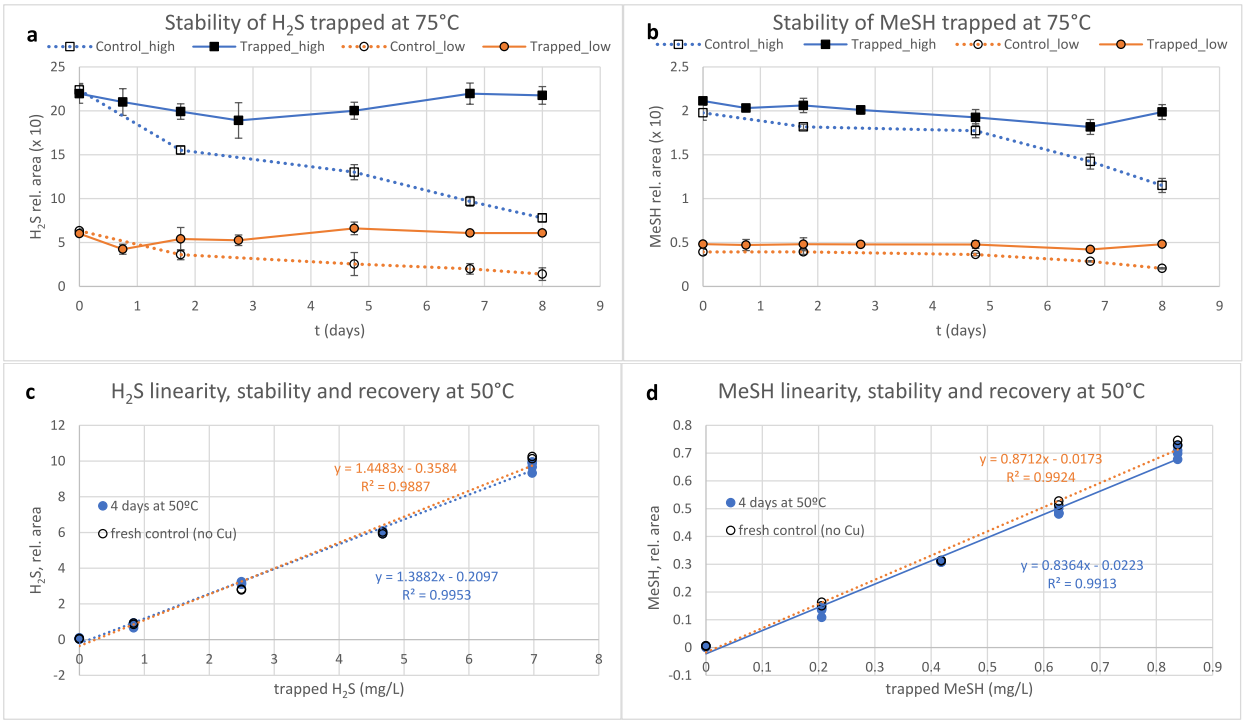

Fig. 2. Stability, linearity and recovery of sulfidic gases trapped in Cu(I) solutions. a) and b) Stability of H_2S (a) or MeSH (b) trapped in aqueous CuCl at two concentrations (1.6 and 0.32 mg/L H_2S and 200 and 50 μg/L MeSH) and stored in anoxia at 75 °C different times. Controls are aqueous solutions with the same concentration of sulfidic gases without copper similarly stored; error bars are standard deviations (n = 2); c) and d) linearity, stability and recovery of increasing concentrations of H_2S (a) and MeSH (d) trapped in aqueous CuCl and stored at 50 °C for 4 days. Controls are fresh aqueous solutions containing calibrated amounts of the sulfidic gases (no copper).

