

Analysis of Aldehydes in Beer Using Solid-Phase Microextraction with On-Fiber Derivatization and Gas Chromatography/Mass Spectrometry

PETR VESELY,^{†,‡} LANCE LUSK,^{*,†} GABRIELA BASAROVA,[‡] JOHN SEABROOKS,[†] AND
 DAVID RYDER[†]

Miller Brewing Company, 3939 West Highland Boulevard, Milwaukee, Wisconsin 53208, and
 Institute of Chemical Technology, Technicka 5, Prague 6, Czech Republic

A new, fast, sensitive, and solventless extraction technique was developed in order to analyze beer carbonyl compounds. The method was based on solid-phase microextraction with on-fiber derivatization. A derivatization agent, *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBOA), was absorbed onto a divinyl benzene/poly(dimethylsiloxane) 65- μ m fiber and exposed to the headspace of a vial with a beer sample. Carbonyl compounds selectively reacted with PFBOA, and the oximes formed were desorbed into a gas chromatograph injection port and quantified by mass spectrometry. This method provided very high reproducibility and linearity. When it was used for the analysis of aged beers, nine aldehydes were detected: 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, pentanal, hexanal, furfural, methional, phenylacetaldehyde, and (*E*)-2-nonenal.

KEYWORDS: Aldehydes; beer analysis; derivatization; SPME; GC/MS

INTRODUCTION

Carbonyl compounds, particularly aldehydes, are considered to play an important role in the deterioration of beer flavor and aroma during storage. Strecker degradation of amino acids, melanoidin-mediated oxidation of higher alcohols, oxidative degradation of lipids, aldol condensation of short-chain aldehydes, and secondary oxidation of long-chain unsaturated aldehydes are mechanisms implicated in their formation (1). Their levels in beer are usually very low, and therefore it has always been a challenge for brewing chemists to develop an analytical method that would enable routine analysis of aldehydes.

Several analytical methods for the determination of aldehydes in beer have been developed, and good results have been obtained using liquid–liquid extraction (2), distillation (3), or sorbent extraction (4). However, these methods are rather complicated and not highly selective.

A simple way to increase the selectivity of extraction techniques is to derivatize the carbonyl compounds. *O*-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine (PFBOA) is commonly used as a derivatization agent in gas chromatography (5). This technique has been applied to the analysis of carbonyl compounds in water and also in beer (6). Although these methods provide good reproducibility, they are time-consuming and require use of solvents, materials for the derivatization, and isolation steps. Martos and Pawliszyn developed an original extraction technique

based on PFBOA on-fiber derivatization of gaseous formaldehyde followed by gas chromatography with flame ionization detection (7).

In this work, we adapted a method for the analysis of beer aldehydes using solid-phase microextraction (SPME) with on-fiber derivatization. This extraction technique does not require solvents, consists of a one-step sample preparation procedure, and provides high sensitivity and reproducibility. It enabled a detailed study of aldehyde level changes during packaged beer storage.

MATERIALS AND METHODS

Chemicals. Carbonyl compound standards, 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, pentanal, hexanal, furfural, methional, phenylacetaldehyde, and (*E*)-2-nonenal, were purchased from Sigma-Aldrich (Milwaukee, WI). A stock solution containing a mixture of the standard compounds in ethanol was prepared in the concentration 100 ppb each. Stock standard solution was prepared daily. An aqueous solution of the derivatization agent *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBOA) (Sigma-Aldrich, Milwaukee, WI) was prepared at a concentration of 6 g/L. PFBOA solution was prepared every 3 months, and it was kept refrigerated.

Beer Samples. American lager beer samples used for the aldehyde analysis were stored at 30 °C for 4, 8, or 12 weeks. Control samples were stored for 12 weeks at 0 °C.

The SO₂ level of the fresh beer was 3.4 ppm, a low level for beer. Knowledge of the SO₂ level in beer is important because SO₂ complexes with aldehydes and only “free” aldehydes are measured by the described method.

SPME Fiber. A 65- μ m poly(dimethylsiloxane)/divinyl benzene (PDMS/DVB) fiber coating (Supelco, Bellefonte, PA; catalog no.

* To whom correspondence should be addressed [phone (414) 931-2732; fax (414) 931-2506; E-mail lusk.lance@mbco.com].

[†] Miller Brewing Co.

[‡] Institute of Chemical Technology.

