



Analytical Methods

Analysis of volatile thiols in alcoholic beverages by simultaneous derivatization/extraction and liquid chromatography–high resolution mass spectrometry

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ABSTRACT

A simultaneous derivatization/extraction method followed by liquid chromatography–electrospray–high resolution mass spectrometry for the determination of volatile thiols in hydroalcoholic matrixes was optimized and used to identify and quantify volatile thiols in wine and beer samples. The method was evaluated in terms of sensitivity, precision, accuracy and selectivity. The experimental LOQs of eleven thiols tested ranged between 0.01 ng/L and 10 ng/L. Intra-day relative standard deviation (RSD) was in general lower than 10% and inter-day RSD ranged between 10% and 30%. Recovery in the model and real matrixes ranged from 45% to 129%. The method was then applied for the analysis of four white wines and six beers. Five out of the eleven reference thiols were identified and quantified in the samples analyzed. The non-target approach, carried out by monitoring the diagnostic ion at m/z 275.9922 $[C_{13}H_{10}ONSe]^+$ in the fragmentation spectrum, allowed detecting, in the same samples, fourteen non-target thiols.

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1. Introduction

Volatile sulfur compounds are major contributors to several food and beverage aromas (McGorin, 2011; Vermeulen, Gijis, & Collin, 2005). Due to their extremely low odor thresholds, they have a significant sensory impact even at very low concentrations. In particular, volatile thiols are well known to be powerful odorants in beverages such as wine (Kotseridis & Baumes, 2000; Tominaga, Baltenweck-Guyot, Peyrot des Gachons, & Dubourdieu, 2000) and beer (Hugues, 2009; Vermeulen, Lejeune, Tran, & Collin, 2006). Among wine varietal thiols, 4-mercapto-4-methyl-pentan-2-one, 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA), are responsible for box tree, exotic fruit and grapefruit aromas, respectively, in wines made from Sauvignon Blanc, Colombard, Scheurebe, Merlot and Cabernet Sauvignon cultivars, among others (Bouchilloux, Darriet, Henry, Lavigne-Cruege, & Dubourdieu, 1998; Darriet, Tominaga, Lavigne, Boidron, & Dubourdieu, 1995; Guth, 1997; Kotseridis & Baumes, 2000; Schneider, Charrier, Razungles, & Baumes, 2006). Other thiols such

as 2-furanmethanethiol (2FMT), 2-methyl-3-furanthiol (2M3FT), and benzenemethanethiol, have been associated with the empyreumatic notes of aged wines (Blanchard, Tominaga, & Dubourdieu, 2001; Tominaga, Blanchard, Darriet, & Dubourdieu, 2000; Tominaga, Guimbertau, & Dubourdieu, 2003). Furthermore, several polyfunctional thiols have been reported in beer, usually associated with sensory defects. In particular, 3-methyl-2-buten-1-thiol (3MBT) imparts the well-known lightstruck and “skunky” off flavors (Goldstein, Rader, & Murakami, 1993; Huvaere, Andersen, Skibsted, Heyerick, & Keukeleire, 2005; Lermusieau & Collin, 2003), while 4-mercapto-4-methyl-2-pentanone (Cosser, Murray, & Holzapfel, 1980) and 3-mercapto-3-methylbutyl-formate (Schieberle, 1991) induce a “ribes, catty” odor. Recently, 3-mercapto-2-methylbutanol and 2-mercapto-3-methylbutanol were associated with onion-like notes in beer (Vermeulen et al., 2006). Moreover, 2M3FT (Lermusieau, Bulens, & Collin, 2001) and 3MH are often present in fresh lager beers, although their sensory impact seems of little relevance (Vermeulen et al., 2006).

Finally, highly volatile thiols, such as hydrogen sulfide (H_2S), methanethiol, ethanethiol and propanethiol, have been identified as responsible for putrefaction, garlic, onion or rotten egg-like notes, in beer (Hugues, 2009) and in wine, as reviewed by Mestres, Busto, and Guasch (2000).

