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Determination of stale-flavor carbonyl compounds in beer by stir bar sorptive extraction with in-situ derivatization and thermal desorption–gas chromatography–mass spectrometry

N. Ochiai[a,*], K. Sasamoto[a], S. Daishima[a], A.C. Heiden[b], A. Hoffmann[b]

[a]*Application Development Department, Yokogawa Analytical Systems Inc., 2-11-13 Nakacho, Musashino-shi, Tokyo, 180-0006 Japan*
[b]*GERSTEL GmbH and Co. KG, Aktienstrasse 232-234, D-45473 Mülheim an der Ruhr, Germany*

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Abstract

A method for the determination of stale-flavor carbonyl compounds including *E*-2-octenal, *E*-2-nonenal, *E*,*Z*-2,6-nonadienal and *E*,*E*-2,4-decadienal in beer was developed using stir bar sorptive extraction (SBSE) with in-situ derivatization followed by thermal desorption–GC–MS analysis. The derivatization conditions with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine and the SBSE conditions–sampling mode, salt addition, sample volume, polydimethylsiloxane volume (sample/polydimethylsiloxane phase ratio) and extraction time—were examined. The method showed good linearity over the concentration range from 0.1 to 10 ng ml^{-1} for all analytes and the correlation coefficients were higher than 0.9993. The limits of detection ranged from 0.021 to 0.032 ng ml^{-1} for all analytes. The recoveries (98–101%) and precision (RSD 2.4–7.3%) of the method were examined by analyzing beer samples fortified at the 0.5-ng ml^{-1} level. The method was successfully applied to low-level concentration samples.

Keywords: Stir bar sorptive extraction; Extraction methods; Thermal desorption; Beer; Food analysis; Aroma compounds; Carbonyl compounds

1. Introduction

It is generally recognized that the flavor stability of beer is highly dependent on storage temperature, pH, oxygen level and exposure to ultraviolet (UV) light [1–4]. Oxidatively produced unsaturated carbonyl compounds play a major role in the development of stale-flavor in beer. *E*-2-Nonenal has received special attention as the major source of the papery/cardboard stale-flavor in beer [1,4–6]. Although several hypotheses, e.g. *Strecker* degradation of amino acids [7], oxidative degradation of iso-humulones [8], oxidation of fatty acids [3,4] and aldol condensations [9], were reported, the mechanism contributing to the generation of *E*-2-nonenal is still not clear. *E*-2-Octenal, *E*,*Z*-2,6-nonadienal and *E*,*E*-2,4-decadienal were also mentioned as the compounds responsible for stale-flavor of beer [10,11]. These compounds have a low odor threshold. The odor threshold concentrations were reported to be as low as 0.1–0.3 ng ml^{-1} [4,12]. Although the human olfactory organ can detect such low levels, sensory

*Corresponding author. Tel.: +81-422-525-645; fax: +81-422-525-966.
E-mail address: nobuo_ochiai@agilent.com (N. Ochiai).

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analysis is not always reliable because there are large differences in the response, not only between individuals, but also of an individual from day to day [13,14]. To examine the staling process of beer, these stale-flavor compounds have to be determined by not only sensory analysis, but also reliable and highly sensitive instrumental analysis. Usually, it is essential to have extraction or enrichment steps with or without derivatization and clean-up steps before HPLC or GC analyses because of the trace level of analytes and complicated matrices such as beer. There are a variety of extraction or enrichment techniques for the determination of stale-flavor carbonyl compounds or *E*-2-nonenal in beer, e.g. liquid–liquid extraction (LLE) [15–18], low-pressure or steam distillation [5,19], low-pressure distillation followed by purge and trap (P&T) with Tenax TA [10], and solid-phase extraction [4,6,20]. A simple and easy method to decrease the interference caused by the beer matrix is to use derivatization of carbonyl compounds. 2,4-Dinitrophenylhydrazine (2,4-DNPH) [5,15], dansylhydrazine [20] and *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA) [4,17] were used as derivatizing agents. Although several methods for the determination of underivatized carbonyl compounds or *E*-2-nonenal were reported, these methods require extensive separation or enrichment steps [10,16,18–20]. Recently, solid phase microextraction (SPME), which is a simple, solvent-free technique requiring only a small sample volume, was applied to the determination of underivatized *E*-2-nonenal and *E,E*-2,4-decadienal in beer [11]. However, the sensitivity is considered to be low when compared with the traditional techniques. The limit of detection (LOD) obtained by the SPME method could not achieve the odor threshold concentrations. Although several studies indicated that the SPME approach with derivatization is applicable to the determination of carbonyl compounds in air, water, sunflower oil and spirits [21–24], this approach is still not applied to the determination of stale-flavor carbonyl compounds including *E*-2-nonenal in beer.

In 1999, a new extraction technique known as stir bar sorptive extraction (SBSE) using stir bars coated with 50–300 μl of polydimethylsiloxane (PDMS) was developed by Baltussen et al. [25]. The extraction mechanism and advantages are similar to those of SPME, and therefore the sensitivity of the technique, but the enrichment factor is ~100 times higher.

The aim of this paper was to apply SBSE with derivatization combined with GC–MS to determine less than sub-ng ml^{-1} levels of stale-flavor carbonyl compounds including *E*-2-octenal, *E*-2-nonenal, *E,Z*-2,6-nonadienal and *E,E*-2,4-decadienal in beer. After extraction, the analytes were thermally desorbed in a thermal desorption system (auto-sampler) followed by GC–MS analysis.