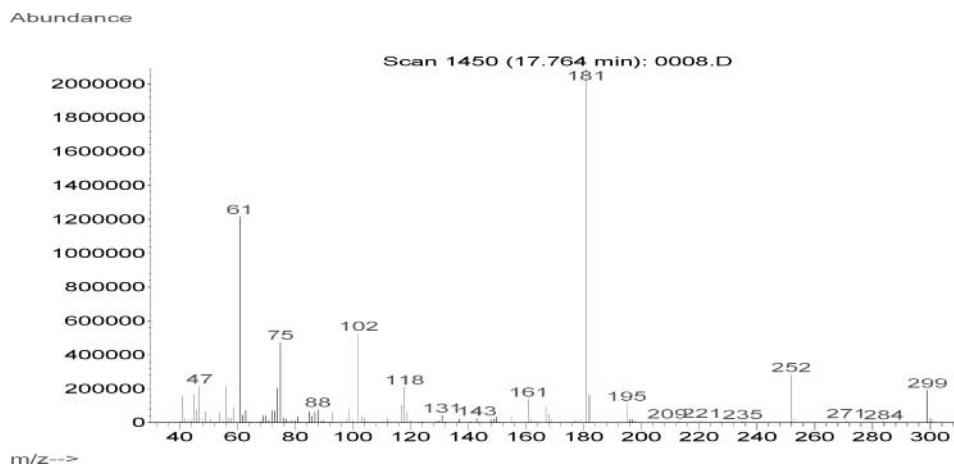


Figure 1. Spectrum of PFBOA derivative of methional.

57328-U) was used in this method. This fiber coating was selected for its ability to retain the derivatizing agent and for its affinity for the PFBOA-aldehyde oxime (7).

Derivatization Procedure. One hundred microliters of PFBOA solution (6 g/L) and 10 mL of deionized water were placed in a 20-mL glass vial and sealed with a magnetic crimp cap (Gerstel, Baltimore, MD). The PDMS/DVB SPME fiber was then placed in the headspace of the PFBOA solution for 10 min at 50 °C. The SPME fiber loaded with PFBOA was then exposed to the headspace of 10 mL of beer placed in a 20-mL glass vial. Different derivatization times and temperatures as well as salt addition were tested in order to obtain the best results. To ensure the reproducibility of the method, an automated process using an MPS2 autosampler (Gerstel, Baltimore, MD) was employed.

GC Conditions. Aldehyde derivatives were analyzed using a HP6890 gas chromatograph equipped with a mass-selective detector (5972A, Agilent Technologies, Palo Alto, CA) and fitted with a DB-5 capillary column, 30 m \times 0.25 mm \times 0.50 μ m (J&W Scientific, Folsom, CA). Helium was the carrier gas at a flow rate of 1.1 mL/min. The front inlet temperature was 250 °C. The injection was in the splitless mode with the purge valve set at 30 s. The oven temperature program used was 40 °C for 2 min, followed by an increase of 10 °C/min to 140 °C and 7 °C/min to 250 °C. The final temperature was held for 3 min.

RESULTS AND DISCUSSION

Identification. Most aldehydes, except formaldehyde, form two geometrical isomers of the derivatives that are represented by two peaks in the chromatogram. Identification of the carbonyl PFBOA derivatives was performed by mass spectrometry using electron impact ionization running in the scan mode. It was confirmed that fragment m/z 181 was the main fragment of all analyzed aldehydes (6). Figure 1 shows as an example the mass spectrum of the PFBOA derivative of methional. To increase the selectivity of the method, all aldehyde analyses were run in the single-ion monitoring (SIM) mode with monitoring for m/z 181.

Beer was also analyzed by gas chromatography/mass spectrometry (GC/MS) without being derivatized by PFBOA in order to ensure that there were no other sources of m/z 181 besides the derivatization agent.

Optimization of Derivatization Procedure. Different parameters that impact the partition of aldehydes between the headspace and the solution, such as derivatization time, temperature, and ionic strength, were tested. The effect of pH was not examined because it was previously shown that the natural pH of beer, 4.5, is sufficiently low for the derivatization reaction (6). Therefore, the pH of standard mixtures was adjusted to 4.5 using 0.1% phosphoric acid. Since methional appeared to be

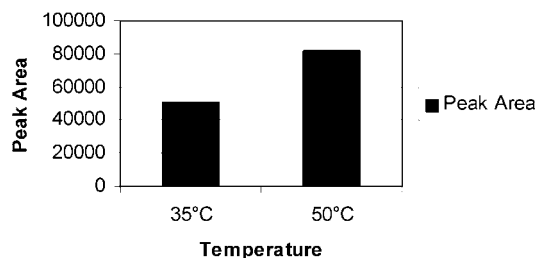


Figure 2. Plot of derivatization temperatures 35 and 50 °C versus detector response area of PFBOA derivative of methional.

