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Quantitative analysis of free and bonded forms of volatile sulfur compounds in wine. Basic methodologies and evidences showing the existence of reversible cation-complexed forms

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ABSTRACT

This paper examines first some basic aspects critical to the analysis of Volatile Sulfur Compounds (VSCs), such as the analytical characteristics of the GC-pFPD system and the stability of the different standard solutions required for a proper calibration. Following, a direct static headspace analytical method for the determination of exclusively free forms of VSCs has been developed. Method repeatability is better than 4%, detection limits for main analytes are below 0.5 $\mu g\,L^{-1}$, and the method dynamic linear range ($r^2 > 0.99$) is expanded by controlling the split ratio in the chromatographic inlet to cover the natural range of occurrence of these compounds in wines. The method gives reliable estimates of headspace concentrations but, as expected, suffers from strong matrix effects with recoveries ranging from 0 to 100% or from 60 to 100 in the cases of H_2S and the other mercaptans, respectively. This demonstrates the existence of strong interactions of these compounds with different matrix components. The complexing ability of Cu^{2+} and to a lower extent Fe^{2+} and Zn^{2+} has been experimentally checked. A previously developed method in which the wine is strongly diluted with brine and the volatiles are preconcentrated by HS-SPME, was found to give a reliable estimation of the total amount (free + complexed) of mercaptans, demonstrating that metal-mercaptan complexes are reversible. The comparative analysis of different wines by the two procedures reveals that in normal wines H_2S and methanethiol can be complexed at levels above 99%, with averages around 97% for H_2S and 75% for methanethiol, while thioethers such as dimethyl sulfide (DMS) are not complexed. Overall, the proposed strategy may be generalized to understand problems caused by VSCs in different matrices.

1. Introduction

Volatile sulfur compounds (VSCs) can be naturally generated in oceans, marshes, grounds and vegetation areas, or can have an anthropogenic origin in processes such as residual water treatments, composting installations, recovery plants of organic matter [1], or the Kraft process to produce pulp, in which MeSH (methanethiol), DMS (dimethylsulfide), COS (carbonyl sulfide) and H_2S (hydrogen sulfide) appear as gaseous wastes [2]. This family of compounds has a relevant importance in the field of food science, since they are present in products such as cheese [3], beer [4] or wine [5–9]. VSCs can communicate unpleasant smells and tastes often described as rotten eggs (H_2S), putrefaction (MeSH), asparagus soup (DMS), garlic (DES), cauliflower (DMDS) or onion (DEDS) [6]. At small concentrations, however, they are normal components of wine, beer, cheese or cooked meat aroma, and can even exert a decisive role on some characteristic sensory descriptors [7,10–12].

These compounds are particularly important in wine because they are associated with off-odor problems commonly defined as reduced character [13]. This reduced character is considered as a wine fault and depreciates the quality of the wine with odors such as those mentioned previously. The exact chemical origin of this reduced character is not completely known, but often appears during aging of the wine in the bottle and occurs more frequently when the wine package presents less permeability towards oxygen [14–16]. Although the production of H_2S and other VSCs during the alcoholic fermentation has been described [17–19], the link between its production and their post-bottling occurrence has not been proven. Different molecules have been proposed as potential

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precursors for the VSCs causing the generic "reduction" problem. However, while the formation of DMS from a major specific precursor, S-methylmethionine, is well documented in the literature [20–22], the processes and the precursors involved in the formation of H_2S, MeSH and other mercaptans likely involved in the reduction problem, have not been unequivocally described.

One of the possibilities to be considered in the genesis of molecules such as H_2S and MeSH, is the potential accumulation of these molecules in wine in the form of odorless complexes with copper or some other metal cations. Small amounts of Cu^{2+} are in fact used in the cellar to eliminate reductive off-odors. While winemakers tend to think that mercaptans are completely removed by precipitation with Cu^{2+} salts [23–25], it may be possible that such precipitation would not be complete, resulting in small amounts of copper (sub ppm levels) complexed with mercaptans, remaining in the wine. At least theoretically, such complexes could be reversed releasing the mercaptans if some competitive reaction, such as the reduction of Cu^{2+} to Cu^0 by wine antioxidants [26] or the competitive complexation of Cu^{2+} by polyphenols [27], takes place during aging. In order to assess the plausibility of this hypothesis, it is crucial to develop analytical methods able to discriminate between bonded and free forms of mercaptans. To the best of our knowledge, there are no previous reports on this question.

For analysis, headspace techniques are generally preferred due to the high volatility of these species and their relatively low solubility in organic solvents. Consequently liquid–liquid extraction is seldom used [28], and most reports use simple static headspace [8,29] or more often, headspace solid phase microextraction [5,30–32]. Most reports propose procedures in which the headspace from undiluted wine is directly analyzed, with [8,29,32] or without [31,33] salting out effects to improve the signal from the analytes. It should be remarked that even using salt addition, strong matrix effects have been noticed in the headspace concentrations as noticed by Lopez et al. [5] or by Ebeler et al. [32], who noted that a standard addition calibration should be carried out to get unbiased estimates of analyte concentrations. Those matrix effects are expected if analytes exert strong interactions towards wine components. In the report from Lopez et al. [5], those matrix effects are avoided by strongly diluting the wine (factor 50) in brine. Such dilution, together with the salting out effect and the extraction process in the fiber, make us think that such procedure could be estimating total amounts of mercaptans. Dilution favours dissociation of any complex; salting out strongly decreases solubility in the liquid phase of neutral molecules, particularly of those with Lewis acid character such as mercaptans [34], which in turn favours transference to the headspace and consequently to the SPME fiber. Otherwise the other procedures in which wine is undiluted are estimating just free forms of these compounds.

