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Sensitive Analysis of Off-flavor Compounds, Geosmin and 2-Methylisoborneol, in Water and Farmed Sturgeon by using Stir Bar Sorptive Extraction Coupled with Thermal Desorption and Gas Chromatography-Mass Spectrometry

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Abstract

The semi-volatile cyclic alcohols 2-methylisoborneol (2-MIB) and geosmin (GSM) impart muddy or musty flavors to water and fish products. A rapid quantitative analytical technique has been developed based on stir bar sorptive extraction (SBSE), coupled with thermal desorption and gas chromatography-mass spectrometry (TD-GC-MS). SBSE is used to extract and concentrate the off-flavor compounds of GSM and 2-MIB in coating material polydimethylsiloxane (PDMS). The analytes are thermally released from SBSE and analyzed by GC-MS. Limits of detection (LOD) and limits of quantification (LOQ) of GSM and 2-MIB are 0.3 ng/L and 1 ng/L, based on the main fragment ions of m/z 112 and m/z 95, respectively. This methodology allows quantitative analysis of 2-MIB and GSM in both water and fish tissue successfully.

Keywords: Off-flavor; Geosmin; 2-Methylisoborneol; Stir Bar Sorptive Extraction (SBSE); Gas Chromatography-Mass spectrometry (GC-MS)

Introduction

Muddy-earthy-musty-type flavours, such as Geosmin (1a,10h-dimethyl-9a-decalol, GSM) and 2-methylisoborneol (1-R-exo-1,2,7,7-tetramethylbicylo-[2,2,1]-heptan-2-ol, 2-MIB), are semi-volatile off-flavor compounds with similar chemical structures that are produced by certain species of actinomycetes, fungi and blue-green algae [1,2]. These off-flavor compounds have been shown to be the main cause of earthy-musty odorants in water from aquaculture facilities, and the occurrence of such flavours has also been reported for a diverse range of freshwater aquaculture species. 2-MIB and GSM tend to bio-accumulate within fish flesh dependent on the concentration of the compound in the water supply, water temperature, fat content and mass of fish, and other abiotic and biotic factors. Although off-flavor compounds are harmless to human health, fish presenting with these flavour characteristics are often referred to as being 'off-flavour' or 'tainted' and are commonly considered to be spoiled or of low quality [3]. Placing tainted fish in the marketplace typically lowers consumer confidence in the cultured product and ultimately results in significantly lower commercial returns. Uptake of these tainting compounds is primarily via the gills and accumulation in the flesh is influenced by the concentration of the compounds in the holding water, water temperature, and the physiology and lipid content of the fish [2,4]. These compounds are known to be particularly problematic in aquaculture systems due to persistent and elevated nutrient loading.

Recently, this issue has been highlighted as the primary cause of an escalation in negative consumer perceptions of aquaculture fish and are ultimately eroding the market value of end products. This lack of information is constraining efforts to understand the mechanisms of off-flavour tainting and the implementation of practices aimed at regaining consumer confidence in the quality of farmed fish. As such, it is essential for fish farm managers to have access to reliable measurement techniques for 2-MIB and GSM in water and in fish, which would allow the optimization of off-flavor depuration protocols and ultimately enhance product quality. The threshold concentration of off-flavor compound odor in water has been reported to be around 30 ng/L for 2-MIB and 10 ng/L for GSM [5-7]. Traditionally, the analysis of off-flavor compounds, as GSM and 2-MIB, in water and fish tissue requires several steps for sample preparation and concentration of the target compounds, like liquid/liquid extraction (LLE), solid phase extraction (SPE) or distillation techniques [6,8]. The approach of solid phase microextraction (SPME) is simple and fast, but it is limited by capacity of sorptive fibers [5,9]. A novel approach using stir bar sorptive extraction (SBSE), coated with polydimethylsiloxane (PDMS), recently has been wide used, as a solvent-less sample preparation method for the extraction and enrichment of organic compounds from different matrices [10,11]. After the extraction, the stir bar is removed from sample and transferred to a thermal desorption instrument where the analytes are thermally released and delivered to the GC column.

In this study, SBSE was used for the sample enrichment of 2-MIB and GSM in water and fish samples, followed by TD-GC-MS analysis. The aim of this study is to develop a sensitive, selective, and simple method determining and monitoring the level of 2-MIB and GSM at low in water and fish.