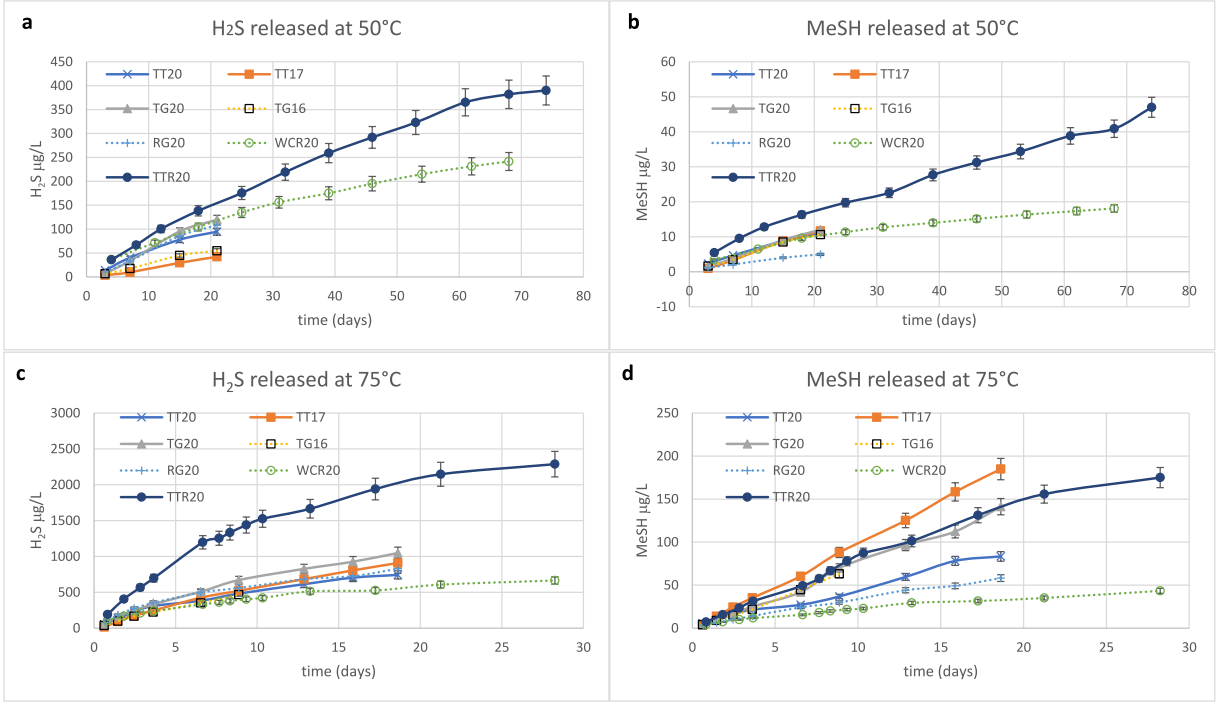

Fig. 3. Release of H_2S and MeSH from 7 different wines stored in the trapping device at 50 °C or 75 °C for different times. Trapping solutions were analyzed and replaced by new ones several times per week. Data are accumulated amounts found in the trapping solutions recovered up to that time: a) H_2S released at 50 °C; b) MeSH released at 50 °C; c) H_2S released at 75 °C; d) MeSH released at 75 °C. Error bars are standard deviation of 2 or 3 independent replicates analyzed twice each. Wine codes: first letter, T = Red, R = Rosé, W = White; second letter, T = tempranillo, G = garnacha, C = chardonnay; a third R means reductive; the number refers to vintage. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

significant at P less than 10^{-12} for H_2S and at P less than 10^{-11} for MeSH), while the RSDs did not change with concentration. For H_2S, the pooled average relative standard deviation was 8.9%, a figure which can be entirely attributed to the repeatability of the analytical measurement of trapped H_2S ($p(F) = 0.98$ for the ANOVA with tube replicates as factor), which suggests that the devices are very repetitive and that imprecision comes mostly from the analytical determination. The same conclusion was reached for MeSH at 50 °C ($p(F) = 0.99$ for the ANOVA with tube replicates as factor), but not at 75 °C. At that temperature the production and trapping of MeSH became significantly more imprecise ($p(F) = 2.9\ 10^{-5}$), so that the pooled average relative deviation was 14.3%.