The quantitative analysis of VSCs has to face additional difficulties related to their high reactivity, particularly towards O_2, which seriously questions the stability of standard calibrated solutions. Results obtained by Hofmann et al. [35] showed that the storage and handling of mercaptans bearing an additional functional group (polyfunctional mercaptans) in organic solvents can generate high amounts of the corresponding disulfides, and that common antioxidants, such as BHA or BHT, had a quite limited ability to avoid the oxidation of these molecules.

The two most commonly used detectors for the analysis of the VSCs are the sulfur chemiluminiscence (SCD) and the flame photometric detectors (FPD), followed by the mass spectrometer [36]. The SCD is more sensitive than the classic FPD, and has no quenching problems, but shows higher drift in sensitivity [37] which apparently is solved in most recent designs [38,39]. Flame Photometric Detectors have a quadratic response towards Sulfur atoms [40,41], which translates into reduced dynamic ranges, although this also means that signal to noise ratios close to the detection limit are higher [42]. One of the major drawbacks of FPDs is quenching in the photometric signal by coelution with organic compounds, CO and CO_2 [43,44]. The pulsed version of FPD, the pFPD detector, has improved selectivity and sensitivity similar to or even better than SCDs [42,45].

The major aim of this paper is to develop reliable analytical methodology for the quantitative determination of free and bonded forms of VSCs. With that purpose, this work addresses first some critical questions about the stability of the calibrated solutions and the response of the detector, secondly develops a gentle direct headspace analytical method, and finally combines this method with another one previously developed [5] in which the wine is strongly diluted with brine, so that complexes are broken.

2. Materials and methods

2.1. Standards, solvents and reagents

Pure standards (>95%): methanethiol and ethanethiol from Fluka (Steinheim, Germany), dimethylsufide from Merck (Darmstadt, Germany), sodium sulfide, ethylmethylsulfide, 1-propanethiol, thiophene, diethyldisulfide, dimethyldisulfide, diethylsulfide from Sigma-Aldrich (Steinheim, Germany).

Analytical quality reagents: acetonitrile from Panreac (Barcelona, Spain), isooctane and methanol (Suprasolv) from Merck (Darmstadt, Germany) and tetrahydrofurane from Sigma-Aldrich (Steinheim, Germany).

Brine containing 350 $g\,L^{-1}$ of NaCl (Panreac, Barcelona, Spain) in Milli-Q water Millipore (Billerica, MA, USA).

Synthetic wine was a pure water solution containing 5 $g\,L^{-1}$ of tartaric acid, 12% v/v ethanol and pH 3.4 adjusted with diluted NaOH (0.1 M).

H_2S was produced by addition of a water solution of Na_2S at pH 9.6.

Metals salts (99%): $CuSO_4{\cdot}5H_2O$, $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$, $FeCl_3{\cdot}6H_2O$, $ZnCl_2$, $MnCl_2{\cdot}4H_2O$, $Ni(NO_3)_2{\cdot}6H_2O$ and $SnCl_2{\cdot}2H_2O$ from Panreac (Barcelona, Spain).

2.2. Wines

The wines selected for this study were five commercial Spanish wines from different Spanish Denominations of Origin, four reds from: Ribera del Duero (wine 1: Tempranillo, 2011, 13% v/v), Campo de Borja (wine 2: Tempranillo and Grenache, 2009, 14% v/v), La Rioja (wine 4: Tempranilo, 2011, 14% v/v) and Campo de Cariñena (wine 5: Grenache and Carignan, 2013, 12% v/v) and one white from Rueda (wine 3: Verdejo, 2012, 12%v/v). All of them were purchased from a wine retailer in Zaragoza.

2.3. Work under inert atmosphere

All the solutions were prepared in the interior of a glove box P[BOX] (Jacomex) containing Argon atmosphere with levels of O_2 and H_2O below 1 ppm (v/v) in normal stationary state, which attending to Raoult's law indicates that maximum level of oxygen in wine should be below 0.05 ppb. Oxygen levels in the gas phase within the chamber were continuously monitored with O2X1 Oxygen Transmitter (GE Panametrics), while oxygen levels in wine and hydroalcoholic solutions were monitored with Optiox SG-9 fluorescence sensor from Mettler-Toledo (Barcelona, Spain) and in all cases levels were not detectable (<0.01 ppm).

All solutions were stored out of the globe box at −20 °C.

Mother solutions were contained in isooctane in 8 mL amber vials equipped with Mininert^{TM} valves and were weight controlled after each use. Diluted working solutions in methanol (acetonitrile