Materials and Methods

Materials

Standard solution (100 µg/mL in methanol) of 2-MIB and GSM were purchased from Sigma–Aldrich (St. Louis, MO, USA). The different concentration of standards was diluted to prepare the working

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standard solutions. All other chemicals were of analytical grade. The commercial stir bars [Twister™] were incorporated in a glass jacket and coated with PDMS (length: 10 mm, thickness: 0.5 mm), as well as the 10 mL vials and related equipment were obtained from Gerstel (Linthicum, MD, USA).

Samples of water and fish (sturgeon) were provided by a local fish farm. Water was collected with fish samples. Skin-off fillets were collected, vacuum sealed in individual plastic bags, and immediately frozen. Fish fillet samples arrived at the laboratory frozen in separate labelled vacuum sealed plastic bags. The fillets were thawed in a cold room (4°C) for 2–3 h before being cut into small pieces for grounding using a Mini-Prep Chopper/Grinder (Cuisinart®, Canada). Ground fish tissue (≤ 1 ± 0.05 g) was put into 10 mL amber vials with 9 mL saturated NaCl solution.

Methods

For sample extraction using SBSE, one stir bar was used for 2 h at 1000 rpm in each screw-capped vial. After extraction, the stir bar was removed with a forceps, rinsed twice with Millipore water, and dried with a lint-free tissue. Two stir bars were placed in a glass thermal desorption tube for TD–GC–MS analysis. The desorption tube was then placed in TDU, where the stir bars were thermally desorbed by programming the TDU from 40°C (held for 0.5 min) to 280°C (held for 3 min) at 240°C/min. Transfer temperature was fixed at 275°C. The desorbed compounds were Cryo focussed in the CIS 4 with a glass wool notched liner at −120 °C. After desorption, the CIS 4 was programmed from 280°C (held for 3 min) at 12°C/s to inject the trapped compounds onto the analytical column. Injection was performed in the programmable temperature vaporization (PTV) solvent vent mode, and purge flow to split vent was 36 mL/min at 1 min.

The separations were carried out on a HP-5ms fused-silica capillary column [30 m (length)×250 µm (I.D.)×0.25 µm (film thickness); Agilent Technologies, Mississauga, ON, Canada]. The oven temperature was programmed from 50°C (held for 1 min) to 150°C (held for 1 min) at 15°C/min, then to 280°C (held for 0.8 min) at 25°C/min, and total run time was 15 min. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The mass spectrometer (5975C, MSD, Agilent Technologies) was operated in the full scan mode. The used stir bars were cleaned by soaking in Milli-Q purified water for 1 h and then a mixture of methylene chloride–methanol (1:1) for 2 h. The stir bars were dried on a clean surface at room temperature for 2–4 h and reconditioned using a Tube Conditioner (TC2, Gerstel, MD, USA) at 280°C for 2 h in a flow of N_2.

Results and Discussion

SBSE is by nature an equilibrium technique and the extraction of compounds from the matrixes into the PDMS phase is controlled by the partitioning coefficients with the octanol–water distribution ($K_{o/w}$) [11]. Both the distribution coefficient ratio and phase ratio control the extraction recovery in SBSE process. High hydrophobicity of compound has high $K_{o/w}$ values with high extraction efficiency. The log$K_{o/w}$ values of 2-MIB and GSM were 3.31 and 3.57 respectively and theoretical recoveries of 2-MIB and GSM for SBSE using smaller sample volumes are higher. Water samples were extracted by SBSE directly and fish samples (fillets) were well grounded. As shown in Figure 1, water sample (1 mL) or fish sample (1 ± 0.05 g) with 9 mL saturated NaCl solution was extracted by one stir bar with a stirring rate was 1000 rpm in 10 mL vial. In process of SBSE of GSM and 2-MIB over time, the equilibrium of extraction reached after 2 hours in our previous study [12-17]. So, a SBSE time of 2 hours was chosen for all experiments, without further tests.

The typical MS spectra and ion extraction chromatograms of 2-MIB and GSM were shown in Figure 2, obtained by 2 hours SBSE of water sample with fortified at 100 ng/L standards followed by TD–GC–MS analysis. The linearity was initially examined over a range of 0.3–100 ng/L (n=3) for 2-MIB and GSM standards, with correlation coefficients (r^2>0.99), as shown in Figure 3. Limit of detections (LODs) were calculated as three times of the signal-to-noise (S/N) of blank sample and limit of quantifications (LOQs) were determined as ten times of S/N of blank. The values of LODs (n=3) and LOQs (n=3) of 2-MIB and GSM were ~0.3 ng/L and ~1 ng/L based on the main fragment ions of m/z 112 and m/z 95, respectively.