The efficiency of the device was demonstrated by incubating 24 h at 50 °C model wines containing known amounts of the sulfidic gases or real wines in highly reductive states, spiked or not with known amounts of the gases. Results (Supp. Mat. Fig. S7) revealed that recoveries worsen at levels above 200 μg/L in all cases, which was tentatively attributed to the solid layer of Cu_2S formed on the surface of the trapping solution. The possibility of avoiding this problem by incorporating magnetic agitation was investigated. However, ordinary PTFE-covered agitation nuclei were found to adsorb significant amounts of Cu_2S, so glass-covered agitation nuclei had to be used, which made it possible to quantitatively transfer up to 500 μg/L of H_2S in one day. However, as glass nuclei break down easily and considering that no wine is going to produce levels of H_2S higher than 200 μg/L per day, agitation was discarded.

Results also revealed that transference is better in wine than in synthetic solution. In wines, the fractions of H_2S transferred to the trap in 1 day are within the 90%-97% range for levels below 200 ug/L, while in synthetic solutions, the fraction transferred are in the 75–80% range (Suppl. Mat. Fig 7). This was attributed to the unavoidable losses of little amounts of H_2S by reaction and adsorption in the surfaces of the glass, particularly in the joints of the ground glass of the stopper, protected with Teflon liners. The many more volatile components present in wine help reducing those losses. These results demonstrate that the device is able to transfer to the trapping solution nearly quantitative amounts of H_2S and MeSH released from wine, providing that the amount released is not higher than 200 μg/L per day.

3.3. Application to the study of the spontaneous release of H_2S and MeSH from wines in accelerated conditions

Seven different wines (five reds, one rosé and one white) were submitted to anoxic storage at 50 °C and at 75 °C in the trapping devices. Trapping solutions were replaced between 1 and 3 times per week in order to prevent the formation of the solid Cu_2S layer and were analysed. The cumulative amounts of H_2S and MeSH released by these wines at both conditions are presented in Fig. 3, while Table 2 summarizes the free and BR contents of these compounds in the original wines plus those found after a standard reductive aging at 50 °C and those accumulated in the traps at selected times.

3.3.1. Production of H_2S

Results reveal that wines actively release significant amounts of H_2S during accelerated anoxic aging. As can be seen in the Fig. 3a, the wine releasing more H_2S at 50 °C (TTR20) did it approximately 3.5 times faster than the one releasing less (TT17). The former released 176 μg/L in 25 days (7.0 μg/L per day in average), while the latter released 42μ/L in 21 days (2.0 μg/L per day in average). It is evident from the two samples studied longer times that the release was still active after more than 2 months of incubation at 50 °C. As can be seen, after nearly two months of storage, TTR20 had released nearly 400 μg/L of H_2S, while WCR20 had accumulated nearly 250 μg/L.

At 75 °C the amounts released were much higher, and after 9 days of anoxic storage, the cumulated released amounts ranged from 404 (sample WCR20) to 1330 μg/L (sample TTR20). At this temperature, the graphs from the two wines monitored longer time, TTR20 and WCR20, showed that the release was much smaller in the last sampling period. These two wines accumulated 2287 μg/L and 664 μg/L after 28 days of anoxic incubation (81.7 and 23.7 μg/L per day, respectively), while in the last week accumulated just 41 and 57 μg/L (5.8 and 8.1 μg/L per day), respectively.

The comparison between the plots in Fig. 3a and 3c shows that there is no overall consistency in the rank order of samples in terms of rate of release of H_2S at both temperatures. The only coincidence is that TTR20 in both cases releases maximum amounts.

