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Analysis of volatile phenols in alcoholic beverage by ethylene glycol-polydimethylsiloxane based stir bar sorptive extraction and gas chromatography–mass spectrometry

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ABSTRACT

An ethylene glycol (EG)/polydimethylsiloxane (PDMS) copolymer based stir bar sorptive extraction (SBSE)-GC–MS method was developed for the analysis of volatile phenols (4-ethylphenol, 4-vinylphenol, 4-ethylguaiacol, and 4-vinylguaiacol) in alcoholic beverages. The beverage samples were diluted with phosphate buffer (1 M, pH 7) and extracted with an EG/PDMS stir bar. Volatile phenols were thermally desorbed and analyzed by GC–MS. Parameters affecting extraction efficiency were studied including ionic strength, pH, extraction time, ethanol content and nonvolatile matrix. Good correlation coefficients with R^2 in the range of 0.994–0.999 were obtained for volatile phenol concentration of 5–500 μg/L. Recovery for all phenols were from 95.7% to 104.4% in a beer matrix and 81.4% to 97.6% in a wine matrix. The method had a standard deviation less than 5.8% for all volatile phenols. The limit of quantification (LOQs) in beer samples was lower than 3 μg/L. The method was further applied to analyze the concentrations of volatile phenols in beer, wine and other alcoholic beverage samples.

1. Introduction

Volatile phenols, specifically 4-vinylguaiacol (4-VG), 4-vinylphenol (4-VP), 4-ethylguaiacol (4-EG) and 4-ethylphenol (4-EP), have a significant impact on the flavor of beer, wine, and other alcoholic beverages [1–4]. Although they contribute to the characteristic aroma of certain types of beer [5] and wine [6], volatile phenols are typically associated with aroma defect at high concentration [7], referred to phenolic, horsy, barnyard, medicinal, smoky, phenolic, and clove-like flavor characteristics.

Volatile phenols in beer and wine originate from hydroxycinnamic acids [8]. In the brewing process, ferulic and *p*-coumaric acid can decarboxylate thermally during wort boiling [9] or enzymatically during fermentation by hydroxycinnamate decarboxylase. This enzyme can be present in various microorganisms including *Saccharomyces cerevisiae*, lactic acid bacteria, acetic acid bacteria, and *Brettanomyces/Dekkera* sp. which can generate 4-vinylphenol and 4-vinylguaiacol [4,10–12]. Both 4-vinylphenol and 4-vinylguaicol have relatively high sensory thresholds (300 ppb) and typically do not cause off-flavors in beer and wine. However, 4-vinylphenol and 4-vinylguaiacol can be reduced to their corresponding ethyl derivatives (4-ethylphenol and 4-ethylguaiacol) by the enzyme vinylphenol reductase which can be produced by the yeast *Brettanomyces/Dekkera* sp. [13,14]. 4-Ethylphenol and 4-ethylguaiacol have lower sensory thresholds and are major compounds responsible for 'Brett character' in wine. Unlike *Brettanomyces/Dekkera* sp., other wine associated yeasts such as *S. cerevisiae*, *Pichia* sp., *Torulaspora* sp., and *Zygosaccharomyces* sp. can produce vinylphenols but not ethylphenols under normal oenological conditions [15].

Analysis of volatile phenols in alcoholic beverages is an active research area [16–23]. High performance liquid chromatography (HPLC) is a frequently used analytical technique [18,19,22,24]. However, HPLC methods have poor sensitivity and require tedious sample preparation prior to chromatographic separation. Great efforts have been made to develop easy and sensitive gas chromatography (GC) methods to analyze volatile phenols in a complex matrix. Headspace solid-phase microextraction (SPME) [20,23] as well as stir bar sorptive extraction (SBSE) [17,25] coupled with GC and GC–MS have been attempted for volatile phenol analysis. Although the SPME technique is simple and sensitive, the presence of other volatile compounds within the sample matrix can compete with fiber's limited active sites and interfere with quantification [20,26]. Stir bar sorptive extraction (SBSE) employs a magnetic stir bar coated with a thick layer of polymer (0.5–1 mm thickness) for volatile extraction which increases phase volume and

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minimizes the absorptive competition [27]. Polydimethylsiloxane (PDMS)-based SBSE techniques have been widely used in trace level volatile analysis in many matrices including alcoholic beverages due to its low affinity to alcohols [17,25,28–31]. However, the non-polar PDMS phase has limited affinity to polar compounds such as phenols [32]. Studies have shown that the extraction efficiency of PDMS stir bar for volatile phenols is not satisfactory especially for 4-vinylphenol [17]. A new type of stir bar coated with ethylene glycol (EG)/polydimethylsiloxane (PDMS) copolymer has been developed recently [33–35]. This EG coating allows binding of polar compounds as well as hydrogen bond donor compounds, such as phenols, to be efficiently extracted [36]. Since the H-bonding is affected by pH, ionic strength, ethanol concentration and other parameters [34], the aim of this study was to determine the effect of these parameters on the absorption of phenols to the EG/PDMS stir bar and to develop a fast, sensitive and reliable method for the quantitative analysis of 4-EP, 4-EG, 4-VP and 4-VG in alcoholic beverages such as beer and wine.