Fish fillets and water samples were analyzed using the developed method to obtain an average value for monitoring and evaluating the process of depuration. The recovery of water samples were shown in Table 1 and results of GSM and MIB in water and fish sampels were displayed in Table 2. The described SBSE–TD–GC–MS method was developed for accurate analyses of the 2-MIB and GSM off-flavor compounds in fish and water.

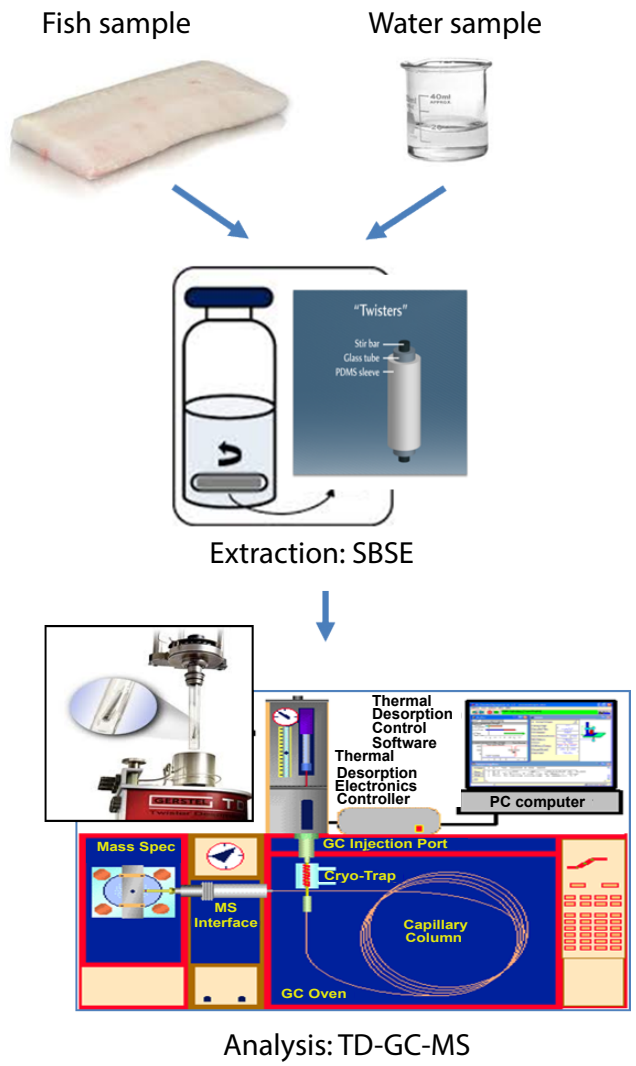

Figure 1: Scheme of SBSE-TD-GC-MS method.

	Recovery (%)	
	Spike-in 100 ng/L	Spike-in 30 ng/L
MIB	98.2 ± 5.0	93.7 ± 1.8
GSM	93.7 ± 1.0	94.0 ± 4.0

Table 1: Recovery (%) of 2-MIB and GSM in water samples using SBSE-TD-GC-MS method.

	2-MIB	GSM
Rt (min)	8.97	12.19
Water (ng/L)	16.2 ± 1.5	10.9 ± 1.1
Fish (ng/Kg)	474.6 ± 35.4	339.6 ± 32.5

Amount of off-flavor: mean (± S.E.) level of triplicates.

Table 2: Results of 2-MIB and GSM (ng/kg) determined using SBSE-TD-GC-MS method.

