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Sensitive quantification of sulfur compounds in wine by headspace solid-phase microextraction technique

Yu Fang, Michael C. Qian *

Department of Food Science and Technology, 100 Wiegand Hall, Oregon State University, Corvallis, OR 97331-6602, USA

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Abstract

A sensitive solid-phase microextraction and gas chromatography-pulsed flame photometric detection technique was developed to quantify volatile sulfur compounds in wine. Eleven sulfur compounds, including hydrogen sulfide, methanethiol, ethanethiol, dimethyl sulfide, diethyl sulfide, methyl thioacetate, dimethyl disulfide, ethyl thioacetate, diethyl disulfide, dimethyl trisulfide and methionol, can be quantified simultaneously by employing three internal standards. Calibration curves were established in a synthetic wine, and linear correlation coefficients (R^2) were greater than 0.99 for all target compounds. The quantification limits for most volatile sulfur compounds were 0.5 ppb or lower, except for methionol which had a detection limit of 60 ppb. The recovery was studied in synthetic wine as well as Pinot noir, Cabernet Sauvignon, Pinot Grigio, and Chardonnay wines. Although the sulfur compounds behaved differently depending on the wine matrix, recoveries of greater than 80% were achieved for all sulfur compounds. This technique was applied to analyze volatile sulfur compounds in several commercial wine samples; methionol concentrations were found at the ppm level, while the concentrations for hydrogen sulfide, methanethiol, and methyl thioacetate were at ppb levels. Only trace amounts of disulfides and trisulfides were detected, and ethanethiol was not detected.

Keywords: Volatile sulfur compound; Quantification; Wine off-flavor; SPME; Pulsed flame photometric detection

1. Introduction

Volatile sulfur compounds are known to have very powerful and characteristic odors, and these compounds can contribute to pleasant or unpleasant aromas of a wine, according to their nature and concentration [1]. Usually when volatile sulfur compounds are present at very low concentrations, they contribute a positive impression to the wine aroma [2]. However, when present at higher concentrations, they are responsible for "reduced", "rotten egg", or "sulfury" off-flavors [3]. Balancing the two can be a significant challenge to winemakers, since many factors such as deficiencies of nutrients (amino acids and vitamins), yeast strains, metal ions, redox potential, and fermentation temperature, can all influence the formation of volatile sulfur compounds [4]. The mechanisms that form these compounds are still poorly understood, which is partially because there is no sensitive, reliable analytical method available to measure them. For this reason, it has become increasingly important to develop a quick and reliable analytical method to quantify volatile sulfur compounds in wine.

Sulfur compounds are present in trace amounts in wine, therefore a pre-concentration step is required before chromatographic analysis [5]. Solvent extraction [6,7] and static headspace extraction [8,9] have been widely used for volatile extraction, but time consumption and lack of sensitivity are the two major downfalls to limit their application for sulfur analysis in wine. In addition, some sulfur compounds are extremely volatile and chemically reactive so it is impossible to use traditional technique to enrich them.

As an alternative to traditional pre-concentration methods, solid-phase microextraction (SPME) has been successfully used to extract volatile compounds, including sulfur compounds, from the headspace of various samples [10–15]. SPME technique has been previously used to analyze volatile

* Corresponding author. Tel.: +1 541 737 9114; fax: +1 541 737 1877.
E-mail address: michael.qian@oregonstate.edu (M.C. Qian).

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sulfur compounds in wines [16–19], but quantification has not been successful due to the challenges involved with sulfur compounds as well as competitive adsorption [20]. A SPME extraction coupled with stable isotope dilution assay was successfully developed to analyze ethanethiol and diethyl disulfide in Sarah wine [21,22], but this technique is time-consuming. Moreover, not all important volatile sulfur compounds, such as hydrogen sulfide and methanethiol, could be quantified by this method.

Due to low concentrations in food, sulfur compounds are typically analyzed by gas chromatography (GC) with sulfur-specific detection, including flame photometric detection (FPD) [8,9], chemiluminescent detection (SCD) [23] and atomic emission detection (AED). Recently, pulsed flame photometric detection (PFPD) has proven to be very sensitive for sulfur compounds [15,24–26]. This technique uses a pulsed flame, rather than a continuous flame as with traditional FPD, to achieve the generation of flame chemiluminescence [27]. With PFPD, light emissions due to hydrocarbons and flame background can be ignored during each pulse of the flame by electronically gating the emission, allowing for only the sulfur portion of the spectrum to be integrated, thereby greatly increasing the selectivity and sensitivity for this detector.