3.3.2. Production of MeSH

The production of MeSH at 50 °C (Fig. 3b) ranged from slightly less than 5 μg/L accumulated after 21 days by TG20 to the 20 μg/L accumulated by TTR20 in 25 days. Five of the wines produced MeSH at relatively similar rates, accumulating 10–11 ug/L in 21 days. At 75 °C (Fig. 3d), the production was much higher, more imprecise and was also poorly related to that observed at 50 °C. At 75 °C, sample TT17 had produced more than 180 μg/L of MeSH after 18 days, while the smallest production was observed in WCR20 and TG20 which accumulated in that time around 33 and 58 μg/L, respectively.

3.3.3. Relationship to the initial VSCs contents and to the accelerated reductive aging assay

The initial contents in free and BR levels of H_2S and MeSH of the seven wines, together with the levels of these compounds accumulated in the standardized accelerated 2 weeks reductive aging test are shown in Table 2. Remarkably, the amounts of H_2S and MeSH accumulated by the different wines, both at 50 °C and 75 °C, do not seem to bear any relationship either with the initial H_2S or MeSH contents (free or BRs) of the wines, or with the levels accumulated in the 2-weeks reductive aging test. Nor do they seem to be related to the other wine chemical parameters, such as redox potential or metal contents (Supp. Mat. Table S1).

3.4. Release of H_2S and MeSH after the direct addition of TCEP

The direct addition to wine of the strong reducing agent, TCEP, together with the Cu(I) complexing agent, bathocuproine, has been proposed for the evaluation of the reductive tendency of wines (Chen et al., 2017; Chen, Jastrzembski, & Sacks, 2018; Kreitman et al., 2017). Here, the effects associated to the direct addition of different amounts of TCEP to the wine were first studied. Results revealed (Supp. Mat. Fig. S8e) that the addition of TCEP has an immediate effect on the redox potential of the wine sample, which drops to values as low as −350 mV (vs. Ag/AgCl), and then increases gradually so that after 4 days the redox potential is in the range −30 mV to −60 mV. However, the effect on the release of free H_2S is not immediate (Supp. Mat. Fig. S8a), and levels measured in the 60 min after the addition of the TCEP, as proposed in the original reference, are between 4 and 30 μg/L, depending on the amount of the TCEP added, but after 24 h, the levels reach a maximum of 100 μg/L and in the following days strongly decrease. The evolution of BR forms was similar (Supp. Mat. Fig. S8c). BR levels measured after 24 h at the maximum level of addition were very close to free levels, which suggests that all H_2S was in free form at that point. These results suggest that the original TCEP procedure, in which free H_2S is measured shortly after TCEP addition, cannot provide an accurate measurement of H_2S precursors. It is also evident that free H_2S in wine is very reactive, which confirms that, for the assessment of the pool of precursors, this gas should be trapped as soon as it is produced.

The mixture TCEP + BCDA (Kreitman et al., 2017) was used in combination with the trapping device and applied to the wines TTR20 and WCR20. Results are summarized in Fig. 4, which shows that the mixture of reagents releases in the white wine up to 350 μg/L of H_2S in 20 days, above the 250 μg/L found in its anoxic incubation at 50 °C for 68 days, and 20 μg/L of MeSH in just 4 days, which is very close to the 18 μg/L released in its anoxic incubation at 50 °C. However, in the red