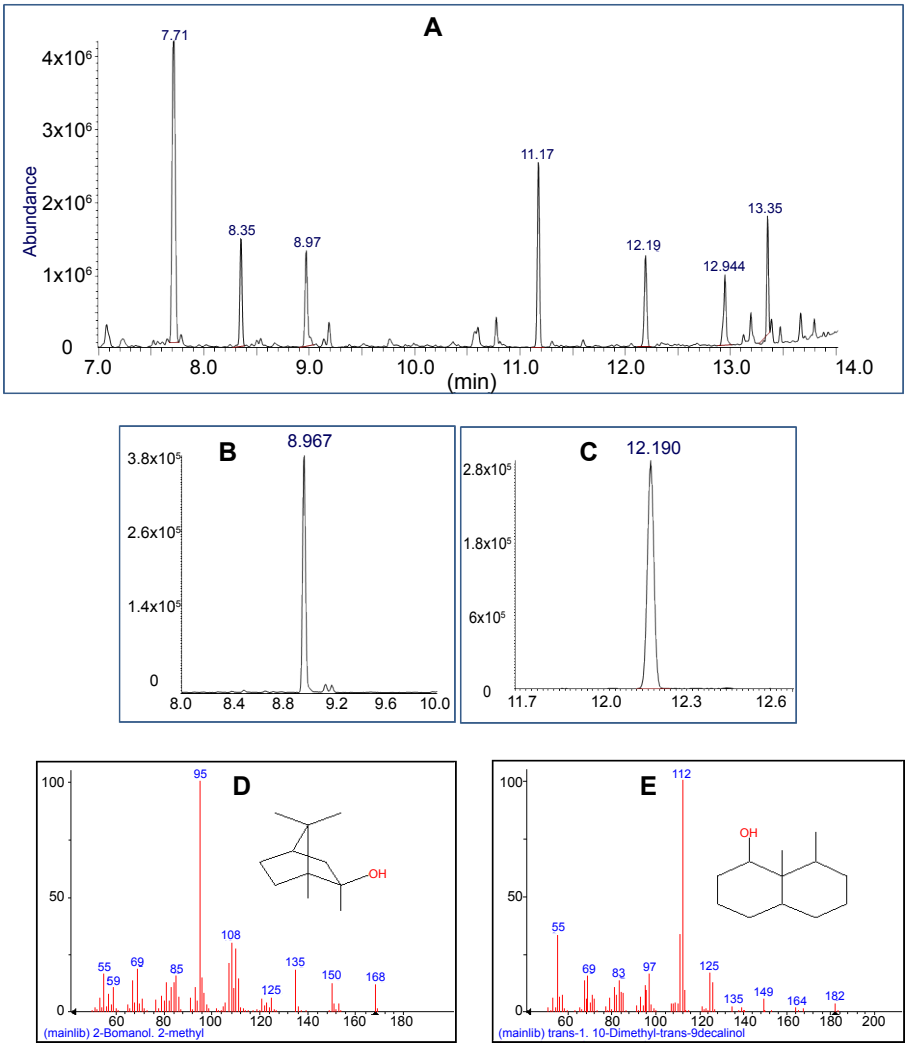

Figure 2: Typical GC-MS chromatography and spectra of GSM and 2-MIB. (A) Total ion chromatography (TIC) of water sample with 2-MIB and GSM spiked, extraction ion chromatograms (XIC) of *m/z* 95 for 2-MIB (B) and *m/z* 112 for GSM (C), spectra of 2-MIB (D) and GSM (E).

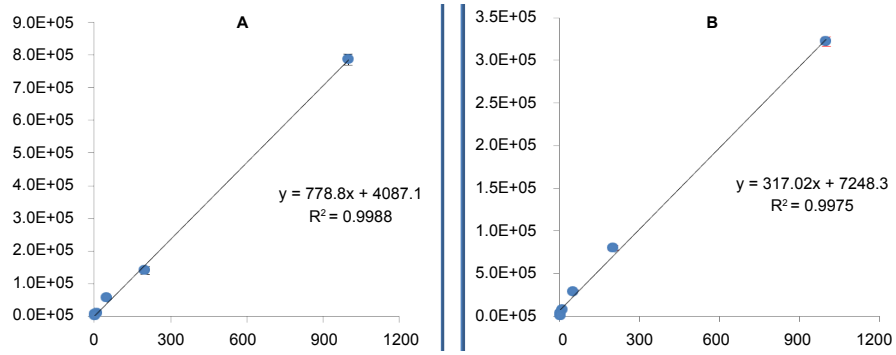

Figure 3: Standard curves of 2-MIB (A) and GSM (B).

Conclusion

The rapid, simple and solvent-less method of SBSE–TD–GC–MS can be applied to detect off-flavor compounds at very low concentration, by using significantly smaller amounts of water and fish samples, without requirement of any pre-concentration, like microwave distillation, or liquid extraction. The method can be reliably utilized by the farmed fish industry for studying accumulation and dissipation of 2-MIB and GSM in fish tissues associated with altering management practices.

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