2. Experimental

2.1. Materials

E-2-Octenal, *E*-2-nonenal, *E,Z*-2,6-nonadienal and *E,E*-2,4-decadienal were purchased from Wako (Osaka, Japan). The stock standard solution containing each carbonyl compound at 1 mg ml^{-1} in 10 ml of ethanol was prepared and kept at −20 °C. The stock standard solution was diluted with ethanol to prepare the working standard solutions. The derivatizing reagent PFBHA (Wako) was dissolved in natural mineral water at 10 mg ml^{-1}. Analytical grade sodium chloride (NaCl) was purchased from Wako. The NaCl was previously heated at 400 °C for 12 h. All beer samples used in this study were Pilsner-type and were obtained in Japan. Beer samples were placed in a refrigerator at −4 °C before use. Accurately weighed amounts of 0.90–6.0-g samples were diluted 10-fold in natural mineral water.

2.2. Apparatus

The stir bars (Twister; the magnetic stirring rod is incorporated in a glass jacket and coated with PDMS) coated with 24 μl of PDMS (length: 10 mm, thickness: 0.5 mm) and 47 μl of PDMS (length: 20 mm, thickness: 0.5 mm) were obtained from GERSTEL (Mülheim an der Ruhr, Germany). With appropriate re-conditioning the stir bars could be used over 30 times (see Section 2.4). For the extractions, 12-, 22-, 32- and 62-ml headspace vials with PTFE-coated silicone septa from Agilent Technologies (CA, USA) and GL Sciences (Tokyo,

Japan) were used. SBSE was performed using a multiple position magnetic stirrer (20 positions) from Shibata Scientific Technology (Tokyo, Japan). The thermal desorption (TD)–GC–MS analysis was performed using a GERSTEL TDS 2 thermodesorption system equipped with a GERSTEL TDS-A autosampler and GERSTEL CIS 4 programmable temperature vaporization (PTV) inlet (GERSTEL) and an Agilent 6890N gas chromatograph with a 5973N mass-selective detector (Agilent Technologies).

2.3. Sample preparation

Prior to use, the stir bars were conditioned for 1 h at 300 °C in a flow of helium. For liquid sampling SBSE, 10–60 ml of diluted samples were placed in headspace vials (12–62 ml) and 0.15–0.90 ml of PFBHA solutions were added. Stir bars were added and then the vials were crimped with PTFE-coated silicone septa. SBSE with derivatization of diluted samples was simultaneously performed at room temperature (24 °C) for 5–180 min while stirring at 1000 rpm. For headspace sampling (headspace sorptive extraction, HSSE) [26], a small hole was drilled in a septum, enclosing and fixing a homemade holder with a stir bar (PDMS). After inserting 9–45 ml of diluted samples, 0.14–0.68 ml of PFBHA solution and PTFE stir bar (for sample agitation), the vials (12–62 ml) were crimped with the septum including the stir bar (PDMS). HSSE with derivatization was performed for 60 min at 24 °C while agitating the samples by stirring at 1000 rpm. NaCl (15%, m/v) was added to evaluate the effect of salt addition on SBSE and HSSE. When adding NaCl before extraction, an ultrasonic bath was used for dissolution of the NaCl before extraction because shaking by hand or with a shaker will cause the stir bar to break or drop into the liquid phase in HSSE.

2.4. TD–GC–MS

After extraction, the stir bars were easily removed with a forceps, dried with a lint-free tissue and placed in a glass thermal desorption tube. The thermal desorption tube was then placed in the thermal desorption unit, where the stir bar was thermally desorbed by programming the TDS 2 from 20 °C (held for 1 min) to 250 °C (held for 5 min) at 60 °C min^{-1}. The desorbed compounds were cryofocussed in the CIS 4 at −50 °C. After desorption, the CIS 4 was programmed from −50 to 250 °C (held for 5 min) at 12 °C s^{-1} to inject the trapped compounds onto the analytical column. Injection was performed in the splitless mode and the split valve was closed for 3 min. The separations were carried out on a HP-5ms fused-silica capillary column (30 m×0.25 mm I.D., 0.25-μm film thickness, Agilent Technologies). The oven temperature was programmed from 40 °C (held for 3 min) to 280 °C (held for 5 min) at 10 °C min^{-1}. Helium was used as the carrier gas at a flow rate of 1 ml min^{-1}. The mass spectrometer was operated in the scan and the selected ion monitoring (SIM) mode with electron ionization (ionization voltage: 70 V). The scan was set from m/z 29 to 400 in 0.45 s. For SIM, eight ions were monitored (m/z 181, 250, 321 for *E*-2-octenal, m/z 181, 250, 335 for *E*-2-nonenal, m/z 181, 264, 265 for *E*,*Z*-2,6-nonadienal, and m/z 181, 276, 347 for *E*,*E*-2,4-decadienal: the underlined number is the m/z of the ion used for determination). Reconditioning of stir bars was done after use by soaking in Milli-Q purified water and a mixture of methylene chloride–methanol (1:1) for 2–4 h; the stir bars were then removed from the solvent and dried on a clean surface at room temperature for 2–4 h. Finally, the stir bars were thermally conditioned for 1 h at 300 °C in a flow of helium.