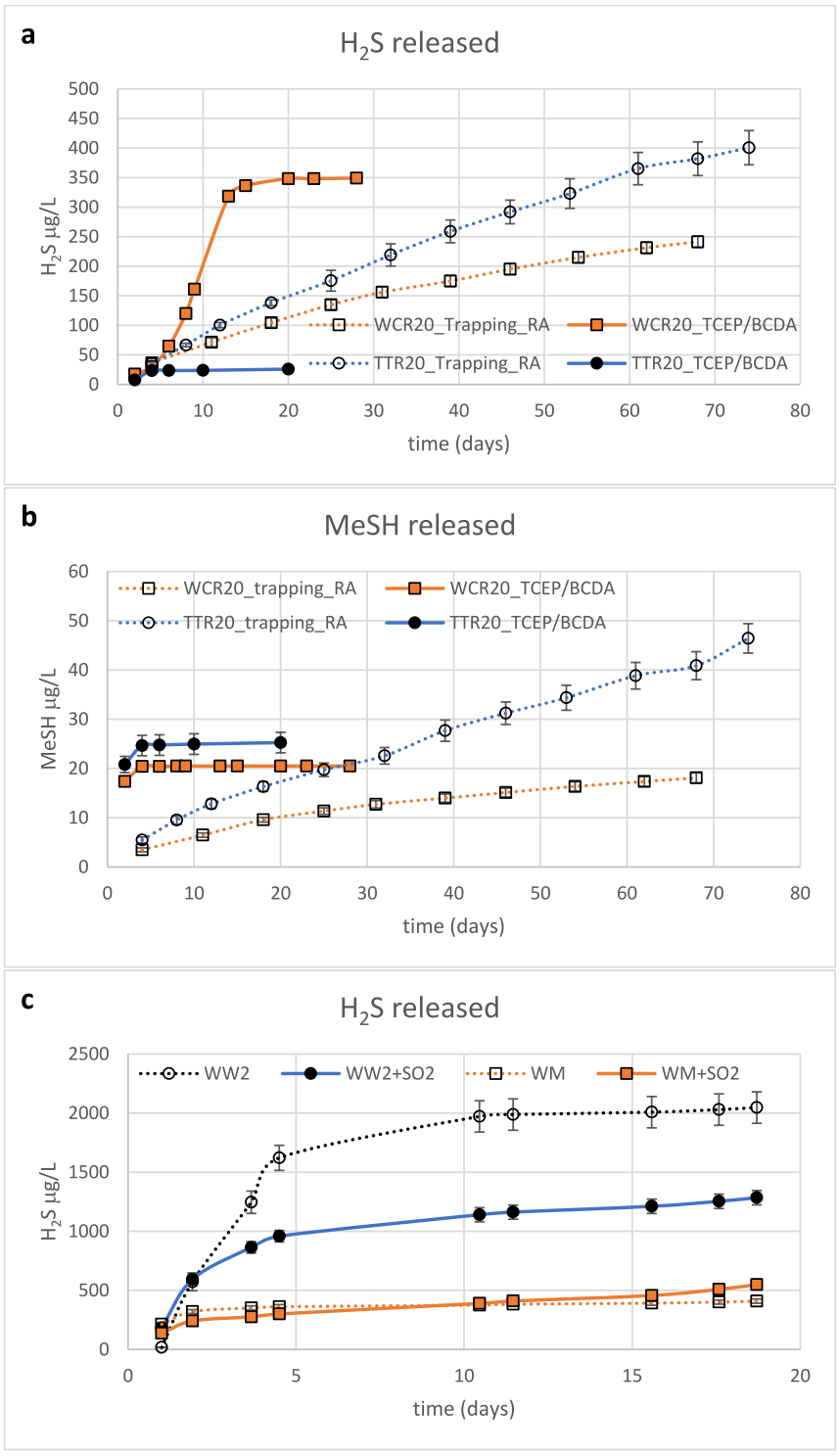

Fig. 4. Release of H_2S and MeSH induced by the addition of TCEP/BCDA; a) and b) Comparison between the amounts of H_2S (a) and MeSH (b) released to trapping solutions by two wines (WCR20, white and TTR20, red) after spiking with TCEP/BCDA and further storage at room temperature, or during accelerated anoxic aging at 50 °C (RA); c) effects of the addition of SO_2 (50 mg/L) on the H_2S released by a white wine (WW2) or a model wine with 400 µg/L H_2S after TCEP/BCDA addition. Error bars are standard deviations of two independent replicates, analyzed each twice. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

wine, the release in the sample with TCEP and BCDA ceased after 4 days and only 23.6 µg/L of H_2S and 25 µg/L of MeSH were recovered, well below the 400 µg/L and 48 µg/L found in the 74 days of anoxic incubation at 50 °C, respectively.