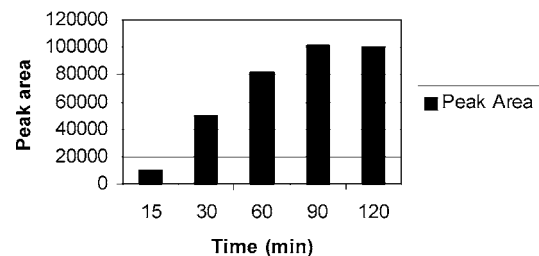


Figure 3. Plot of time of derivatization versus detector response area of PFBOA derivative of methional.

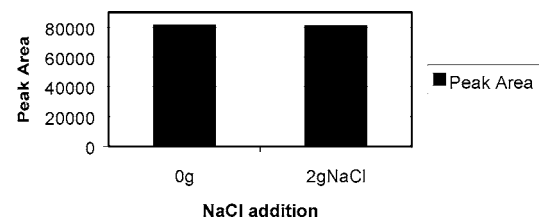


Figure 4. Plot of NaCl concentration versus detector response area of PFBOA derivative of methional.

the most problematic aldehyde to detect, optimization was carried out in a 5% ethanol (pH 4.5) solution spiked with 5 ppb of methional.

The effect of temperature on the extraction of methional from ethanol solution and its derivatization on a PFBOA-loaded fiber was examined for 35 and 50 °C (Figure 2). Increasing the extraction temperature caused an increase in the peak area of the derivatized methional. On the basis of this result, subsequent derivatizations were conducted at 50 °C.

The optimal derivatization time was also tested. The ethanol solution spiked with 5 ppb of methional was exposed for 15, 30, 60, 90, and 120 min at 50 °C. It was determined that the time to reach equilibrium between stationary phase and sample headspace was 90 min (Figure 3). A derivatization time of 60

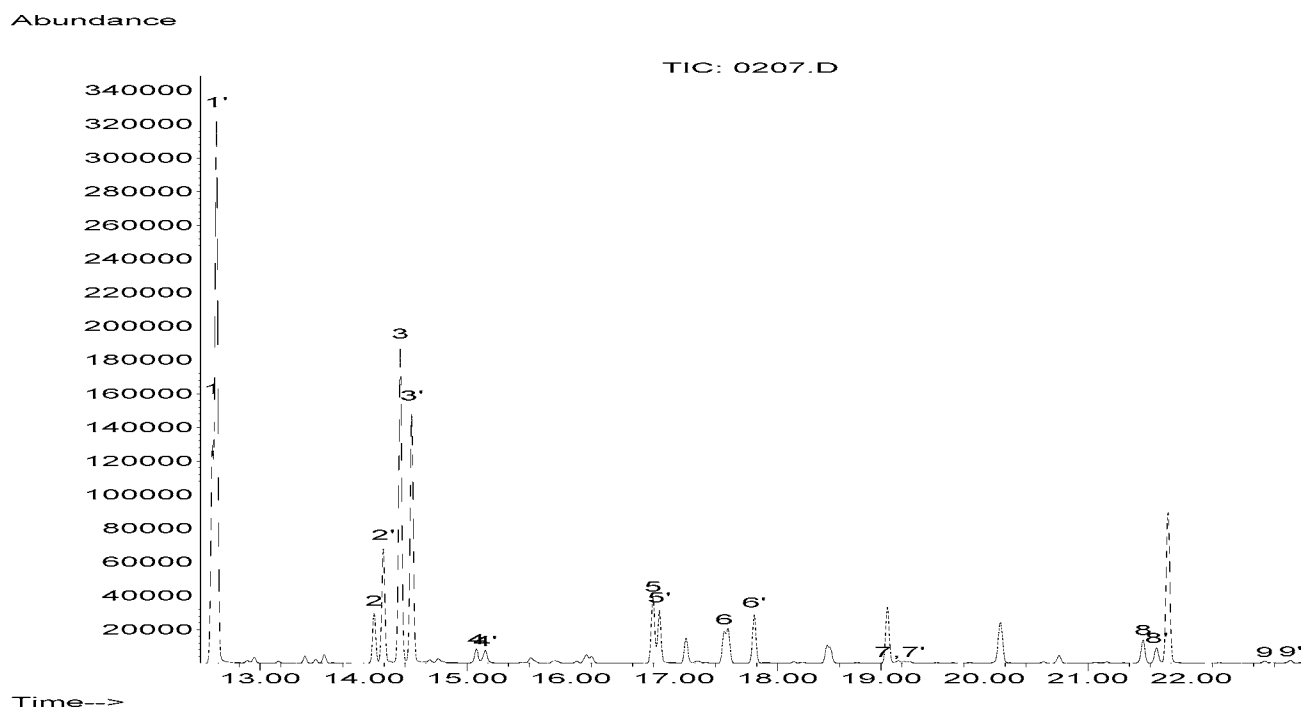


Figure 5. Gas chromatogram of a beer sample derivatized with PFBOA. Peaks: 1,1', 2-methylpropanal; 2,2', 2-methylbutanal; 3,3', 3-methylbutanal; 4,4', pentanal; 5,5', hexanal; 6,6', furfural; 7,7', methional; 8,8', phenylacetaldehyde; 9,9', (*E*)-2-nonenal.