3. Results and discussion

3.1. Derivatization

When derivatization is used in combination with SBSE, there are two approaches as in the case of SPME [27]. One is in-situ derivatization [28], and the other is on-stir bar derivatization [29]. The former is analogous to well-established approaches used in solvent extraction. The latter is simultaneous derivatization and extraction, performed directly in the PDMS coating. Since the derivatization of carbonyl compounds with PFBHA can be performed in aqueous phase, SBSE with in-situ derivatization and SBSE with on-stir bar derivatization were compared for the extraction efficiency. A 10-ml volume of the fortified sample (10 ng ml^{-1} for all analytes) and a

stir bar coated with 24 μl PDMS were used. For the on-stir bar derivatization, stir bars were stirred for 60 min in PFBHA solution before extraction. Because high temperatures can promote the additional formation of stale-flavor carbonyl compounds in beer [1,5], five extraction times between 15 and 180 min were examined at low temperature (24 °C), and duplicate analyses were performed at each extraction time. Fig. 1 shows typical mass spectra of PFBHA derivatives of *E*-2-octenal, *E,Z*-2,6-nonadienal, *E*-2-nonenal and *E,E*-2,4-decadienal obtained by SBSE with in-situ derivatization. Fig. 2 shows the extraction efficiency of stale-flavor carbonyl compounds as a function of extraction time. Since two geometrical isomers of the PFBHA derivatives were formed, the sum of the isomer peak area for each analyte was taken. For all analytes, the responses obtained by the two derivatization modes were similar between 5 and 60 min, and the time needed to reach extraction equilibrium was 60 min for on-stir bar derivatization and 120 min for in-situ derivatization. The responses obtained after extraction equilibrium (120 min) by in-situ derivatization were, however, higher at 1.3–1.5, 1.3–1.4, 1.1–1.2 and 1.1–1.2 times larger for *E*-2-octenal, *E,Z*-2,6-nonadienal, *E*-2-nonenal and *E,E*-2,4-decadienal, respectively. This is due to the conversion of carbonyl compounds into oximes by the derivatization with PFBHA. This substantially increases the affinity of the compound to the PDMS, as indicated by an increase in the octanol–water distribution coefficient ($k_{o/w}$). The distribution coefficients of the analytes between water and PDMS are correlated with their $k_{o/w}$ [30–32]. Log $k_{o/w}$ for *E*-2-octenal, *E*-2-nonenal, *E,Z*-2,6-nonadienal and *E,E*-2,4-decadienal are calculated to be in the range 2.57–3.33, and log $k_{o/w}$ for their oximes are calculated to be in the range 5.36–6.13 [33]. Consequently, extraction of larger amounts can be predicted for their oximes. Extraction amounts of their oximes at full equilibration with this sample/PDMS phase ratio ($\beta = 417$) are calculated to be 2.1, 1.6, 1.4 and 1.2 times larger for *E*-2-octenal, *E,Z*-2,6-nonadienal, *E*-2-nonenal and *E,E*-2,4-decadienal, respectively [25]. However, derivatization and extraction steps for in-situ derivatization were simultaneously performed for time saving. According to Ojala et al. [17], the reaction time needed to complete the derivatization of carbonyl compounds with PFBHA before the extraction step is 5 h in the case of beer samples. The extraction amounts obtained by the in-situ derivatization were, therefore, smaller than that of their oximes because carbonyl compounds were extracted not only as their oximes, but also as underivatives, and the underivatives were then derivatized in the PDMS.

Because of the larger extraction amount for all

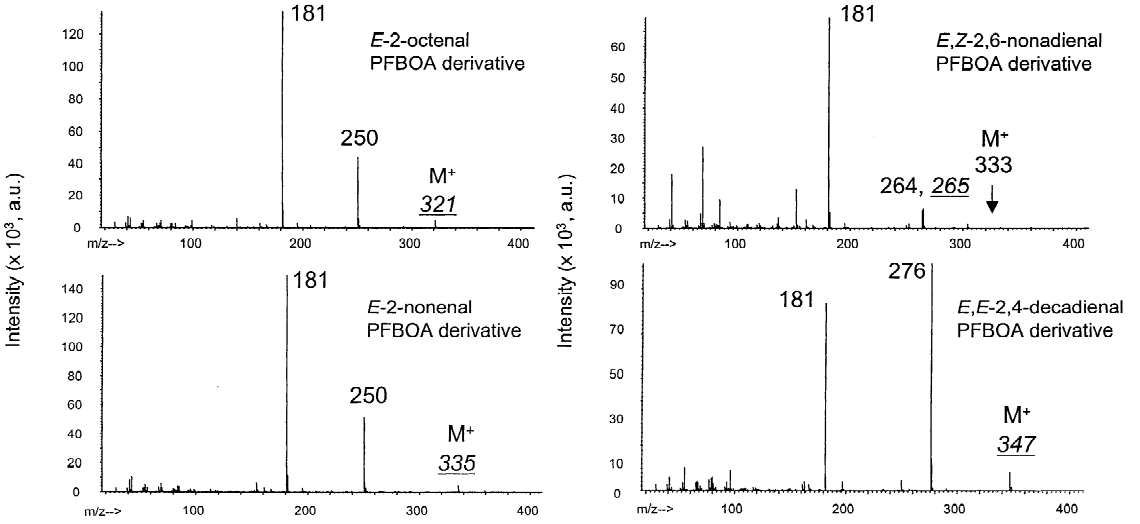

Fig. 1. Mass spectra of the PFBHA derivative of *E*-2-octenal, *E*-2-nonenal, *E,Z*-2,6-nonadienal and *E,E*-2,4-decadienal. The numbers in italic and underlined were the *m/z* of the ions used for determination.