In this study, a quick, sensitive method was developed to quantify the trace amounts of volatile sulfur compounds in wines by SPME and GC-PFPD. Parameters for SPME extraction were optimized to increase sensitivity, and highly reactive sulfur compounds were stabilized during the analysis. The technique was used to measure the concentrations of volatile sulfur compounds in several commercial wines.

2. Experimental

2.1. Chemicals

Sodium sulfide, methanethiol (MeSH), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), and isopropyl disulfide (IsoProDS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanethiol (EtSH), diethyl sulfide (DES), methyl thioacetate (MeSOAc), ethyl thioacetate (EtSOAc), 3-methylthiopropanol (methionol), and 4-methylthiobutanol were obtained from Johnson Mattey Catalog Company Inc. (Ward Hill, MA, USA). Ethyl methyl sulfide (EMS), dimethyl sulfide (DMS), diethyl disulfide (DEDS) were supplied by TCI America (Portland, OR, USA). Methanol and L-tartaric acid were obtained from J.T. Baker (Phillipsburg, NJ, USA), and the ethanol was from AAPER Alcohol and Chemical Co. (Shelbyville, KY, USA).

2.2. SPME extraction condition

An automatic headspace sampling system (CombiPAL autosampler equipped with a SPME adapter, from CTC Analytics, Zwingen, Switerland) with an 85 μm Carboxen-PDMS StableFlex SPME fiber (SUPELCO, Bellefonte, PA, USA) was used for extraction of sulfur compounds. Five milliliters of samples were placed in 20 mL autosampler vials. The vials were tightly capped with Teflon-faced silicone septa, and placed in an automatic headspace sampling system. The SPME conditions were set as following: samples were equilibrated at 30 °C for 30 min with 500 rpm agitation; and extracted for 15 min with 250 rpm agitation (on for 8 s, off for 2 s) at the same temperature.

2.3. Detection of volatile sulfur compound by GC-PFPD

The analyses were made on a Varian CP-3800 gas chromatography equipped with a PFPD detector (Varian, Walnut Creek, CA, USA) operating in sulfur mode. After extraction, the SPME fiber was directly injected into the GC injection port with the splitless mode at 300 °C and kept for 7 min. The separation was performed using a DB-FFAP capillary column (30 m × 0.32 mm I.D., 1 μm film thickness, from Agilent, Palo Alto, CA, USA). The oven temperature was programmed as follows: 35 °C (initial hold 3 min), ramp at 10 °C/min to 150 °C (hold for 5 min), and then ramp at 20 °C/min to 220 °C (final hold 3 min). The carrier gas was nitrogen with a constant flow rate of 2 mL/min. The temperature of the detector was 300 °C, and the detector was supplied with 14 mL/min hydrogen, 17 mL/min air 1, and 10 mL/min air 2. The detector voltage was 500 V, the gate delay for sulfur compounds was 6 ms, and the gate width is 20 ms. All sulfur compounds were identified by comparing their retention times with those of the pure standards. The sulfur responses of specific compounds were calculated by the square root of peak area.

2.4. Quantification of volatile sulfur compounds

2.4.1. Synthetic wine

The synthetic wine was made according to Mestres et al. [16] where 3.5 g L-tartaric acid was dissolved into 1 L of 12% ethanol solution, and the pH was adjusted to 3.5 with 1 M NaOH.

2.4.2. Sulfur standards and internal standard preparation

Hydrogen sulfide (H_2S) was generated by adding sodium sulfide solution into synthetic wine. Different concentrations of sodium sulfide solutions were made by dissolving the salt in distilled water (pH 7). The solutions were stored at 4 °C. Before analysis, the sodium sulfide solutions were directly added into sample vials containing synthetic wines (pH 3.5). The concentrations of H_2S were calculated based on the amounts of sodium sulfide added into the synthetic wines. The MeSH standard was prepared by bubbling pure MeSH gas directly into cooled methanol (−15 °C). Its concentration was calculated by weight. Standard solutions of 2000 ppm (w/w) of DMS, DMDS, DMTS, EtSH, DES, DEDS, MeSOAc, EtSOAc and methionol were individually