2. Experimental

2.1. Chemicals and reagents

Standards of 4-ethylphenol, 4-ethylguaiacol, 4-vinylphenol (10 wt.% in propylene glycol), 4-vinylguaiacol, and 3, 4-dimethylphenol were obtained from Sigma–Aldrich (St. Louis, MO, USA). Absolute ethanol (200 proof, Koptec USP) was purchased from VWR (Radnor, PA, USA). Stock solution containing mixtures of each standard (9460 mg/L of 4-ethylguaiacol, 9830 mg/L of 4-ethylphenol, 11,050 mg/L of 4-vinylguaiacol and 9950 mg/L of 4-vinylphenol) was prepared in absolute ethanol and stored at −4 °C. An internal standard solution (IS) was made by dissolving 0.1192 g of 3,4-dimethylphenol in 10 mL absolute ethanol and diluting 200 times to 59.6 mg/L and keeping at −4 °C until use. Phosphate buffer (1 M, pH 7) was made by mixing 1 M K_2HPO_4 solution and 1 M KH_2PO_4 solution to give the required pH. Beer and wines samples were obtained commercially and stored at 4 °C until use.

2.2. Sample preparation

Four milliliters of sample was diluted with 16 mL of phosphate buffer (1 M, pH 7) in a 20 mL glass vial. An aliquot of 20 μL of 59.6 mg/L IS was then added. An EG/PDMS stir bar (1 cm length, 0.5 mm thickness, GERSTEL, Inc., U.S.A, Linthicum, MD, USA) was put in the vial and stirred for 3 h at 1000 rpm at room temperature. After extraction, the stir bar was rinsed with Milli-Q water, dried with a Kimwipe tissue and placed in the sample holder of the thermal desorption unit (TDU) for GC–MS analysis.

2.3. SBSE-GC–MS analysis

The SBSE-GC–MS analysis was performed on an Agilent 7890 GC-5975 MSD system equipped with a Multi-purpose Sampler (MPS, GERSTERL, Inc., U.S.A). The analytes were thermally desorbed in the TDU in splitless mode. The temperature of the TDU ramped from 30 °C to 220 °C at a rate of 120 °C/min, and held at the final temperature for 3 min. The desorbed analytes from the TDU were re-cryofocused in a programmed temperature vaporizing (PTV) injector with a Tenax TA 60/80 packed liner (CIS-4, GERSTEL, Inc., U.S.A) at −80 °C with liquid nitrogen. After desorption, the CIS was heated to 220 °C at a rate of 10 °C/s. The solvent vent injection mode with a split vent purge flow of 50 mL/min was employed for the CIS-4 injector. Separation was performed on a ZB-WAX column (30 m × 0.25 mm ID, 0.5 μm film thickness, Phenomenex, Torrance, CA, USA) with helium as carrier gas at a constant flow rate of 2.0 mL/min. Initial oven temperature was 80 °C and held for 2 min, then ramped to 230 °C at a rate of 5 °C/min and held for 5 min. The mass spectrometric detection was performed in scan mode from m/z 45–350 with electron ionization (EI) energy of 70 eV. The MS transfer line and ion source temperature was 280 °C and 230 °C, respectively. Selective mass ions were used to quantify the volatile phenols.

2.4. Method development

The variables affecting the SBSE extraction process were studied in terms of ionic strength, pH and extraction time. The experiments were performed with the standard solution at a concentration of 100 μg/L. The influence of ionic strength on extraction efficiency was investigated by adjusting the concentrations of phosphate buffer at 0.1 M, 0.5 M and 1 M, respectively, at pH 7. The sample was extracted for 3 h. Using the optimized buffer concentration (1 M), the effect of phosphate buffer pH (pH 2–8) on extraction efficiency was studied. The sample was extracted for 3 h. Using the optimized pH (pH 7) and ionic strength (1 M) of phosphate buffer, the effect of extraction time (0.5, 1, 2, 3, 4, 5, 6, 16 and 24 h) was evaluated.

To study the interaction of volatile phenols with the nonvolatile matrix, a commercial beer sample was chosen because of its complicated nonvolatile composition. Beer samples were diluted with phosphate buffer (1 M, pH 7) at 2, 4, 5, 10 and 20-fold dilution ratios in 20 mL. The same amount of internal standard solution (20 μL) was added to each sample.

2.5. Method validation

2.5.1. Standard calibration curve

Stock solutions of the phenolic compounds were prepared at concentrations ranging from 0.4 mg/L to 40 mg/L from the previously described stock mixture in ethanol. Calibration working solutions were prepared by spiking 50 μL of each stock solution into a 20 mL vial containing 4 mL of 5% ethanol and 16 mL of phosphate buffer (1 M, pH 7). An aliquot of 20 μL of 59.6 mg/L IS solution was added. The standard solution was analyzed using SBSE-GC–MS described previously. Selected ions were used to build the calibration curve by Chemstation software.

2.5.2. Method reproducibility

The reproducibility of the method was evaluated by analyzing the same sample seven times under the optimized condition and calculating the relative standard deviation (R.S.D.).

2.5.3. Limit of detection and quantification (LOD and LOQ)

Ethanol solutions (5%, v/v) spiked with decreasing levels of phenolic compounds were analyzed by SBSE-GC–MS. The LODs were established as the amount of analytes that gave a signal to noise ratio of 3 (S/N = 3). The limit of quantification (LOQ) was determined as the concentration that gave a signal to noise ratio of 10 (S/N = 10).

2.5.4. Recovery

Three commercial beers and wines were used to study recovery. The samples were analyzed first to obtain the concentration (C_1). The samples were then spiked with volatile phenols at concentration of 100 μg/L and analyzed again (C_2). The recovery was calculated as recovery% = $(C_2 - C_1)/100$ μg/L × 100%. Triplicate analysis was performed for each sample.