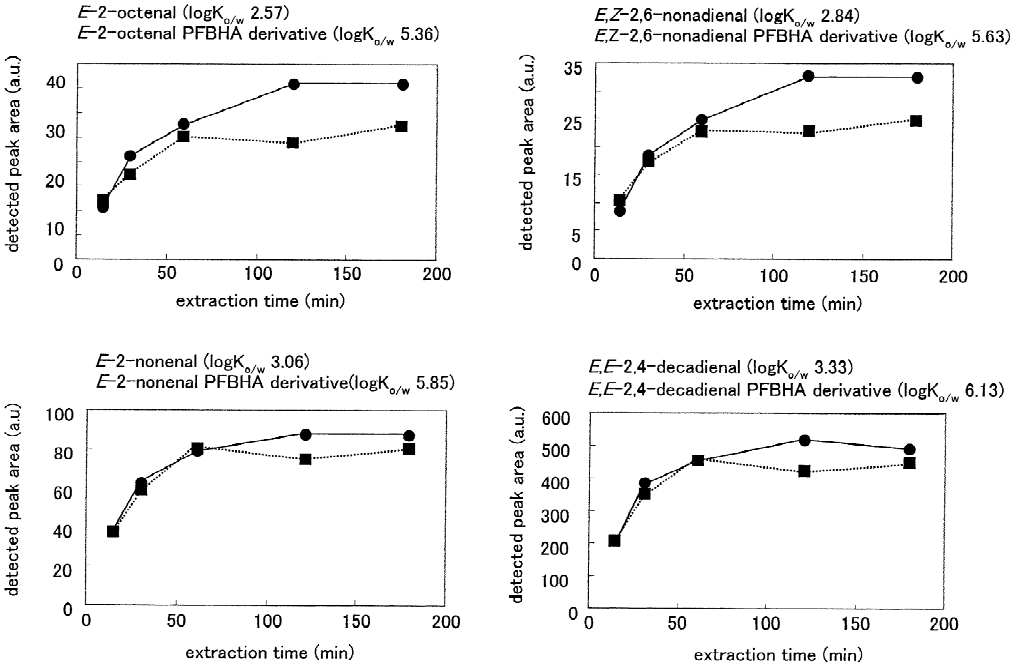

Fig. 2. Comparison of the derivatization approaches for stale-flavor carbonyl compounds in fortified samples by SBSE with in-situ derivatization and SBSE with on-stir bar derivatization. ●, SBSE with in-situ derivatization; ■, SBSE with on-stir bar derivatization. For the on-stir bar derivatization, the stir bar was stirred for 60 min in PFBHA solution before extraction.

analytes at extraction equilibrium and the simplicity of sample preparation, in-situ derivatization with SBSE was selected as the derivatization mode for further experiments.

3.2. Optimization of extraction conditions

To optimize extraction conditions for SBSE with in-situ derivatization, sampling mode, salt addition, sample volume, PDMS volume (sample/PDMS phase ratio) and extraction time were examined. To minimize the additional formation of stale-flavor carbonyl compounds, an extraction temperature of 24 °C was used. Because the LODs by liquid and headspace SPME with in-situ derivatization using PFBHA for the determination of carbonyl compounds in water were similar [22], the extraction modes were first examined with parameters, such as sample volume and salt addition. Fortified samples (10 ng ml^{-1} for all analytes) and a stir bar coated with 24 μl PDMS were used. Each analysis was carried out in duplicate. The extractions were performed for 60 min. NaCl (15%, m/v) was added to study the effect of salt addition. The effect of sample volume on SBSE was investigated as follows: 10 ml of sample was placed in a 12-ml vial, 20 ml in a 22-ml vial, 30 ml in a 32-ml vial and 60 ml in a 62-ml vial. The effect of sample volume on HSSE was evaluated by using a constant percentage headspace (~27–28%), but with different vials of 12, 22, 32 and 62 ml. In this case, sample volume was 9, 16, 23 and 45 ml, respectively. Table 1 shows the typical results (values are relative peak areas normalized by the peak area obtained for SBSE using a 10-ml sample with no salt addition, the sum of the isomer peak area for each analyte was taken) Because the responses obtained by SBSE with 15% salt addition using a 10-ml sample was much lower, being 0.16–0.67 times those obtained with no salt addition for all analytes, salt addition was not examined for 20–60-ml samples for SBSE. The responses obtained by HSSE with 15% salt addition were 1.1–2.6 times larger than those obtained with no salt addition for *E*-2-octenal and *E,Z*-2,6-nonadienal, however, for

Table 1
Comparison of the responses of stale-flavor carbonyl compounds in beer by SBSE and HSSE with in-situ derivatization

Compound	SBSE					HSSE							
	Sample volume (ml)					Vial volume (ml)							
	10	10[a]	20	30	60	12	12[a]	22	22[a]	32	32[a]	62	62[a]
E-2-Octenal	1.0	0.67	3.4	5.2	6.7	2.3	2.6	1.4	2.3	1.8	3.7	2.9	3.6
E,Z-2,6-Nonadienal	1.0	0.60	3.6	5.3	6.9	1.0	1.5	0.53	1.1	0.72	1.9	1.3	1.6
E-2-Nonenal	1.0	0.27	4.5	7.8	13	2.5	1.8	1.7	1.5	2.2	2.5	4.3	2.3
E,E-2,4-Decadienal	1.0	0.16	4.3	8.7	14	0.61	0.55	0.42	0.36	0.41	0.65	1.1	0.48

Values are relative peak areas normalized by the peak area obtained for SBSE using a 10-ml sample without salt addition. A constant percentage (~27%) of headspace was used for HSSE.
[a] NaCl was added to furnish 15% (m/v) solutions.