Furthermore, levels released by TCEP are strongly affected by SO_2 levels, as shown in Fig. 4c. The addition of 50 mg/L SO_2 to the wine nearly halved levels of H_2S released. On the contrary, in model wine, there is evidence that SO_2 is being reduced to H_2S.

3.5. Discussion

The high reactivity of H_2S and MeSH implies that to have a reliable estimation of their actual production in a complex chemical system, it is compulsory to trap them as soon as they are produced. The trapping reaction has to be completely reversible, so that the trapped components could be determined as their free forms. Results demonstrates that only Cu(I) fulfills the requisites, requiring the concourse of the strong reducing agent -TCEP, plus of a strong dilution with brine, to quantitatively release the trapped molecules which can be then easily

determined by GC-SCD. The different experiments carried out demonstrated that trapping is very fast and quantitative, and that trapped molecules remain stable for weeks at room or fridge temperatures or for more than 8 days at 75 °C, at least while anoxic conditions are guaranteed. These analytical characteristics, make the trapping system well suited to monitor reductive off-odors during the anoxic aging of wines.

Reductive off-odors in wines are essentially related to the levels of free H_2S and MeSH accumulated by the wines along their anoxic storage. Such accumulation should be the result of at least four more or less interrelated groups of factors:

1. The rates of the spontaneous and not well-known slow redox reactions, some of them related to, or mediated by, SO_2 (Ontanon et al., 2020), taking place in wine and responsible for a small supply of electrons and, eventually, for the reduction of sulfur atoms in (-I), or even (0), oxidation states to (-II) and to the release of H_2S and MeSH. These poorly known reactions may be named as "intrinsic wine reductive ability";
2. The presence of metal cations, notably Cu, and to a lesser extent also Zn and Fe, able to form strong complexes with H_2S and sulfhydryls, and likely also forming polyatomic structures in the nanoparticle range. This group of factors could be named as "wine sulfhydryl trapping ability";
3. The presence of a net of oxidized forms of sulfur, in the form of (di) organopolysulfides, polysulfides and maybe also of their monosulfonated forms (Kreitman et al., 2019; Müller et al., 2022). This would be the "pool of wine H_2S and sulfhydryl precursors";
4. The presence in wine of electrophiles able to react to H_2S and sulfhydryls, such as anthocyanins, vinylphenols or some conjugated carbonyls (Nikolantonaki, Chichuc, Teissedre, & Darriet, 2010). This should be related to the "wine electrophilic reactivity".

While the four factors take place during the standard anoxic aging of wine, such as during storage in the bottle, only some of them will take part in the different accelerated procedures used to study reductive off odors. The relative rates at which these factors intervene in the process may also be different in the different accelerated procedures. Thus, the number of factors intervening and their relative rates should explain the different results obtained with different procedures.

During the standard accelerated reductive aging at 50 °C for 2 weeks (RA assay), all four factors are present and their relative rates could be similar to those occurring during normal reductive aging at room temperature. This would explain why the levels of free H_2S and MeSH that accumulate in such test correlate well with those accumulated during normal storage in the bottle (Franco-Luesma & Ferreira, 2016a). Accordingly, this should be the procedure of choice to assess the probability that a given wine develops reductive off-odors.

However, if the anoxic incubation is carried out in the trapping device proposed in the present paper (trapping RA assay), then the reactions that sulfidic gases undergo with the compounds in the fourth category are avoided, while the other three factors remain active. This suggests that the results obtained in the trapping-RA should refer to the amount of sulfhydryl compounds able to be released by a wine during the anoxic storage, rather than to the amount that it can accumulate. From this point of view, results obtained should not be good indicators of the probability that a given wine undergoes reductive problems. However, results of this test may be essential to progress in the understanding of the chemistry of the reductive off-odors, since they reflect more accurately the real dimensions of the pool of oxidized precursors that the wine can reduce and transform into volatile species; i.e., results of such a test should reflect the intrinsic ability of wine to release H_2S and MeSH.