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The analytical assay of volatile thiols in food and beverages is particularly difficult due to the complexity of the matrixes, together with the typically low concentrations and high reactivity of the thiols. The most widely used analytical methods are based on the liquid–liquid extraction of thiols from a wine or beer matrix, followed by derivatization with *p*-hydroxymercurybenzoate (Tominaga, Murat, & Dubourdieu, 1998; Vermeulen et al., 2006) or by reversible covalent chromatography (Schneider, Kotseridis, Ray, Augier, & Baumes, 2003). More recently, 2,3,4,5,6-pentafluorobenzyl bromide (PFBBr) has been used to derivatize thiols on solid-phase microextraction (SPME) fibers (Mateo-Vivaracho, Cacho, & Ferreira, 2007) or in the wine matrix, followed by isolation of the derivatives either via solid-phase extraction (SPE) (Mateo-Vivaracho, Cacho, & Ferreira, 2008) or SPE followed by SPME (Rodríguez-Bencomo, Schneider, Lepoutre, & Rigou, 2009). The best quantification performance achieved with these methods uses stable isotopic dilution, which involves the synthesis of deuterated thiols.

These procedures require relatively large sample amounts, they are laborious and time-consuming, and some of them involve several sample-manipulation steps during which volatile thiols can be lost or undergo degradation.

In order to devise a simple, reliable, selective and sensitive method for the analysis of volatile thiols at trace levels in hydroalcoholic matrixes such as wine and beer, a simultaneous derivatization/extraction method followed by liquid chromatography-electrospray-high resolution mass spectrometry (LC-ESI-HRMS) was developed using ebselen as the derivatization reagent. This choice was based on recent reports of its high selectivity, fast reaction and high derivatization yield for some biological thiols (Xu et al., 2010), and volatile thiols in lipid matrixes (Vichi, Cortés-Francisco, & Caixach, 2013). HRMS was chosen to provide the highest chemical selectivity. The analytical conditions were optimized in model systems and real wine and beer samples, both spiked with eleven representative volatile thiols. The method was first evaluated in terms of sensitivity, precision, accuracy and selectivity, and then applied to real samples. Moreover, a non-target approach based on the formation of a diagnostic product ion was evaluated using real wine and beer samples.

2. Materials and methods

2.1. Chemicals and reagents

Mass spectrometry grade dichloromethane and methanol (MS SupraSolv[®]) were purchased from Merck (Darmstadt, Germany). Water was of ultrapure milli-Q grade. Ammonium formate was from Sigma–Aldrich (St. Louis, MO, USA). Nitrogen (Alphagaz N₂, purity 99.999%, Air Liquid) was used in the Orbitrap-Exactive as nebulization and fragmentation gas.

Ebselen (2-phenyl-1,2-benziselenazol-3(2H)-one, purity 98%), absolute ethanol (purity 99.8%), ethylenediaminetetraacetic acid (EDTA) (purity 98.5%); 3-mercaptohexyl acetate (3MHA) (purity 98%); 3-mercaptohexanol (3MH) (purity 98%); 3-mercapto-3-methylbutan-1-ol (3MMB) (purity 98%); 2-furanmethanethiol (2FMT) (purity 97%); *p*-mentha-8-thiol-3-one (MT) (purity 98%); 1-hexanethiol (HT) (purity 98%); 4-methoxy- α -toluenethiol (IS, purity 90%) were purchased by Sigma–Aldrich (St. Louis, MO, USA). 4-mercapto-4-methylpentan-2-ol (4MMP) (purity 98%), 4-methoxy-2-methyl-2-butanethiol (4MMB) (purity 98%); 3-mercaptohexyl hexanoate (3MHH) (purity 98%); 1-phenylethyl mercaptan (1PEM) (purity 98%) were from Endeavour Speciality Chemicals Ltd (Northants, UK). 3-methyl-2-butene-1-thiol preparation kit was provided by Toronto Research Chemicals Inc. (North York, Canada). Molecular structures of the reference thiols are shown in Fig. S1 (Supplementary information).