E-2-nonenal and *E,E*-2,4-decadienal, the responses were 0.4–0.9 times larger (except for the 32-ml vial). For all analytes in SBSE, the responses significantly increased when sample volume increased from 10 to 60 ml. Although higher responses were obtained by the largest sample, this was not the case in HSSE. For all analytes, the highest responses were obtained by SBSE using a 60-ml sample: the responses were 6.7–14 times larger than those obtained for 10-ml samples with no salt addition for all analytes. Because of the highest sensitivity and the simplicity of sample preparation, SBSE was selected as the extraction mode for further experiments.

The effect of sample and PDMS volume (sample/PDMS phase ratio) and the extraction time profile (equilibration curve) were determined by use of 30- and 60-ml fortified samples (10 ng ml^{-1} for all analytes) and a stir bar coated with 24 and 47 μl PDMS. Each analysis was carried out in duplicate. Seven extraction times between 5 and 180 min were examined. Fig. 3 shows the results (the sum of the isomer peak area for each analyte was taken). For all analytes, although the extraction equilibrium was reached at 120 min for 10-ml sample with 24 μl PDMS (sample/PDMS phase ratio; $\beta = 417$) (see Section 3.1; Fig. 2), the equilibrium was not reached within 180 min for 30- or 60-ml sample with 24 or 47 μl PDMS. As previous studies indicated, this is due to the increase of β-values in the range 638–2500. The time needed to reach the equilibrium depends on not only $\log k_{o/w}$ but also on the phase ratio [34,35]. For all analytes, the highest responses were obtained by 60-ml sample with 47 μl PDMS ($\beta = 1276$) at all extraction times, and the second highest responses were obtained by 30-ml sample with 47 μl PDMS ($\beta = 638$) at all extraction times (except for *E*-2-nonenal and *E,E*-2,4-decadienal in 180 min). The responses obtained by 30-ml sample with 47 μl PDMS, however, were similar to those of 60-ml sample with 47 μl PDMS for *E*-2-octenal and *E,Z*-2,6-nonadienal in 5–60 min (0.93–1.0 times).

To obtain maximum sensitivity with this method, an extraction time greater than 180 min using 60-ml sample with 47 μl PDMS is required. Although several samples can be extracted in parallel using SBSE, the time and sample throughput should be considered in the selection of the optimum extraction time. In practice, full equilibration is not essential for an accurate determination. A timed stirring period can be used for calibration, as is the case with SPME [27]. However, a relatively short extraction time will not only result in a loss of sensitivity but also of precision [27]. Consequently, a 60-min extraction time using 30- or 60-ml sample with 47 μl PDMS can be selected as optimum extraction condition for time saving and precision. Although the responses of the 30-ml sample were 0.80–0.94 times those of the 60-ml sample for all analytes, the equilibration curve could be nearer to the equilibrium at 60 min because of the lower β-value. A 60-min extraction time using a 30-ml sample with 47 μl PDMS, therefore, was selected for further experiments.

3.3. Method validation and determination of stale-flavor carbonyl compounds in beer

As previous studies indicated, the effect of the sample matrix in SBSE could be compensated by use

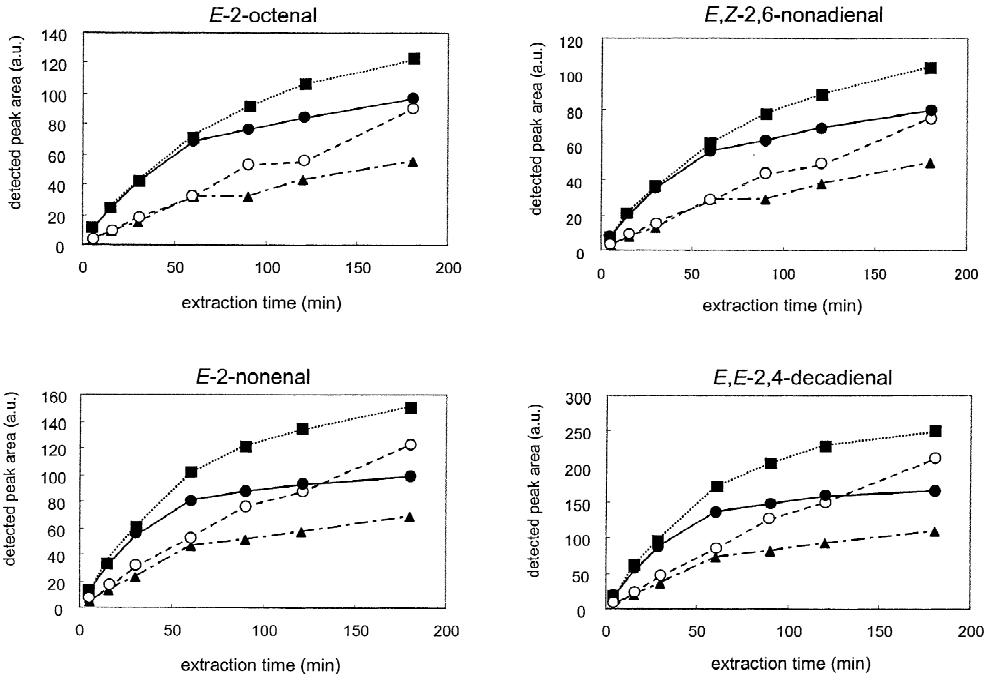

Fig. 3. Extraction time profiles and the effect of sample and PDMS volume (sample/PDMS phase ratio) for the stale-flavor carbonyl compounds in fortified sample by SBSE with in-situ derivatization. ▲, 30-ml sample with 24 μl PDMS (β=1250); ●, 30-ml sample with 47 μl PDMS (β=638); ○, 60-ml sample with 24 μl PDMS (β=2500); ■, 60-ml sample with 47 μl PDMS (β=1276).