Table 1. Correlation Coefficient (R^2), Coefficient of Variation (CV), and Relative recovery (RR) of Analyzed Aldehydes

	R^2	CV (%)	RR (%)
2-methylpropanal	0.9639	4.7	110
2-methylbutanal	0.9723	4.6	104
3-methylbutanal	0.9706	5.0	109
pentanal	0.9951	3.9	114
hexanal	0.9925	4.3	103
furfural	0.9892	5.1	99
methional	0.9983	2.4	90
phenylacetaldehyde	0.9839	5.3	98
(<i>E</i>)-2-nonenal	0.9944	8.0	89

Table 2. Aldehyde Level Changes (ppb) in Beer during 12 Weeks Storage at 30 °C Compared to Storage at 0 °C

	0 °C	30 °C			FT ^a
	12 w	4 w	8 w	12 w	
2-methylpropanal	6.1	20	30.6	42.4	1 000
2-methylbutanal	1.8	3.1	4.2	5.2	1 250
3-methylbutanal	12.2	17.2	20.7	24.4	600
pentanal	0.3	0.6	0.7	0.8	500
hexanal	1	1.8	2.1	2.5	350
furfural	28.8	202.8	362	458.3	150 000
methional	2.8	3.6	4.1	4.6	250
phenylacetaldehyde	6.6	9.9	10.1	12.7	1 600
(<i>E</i>)-2-nonenal	0.01	0.02	0.02	0.03	0.11

min at 50 °C appeared to be a good compromise between the time of reaction and analyte response.

Figure 4 shows that addition of salt (2 g of NaCl in 10 mL of methional solution) did not have any effect on the extraction and derivatization procedure (60 min, 50 °C).

Calibration. For the calibration purposes, the sum of the peak areas of the two geometrical isomers was used for calculations. A six-point calibration curve for nine carbonyl compounds was measured. The calibration range was 0.1–50 ppb, except for (*E*)-2-nonenal, where the calibration range was 0.01–5 ppb. The matrix used for calibration solutions was 5% ethanol solution, pH 4.5. Correlation coefficient (R^2) values indicate that this method can be used for analysis of aldehydes in a wide range of concentrations (**Table 1**).

Method Validation. Reproducibility of the method was determined by repeatedly analyzing one beer sample 10 times. **Table 1** shows that the method provides very good reproducibility, with coefficients of variations for monitored aldehydes below 5.5%, except for (*E*)-2-nonenal. The higher coefficient of variation for (*E*)-2-nonenal may be due to extremely low levels of this aldehyde in the analyzed beer.

Accuracy of the method was assessed using the method of standard addition by spiking beer with 10 ppb of the standard aldehyde. The recovery was defined by comparing the measured

^a Flavor threshold in American-style beer (17).

concentration with the expected concentration (control level + 10 ppb) (**Table 1**).

Beer Analysis. Nine aldehydes were detected in analyzed beer (**Figure 5**). The resolution of two peaks, representing two geometrical isomers of each aldehyde, was good, except for furfural, where the first peak was clustered with a peak of an uncharacterized compound.

The aldehydes 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, methional, and phenylacetaldehyde are so-called Strecker aldehydes, formed as a result of a reaction between dicarbonyl products of the Amadori pathway and amino acids, having one less carbon atom than the amino acid (1). According to Schieberle and Komarek (8), the increase of Strecker aldehydes and some esters might play a central role in flavor changes during beer aging. The same authors exclude (*E*)-2-nonenal, a degradation product of linoleic acid, as a key contributor to the stale flavor of beer. Other aldehydes related to the autoxidation of linoleic acid are pentanal and hexanal (1). Furfural, a product of the Maillard reaction, is a known heat exposure indicator that does not impact beer flavor due to its high flavor threshold (9).

During long-term storage at elevated temperatures, American-style beers develop a stale flavor (10). Analyzed beer samples

were stored at 30 °C for 4, 8, and 12 weeks. Levels of all aldehydes increased during beer storage compared to the control sample (**Table 2**). Although the increase after 12 weeks at 30 °C was significant (16-fold increase for furfural, 7-fold increase for 2-methylpropanal), none of the analyzed aldehydes exceeded their flavor threshold in beer (11). However, it is probable that additive or synergistic effects take place when aldehydes contribute to the stale flavor of aged beer.

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