2.2. Model wine and model beer

Model wine was prepared according to Labanda, Vichi, Llorens, and López-Tamames (2009), by dissolving 5 g/L of glycerol, 10 g/L of glucose, 5 g/L of tartaric acid, 10 mg/L of albumin, 300 mg/L of pectin, 100 mg/L of tannic acid, 150 mg/L of sodium metabisulfite and 120 mL of absolute ethanol in deionized water. The pH of the model wine was 3.0.

Model beer was prepared according to Eagles and Wakeman (2002) with some modifications: 1.5 g/L of glycerol, 1.5 g/L of maltose 100 mg/L of citric acid, 100 mg/L of albumin, 300 mg/L of pectin, 50 mg/L of tannic acid and 50 mL/L of absolute ethanol in deionized water. The pH of the model beer was 4.2.

2.3. Wine and beer samples

The method was applied to distinct commercial samples of wine and beer provided by local retailers. Wine samples were: Albariño (2012) Denomination of Origin (D.O.) Rias Baixas (Spain) (sample W1); Sauvignon Blanc (2012) D.O. Rueda (Spain) (W2); Riesling (2012) D.O. Penedés (Spain) (W3); Sauvignon Blanc/gewürztraminer (2012) D.O. Penedès (Spain) (W4). Two lager beer samples exposed to light (B1, B2), one lager (B3), one double-malt (B4), one alcohol free (B5) and one stout (B6) commercial beers were analyzed.

2.4. Derivatization/extraction conditions

The derivatization and extraction conditions were optimized in model and real wine and beer samples spiked with reference thiols at 5 ng/L. The most suitable conditions were determined by comparing absolute peaks areas. Derivatization/extraction conditions were finally fixed as follows: 20 mL of sample were placed into a screw cap-tube, added with 400 μ L of EDTA 30 mg/mL and spiked with 4-methoxy- α -toluenethiol (IS) to a final concentration of 10 ng/L. 6 mL of ebselen 0.1 mM in dichloromethane was then added and the mixture was vortex-mixed during 1 min. The sample was maintained under nitrogen atmosphere during this process. After centrifugation (4000 rpm, 15 min), 1 mL of the dichloromethane phase was collected, dried under nitrogen flow and redissolved in 0.1 mL of methanol.

2.5. High performance liquid chromatography (HPLC)

The HPLC system consisted of a Surveyor MS Plus pump coupled to an Accela Open automatic sampler (Thermo Fisher Scientific, San Jose, California) equipped with 10 μ L loop. The chromatographic separation was performed on a Luna C18 (150 \times 2.1 mm, 5 μ m) analytical column (Phenomenex, Torrance, CA). Elution was performed at a flow rate of 200 μ L/min, using water (solvent A) and methanol (solvent B), both containing ammonium formate 10 mM. The solvent gradient changed according to the following conditions: 50% (A)–50% (B) during 2 min, to 100% (B) in 18 min; 100% (B) during 13 min, then to 50% (A)–50% (B) in 1 min, followed by 6 min of maintenance.

2.6. High resolution mass spectrometry (HRMS)

Mass spectrometric analysis was carried out with an Orbitrap-Exactive-HCD (Thermo Fisher Scientific, Bremen, Germany) equipped with an electrospray source (H-ESI II). The ionization conditions, in positive mode, were according to Vichi et al. (2013): spray voltage 3.75 kV, capillary voltage 25 V, skimmer voltage 16 V, tube lens voltage 80 V. Sheath gas flow rate was set at 40 arbitrary units (au), auxiliary gas flow rate was 10 au, capillary temperature was 275 °C, and heater temperature was 30 °C. The

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