of the standard addition calibration method or internal standard method [28,36]. In this study, the standard addition calibration method was used. For both calibration and determination, the sum of the isomer peak area for each analyte was taken. To validate the method, the linearity was firstly examined by analyzing fortified samples. The five data points for the standard addition calibration graphs were linear over the range 0.10–10 ng ml^{-1} with correlation coefficients better than 0.9993. The LOD was determined as three times the standard deviation (for six replicates) for an analyte concentration no higher than ten times the LOD [27]. The LOD were calculated to be 0.021–0.032 ng ml^{-1} by repeated analysis (n=6) of fortified sample spiked at 0.10 ng ml^{-1} (lowest concentration of the calibration graphs). The recoveries of the method were assessed by replicate analysis (n=6) of fortified samples spiked at the 0.50-ng ml^{-1} level. The recoveries were calculated by comparing the results determined with the spiked concentrations. The results showed that recoveries were good for all analytes in the range 98–101% (RSD 2.4–7.3%, n=6). Validation of the method is summarized in Table 2. Fig. 4 shows the SIM chromatograms obtained by use of the proposed method after a 60-min extraction of fortified samples spiked at the 0.50-ng ml^{-1} level.

The proposed method was applied to the determination of stale-flavor carbonyl compounds in normal and forced-aging beer samples. A forced-aging beer sample was maintained at 60 °C for 1 day. This condition is generally equivalent to an aging period of 6 months at 18 °C and 3 weeks at 37 °C [1]. Fig. 5 shows typical SIM chromatograms of a normal beer sample. Although *E*-2-nonenal is usually present at less than 0.10 ng ml^{-1} in beer, well-defined SIM chromatograms of the isomer peaks of PFBHA derivatives were obtained without interference from matrix compounds and could be used for the determination. Three kinds of normal beer samples were found to contain *E*-2-nonenal, and the concentrations were in the range 0.065–0.072 ng ml^{-1} (RSD 5.4–6.4%, n=3). Fig. 6 shows SIM chromatograms of a forced-aging beer sample. A

Table 2
Method validations for the determination of stale-flavor carbonyl compounds in beer by SBSE with in-situ derivatization and TD–GC–MS

Compound	Correlation coefficient (r^2) (0.1–10 ng ml^{-1})[a]	LOD/ng ml^{-1}[b]	Recovery (%)[c]	RSD (%), $n=6$[c]
E-2-Octenal	0.9994	0.021	100	3.7
E,*Z*-2,6-Nonadienal	0.9995	0.032	101	7.3
E-2-Nonenal	0.9993	0.023	99	2.4
E,*E*-2,4-Decadienal	0.9996	0.023	98	3.4

[a] Linear range of the standard addition calibration graph.
[b] The LODs were calculated as three times the standard deviation (3 s) of replicate analyses ($n=6$) of fortified samples spiked at the lowest concentrations of the calibration graph.
[c] The recoveries and precision were also examined by replicate analyses ($n=6$) of fortified samples spiked at 0.5 ng ml^{-1}. For both calibration and determination, the sum of the isomer peak area for each analyte was used.

forced-aging beer sample was found to contain *E*-2-octenal (0.070 ng ml^{-1}, RSD 8.3%, $n=3$), *E*,*Z*-2,6-nonadienal (0.069 ng ml^{-1}, RSD 4.2%, $n=3$) and *E*-2-nonenal (0.44 ng ml^{-1}, RSD 3.0%, $n=3$); *E*-2-nonenal showed a dramatic increase and surpassed its odor threshold concentration of 0.1 ng ml^{-1}.

4. Conclusion

The determination of stale-flavor carbonyl compounds—*E*-2-octenal, *E*,*Z*-2,6-nonadienal, *E*-2-nonenal and *E*,*E*-2,4-decadienal—in beer using SBSE with in-situ derivatization followed by TD–GC–MS was described. In-situ derivatization, liquid sampling (SBSE), a sample volume of 30 ml, a PDMS volume of 47 μl and extraction time of 60 min were selected as optimum extraction conditions. The proposed method has many practical advantages, e.g. small sample volume and the simplicity of extraction. It is also solvent-free and the sensitivity is high. The method can be performed in