It can be also suggested that differences between results obtained with the two tests; the standard RA -measuring "accumulation" and the trapping-RA -measuring "release" at 50 °C, should be related to the different electrophile content of the wines. It can be then hypothesized that the sample TG20, which as shown in Fig. 3 and Table 2, releases nearly 120 μg/L but accumulates after RA only 2.5 μg/L of free H_2S, contains numerous electrophiles, while the sample GR20, which releases 108 μg/L, but accumulates the maximum observed level, 19.14 μg/L, contains comparatively lower amounts of electrophiles. Unfortunately, no specific test was conducted to check such hypothesis in the present experiment.

The lack of consistency between the rank order of samples in term of release of H_2S and MeSH at 50 and 75 °C seen in Fig. 3 and Table 2, suggests that temperature has a deep effect on the relative rates at which the three different aforementioned processes intervene. The much higher release at 75 °C may be attributed to a number of causes, including much increased rates of self-oxidation reactions supplying electrons and to an increased ability to cleave and reduce oxidized forms of H_2S and MeSH. However, the magnitude of the differences makes us think that at 75 °C high amounts of H_2S and MeSH are produced by processes that at smaller temperatures take place just marginally, such as the metal-catalyzed degradation of cysteine and methionine, whose existence has been proved (Ferreira et al., 2018). If this is the case, the amounts released at 75 °C will not be good assessments of the pool of oxidized forms of sulfur potential precursors of reductive off-odors. This question will have to be addressed in future research.

Regarding the method using TCEP and BCDA, the marked H_2S release profile over time (Supp. Mat. Fig. S8) demonstrates that it can only be of any use if used in combination with the trapping system, and not by means of the originally proposed procedure. With the trapping procedure, only the factors within the 3rd category in the previous list would be present, so this strategy should be particularly suited to the direct assessment of the "pool of wine H_2S and sulfhydryl precursors". However, results obtained show, on the one hand, that it does not work in red wine (Fig. 4a), and on the other, that the response obtained in white wine is SO_2-dependent (Fig. 4c). Therefore, in any case, it must be concluded that this strategy, as it stands, is far from providing reliable data. A systematic re-optimization and validation would be required. It should be recalled that an assay able to directly assess the "pool of wine H_2S and thiol oxidized precursors" is, together with the standard RA and the trapping-RA assays, essential to bring light into the complex chemical processes involved in the reductive off-odors of wines.

4. Conclusions

Solutions containing CuCl at 100 mg/L can be satisfactorily used for the effective and long-term trapping of sulfidic gases at temperatures as high as 75 °C. Trapped molecules can be quantitatively recovered by a combination of TCEP (30 mg each 0.5 mL of trapping solution) and a 1:24 dilution with brine.

These trapping solutions, placed within the proposed trapping device, constitute a highly effective device for the continuous trapping of sulfidic vapors emanated from wine, making possible to propose a trapping-RA assay.

Wines can release large amounts of H_2S and MeSH during very long times when stored in anoxic conditions (up to 400 μg/L of H_2S and 58 μg/L of MeSH at 50 °C for 68 days, and between 3 and 5 times more at 75 °C for 28 days).

The fact that levels released at 50 °C are not correlated to the levels accumulated by the wines during standard reductive aging, suggests that the wine content in electrophiles plays a role in the wine tendency to accumulate free H_2S and MeSH. The much higher release observed at 75 °C suggests that at this temperature the formation of H_2S and MeSH takes place by different mechanisms.

Studies carried out with the TCEP-BCDA assay have shown that the test requires a deep re-optimization.

Overall, the trapping procedure has proved to be a most useful device for the development of bench and laboratory tests to understand the production of H_2S and sulfhydryls in wine and in many other different biologically relevant systems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.136092.

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