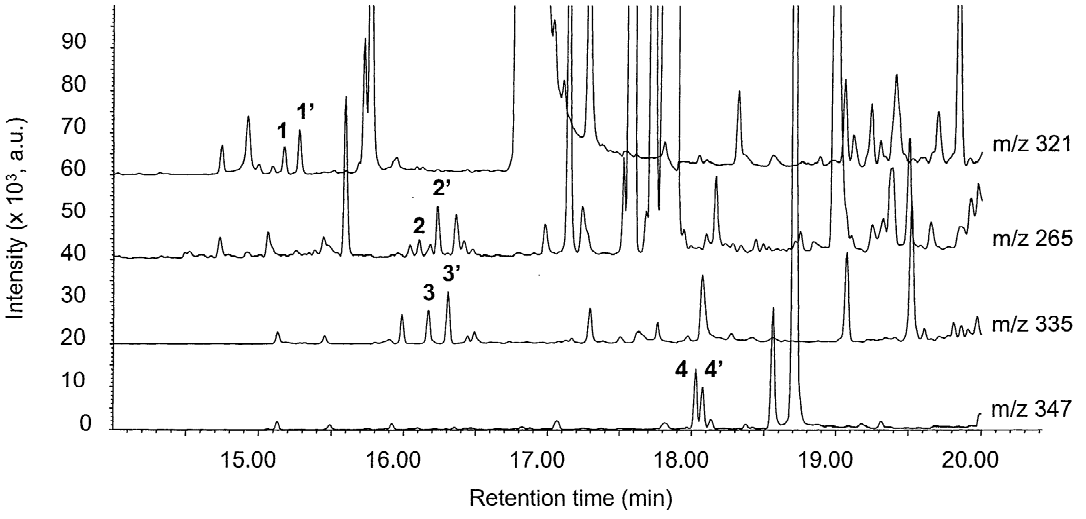

Fig. 4. SIM chromatograms obtained by SBSE with in-situ derivatization and TD–GC–MS of fortified samples spiked at 0.5 ng ml^{-1}. The extraction time was 60 min. 1,1′ = *E*-2-Octenal, 2,2′ = *E*,*Z*-2,6-nonadienal, 3,3′ = *E*-2-nonenal, 4,4′ = *E*,*E*-2,4-decadienal. Two geometrical isomers of the PFBHA derivatives were formed.

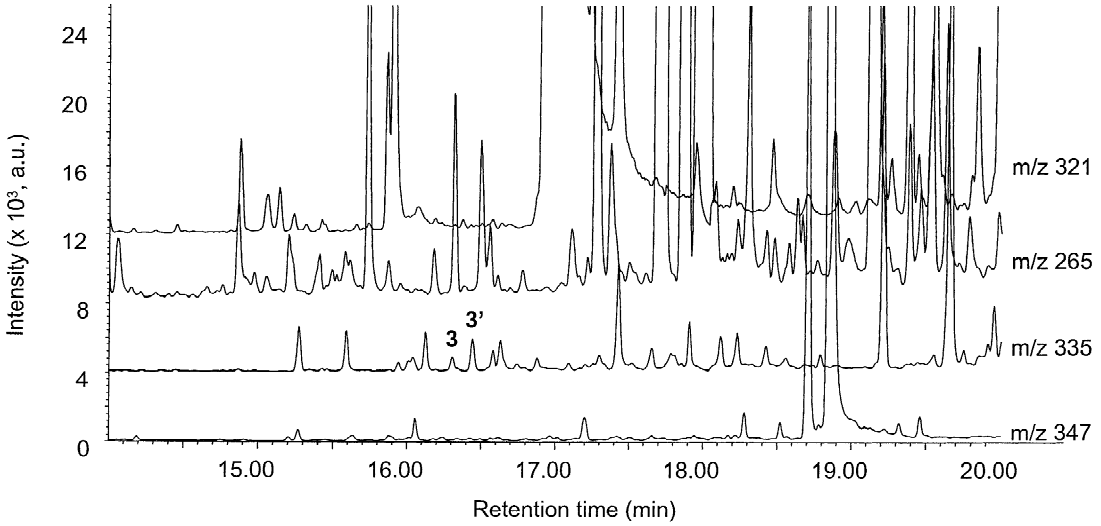

Fig. 5. SIM chromatograms obtained by SBSE with in-situ derivatization and TD–GC–MS of a normal beer sample. The extraction time was 60 min. 3,3′ = *E*-2-Nonenal. Two geometrical isomers of the PFBHA derivatives were formed.

parallel at room temperature. The LODs (0.021–0.032 ng ml^{-1}) were below odor threshold concentrations of *E*-2-octenal and *E*-2-nonenal. The recovery of the method was good (98–101%) and the precision acceptable (RSD 2.4–7.3%) for the beer samples fortified at the 0.5-ng ml^{-1} level. Also,

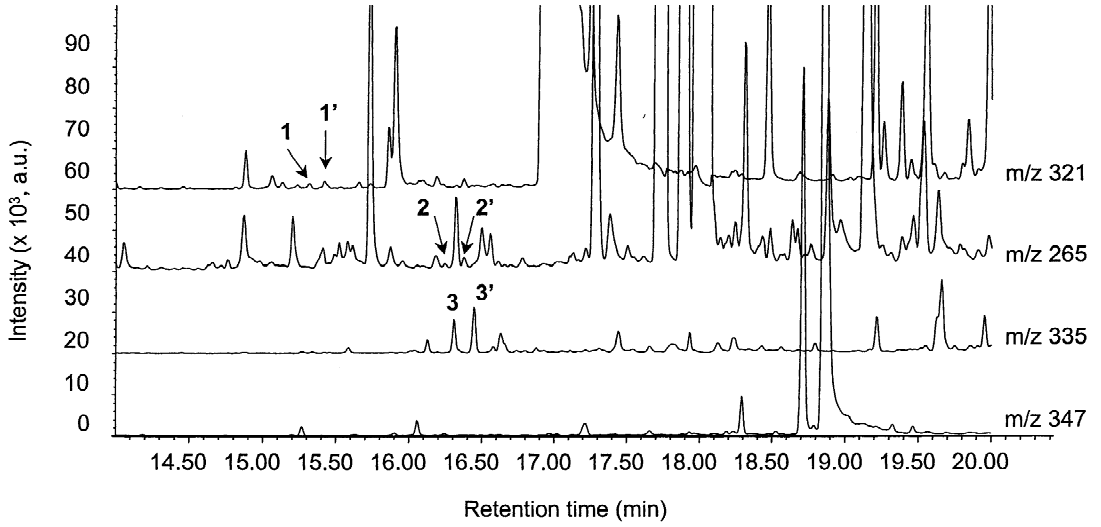

Fig. 6. SIM chromatograms obtained by SBSE with in-situ derivatization and TD–GC–MS of a forced-aging beer sample. A forced-aging beer sample was maintained at 60 °C for 1 day. The extraction time was 60 min. 1,1′ = *E*-2-Octenal, 2,2′ = *E*,*Z*-2,6-nonadienal, 3,3′ = *E*-2-nonenal. Two geometrical isomers of the PFBHA derivatives were formed.

the method allowed the determination of sub-ng ml^{-1} levels (0.065–0.44 ng ml^{-1}) of *E*-2-octenal, *E*,*Z*-2,6-nonadienal and *E*-2-nonenal in normal or forced-aging beer samples with a low RSD (ranging from 3.0 to 8.3%).

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References

[1] K. Greenhoff, R.E. Wheeler, in: Proceedings of the European Brewery Convention Copenhagen, Fachverlag Hans Carl, Nuremberg, Germany, 1981, p. 405.
[2] H. Kaneda, M. Takahio, T. Tamaki, T. Osawa, J. Inst. Brew. 103 (1997) 21.
[3] C.W. Bamforth, MBAA Tech. Q. 37 (2000) 165.
[4] B.W. Drost, R. van den Berg, F.J.M. Freijee, E.G. van der Velde, M. Hollemans, J. Am. Soc. Brew. Chem. 48 (1990) 124.
[5] K. Greenhoff, R.E. Wheeler, J. Inst. Brew. 86 (1981) 35.
[6] D.J. Evans, D.J.M. Schmedding, A. Bruijnje, T. Heideman, B.M. King, N.M. Groebeek, J. Inst. Brew. 105 (1999) 301.
[7] M.T. Walters, J. Inst. Brew. 103 (1997) 111.
[8] N. Hashimoto, T. Eshima, J. Am. Soc. Brew. Chem. 35 (1977) 145.
[9] K. Wackerbauer, R. Hardt, Brauwelt Int. 4 (1997) 320.
[10] K. Harayama, F. Hayase, H. Kato, Biosci. Biotechnol. Biochem. 58 (1994) 1595.
[11] Z. Penton, Chem. NZ 62 (1998) 41.
[12] M. Meilgaard, A. Elizondo, E. Moya, MBAA Tech. Q. 7 (1970) 143.
[13] J.E. Amoore, J. Am. Water Works Assoc. 78 (1986) 70.
[14] P.E. Persson, Water Res. 14 (1980) 1113.
[15] P.S. Wang, K.J. Siebert, MBAA Tech. Q. 11 (1974) 110.
[16] J. Strating, W.M. Westra, L.C. Verhagen, F.Ph. Slotema, MBAA Tech. Q. 16 (1979) 176.
[17] M. Ojala, T. Kotiaho, J. Siirila, M.L. Sihvonen, Talanta 41 (1994) 1297.
[18] S. Sakuma, M. Kowaka, J. Am. Soc. Brew. Chem. 52 (1994) 37.
[19] J.R. Santos, J.R. Carnerio, L.F. Guido, P.J. Almeida, J.A. Rodrigues, A.A. Barros, in: Proceedings of the 25th ISCC, Riva Del Garda, 2002, I.O.P.M.S, Kortrijk, Belgium, 2002, CD-ROM, paper F12.
[20] L.C. Verhagen, J. Strating, U.R. Tjaden, J. Chromatogr. 393 (1987) 85.
[21] P.A. Martos, J. Pawliszyn, Anal. Chem. 70 (1998) 2311.
[22] M. Bao, F. Pantani, O. Griffini, D. Burrini, D. Santianni, K. Barbieri, J. Chromatogr. A 809 (1998) 75.
[23] A. Keszler, K. Heberger, J. Chromatogr. A 845 (1999) 337.
[24] W. Wardencki, J. Orlita, J. Namiesnik, Fresenius J. Anal. Chem. 369 (2001) 661.
[25] E. Baltussen, P. Sandra, F. David, C. Cramers, J. Microcol. Sep. 11 (1999) 737.
[26] B. Tienport, F. David, C. Bicchi, P. Sandra, J. Microcol. Sep. 12 (2000) 577.
[27] J. Pawliszyn, Solid Phase Microextraction Theory and Practice, Wiley–VCH, New York, 1997.
[28] B. Tienport, F. David, K. Desmet, P. Sandra, Anal. Bioanal. Chem. 373 (2002) 46.
[29] F. David, P. Sandra, in: Abstracts of PITTCON 2001, The Pittsburgh Conference, Pittsburgh, PA, 2001, p. 740.
[30] J. Dugay, C. Miege, M.C. Hennion, J. Chromatogr. A 795 (1998) 27.
[31] L.S. DeBruin, P.D. Josephy, J. Pawliszyn, Anal. Chem. 70 (1998) 1986.
[32] J. Beltran, F.J. Lopez, O. Cepria, F. Hernandez, J. Chromatogr. A 808 (1998) 257.
[33] W.M. Meylan, P.H. Howard, J. Pharm. Sci. 84 (1995) 83.
[34] N. Ochiai, K. Sasamoto, M. Takino, S. Yamashita, S. Daishima, A.C. Heiden, A. Hoffmann, Analyst 126 (2001) 1652.
[35] C. Bicchi, C. Cordero, C. Iori, P. Rubiolo, P. Sandra, in: Proceedings of the 25th ISCC, Riva Del Garda, 2002, I.O.P.M.S, Kortrijk, Belgium, 2002, CD-ROM, paper C20.
[36] N. Ochiai, K. Sasamoto, M. Takino, S. Yamashita, S. Daishima, A.C. Heiden, A. Hoffmann, Anal. Bioanal. Chem. 373 (2002) 56.