ELSEVIER

Journal of Chromatography A, 872 (2000) 203–213

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Determination of sulphur compounds in beer using headspace solid-phase microextraction and gas chromatographic analysis with pulsed flame photometric detection

Peter G. Hill[a,*], Roger M. Smith[b]

[a]*Brauerei Beck & Co, Am Deich 18/19, 28199 Bremen, Germany*

[b]*Department of Chemistry, Loughborough University, Loughborough, Leics. LE11 3TU, UK*

Received 15 September 1999; received in revised form 15 December 1999; accepted 15 December 1999

Abstract

A simple and sensitive method for the analysis of volatile and semi-volatile sulphur compounds in beer at trace levels was developed using headspace solid-phase microextraction (SPME) and gas chromatography with pulsed flame photometric detection. Different SPME fibres were tested and a Carboxen–polydimethylsiloxane coated fibre was found to be the most appropriate. The adsorption and desorption conditions were optimised. The effect of ethanol concentration in the sample on the extraction of analytes was examined. A 60 m non-polar capillary column preceded by a 10 m length of a polar column was found to be capable of separating a wide range of C_1–C_6 sulphur compounds. The pulsed flame photometric detector enabled increased sensitivity to be obtained over previous methods, such as dynamic headspace followed by conventional flame photometric detection or sulphur chemiluminescent detection, with high sulphur selectivity. Two sulphur compounds, 2-methyl-1-butanethiol and 3-methylthiophene, were identified in beer for the first time.

Keywords: Beer; Food analysis; Headspace analysis; Extraction methods; Detection, GC; Sulphur compounds

1. Introduction

Although sulphur compounds contribute in a positive way to the aroma and taste of many foodstuffs [1], because of their low sensory thresholds and powerful, often unpleasant characteristics they are frequently the cause of off-flavours and odours. In uncooked foods they are especially important in a wide range of vegetables such as leeks, onions, garlic, broccoli and cabbage [2,3]. In cooked foods, sulphur compounds are often products of the Maillard reaction, a non-enzymatic browning reaction [4,5]. Sulphur compounds formed in this way by the roasting, baking or cooking of food are of great importance in bread, roast beef, coffee and UHT milk.

A wide variety of sulphur compounds have been reported in beer [6], the main volatile sulphur components being dimethyl sulphide (DMS) [7] and methionol [8]. Many other sulphur compounds are only found at trace levels [9–12]. In Germany, the

*Corresponding author.
E-mail address: phill@becks.de (P.G. Hill)

0021-9673/00/$ – see front matter
PII: S0021-9673(99)01307-2

Purity Law of 1516 states that only malt, hops and water may be used in the brewing of beer, and these three ingredients are all possible sources of sulphur compounds [13,14]. However, the majority of the volatile and semi-volatile sulphur compounds do not come directly from the raw materials but are formed during fermentation. Non-volatile sulphur-containing compounds are chemically broken down and converted into more volatile compounds [15–17]. A further, but fortunately rarely found route for the formation of sulphur compounds is through bacterial infection [18–20], which leads to spoilage of the beer.

In view of the importance of sulphur compounds in beer flavour, and their possible impact as off-flavours and odours, a sensitive method of analysis for the routine determination of these substances is required. Many non-chromatographic methods have been used previously [21] but they do not provide the levels of sensitivity and selectivity required. The current method of choice in the brewing industry is dynamic headspace sampling followed by capillary gas chromatography (GC) coupled to flame photometric detection (FPD) or sulphur chemiluminescent detection (SCD) [12,22,23]. In a review in 1988, Peppard [22] noted several disadvantages with dynamic headspace coupled to FPD, including adsorption losses, the introduction of artefacts and signal quenching. Peppard concluded that ''the continued development of new and improved analytical techniques is therefore clearly necessary whilst so many of the questions relating to sulphury flavours in beer remain unanswered''.

This paper reports the development of an improved analytical technique to allow the simple and relatively inexpensive routine analysis of volatile and semi-volatile sulphur compounds in beer. The proposed method examines the use of solid-phase microextraction (SPME) [24], which has been applied in a number of areas of the food and beverage industry [25–30]. Different columns were examined for the optimum separation of the sulphur compounds. The method also examined the use of the relatively new pulsed flame photometric detection (PFPD) system developed by Amirav and co-workers [31–33] for the selective detection of sulphur analytes.

2. Experimental

2.1. Chemicals

The sulphur compounds studied were 2-acetylthiophene (2-AcThPh) [88-15-3], 1-butanethiol (1-BuSH) [109-79-5], carbon disulphide (CS_2) [75-15-0], cyclopentylmercaptan [1679-07-8], diethyl disulphide (DEDS) [111-81-6], diethyl sulphide (DES) [352-93-2], dimethyl disulphide (DMDS) [624-92-0], dimethyl sulphide (DMS) [75-18-3], dimethyl trisulphide (DMTriS) [3658-80-8], dimethyl tetrasulphide (DMTetraS) [5756-24-1], ethylmercaptan (EtSH) [75-08-1], ethylene sulphide (thiirane) [420-12-2], ethyl-3-(methylthio)propionate [13327-56-5], ethyl thioacetate (EtSAc) [625-60-5], methylmercaptan (MeSH) [74-93-1], methional [3268-49-3], methionol [505-10-2], 2-methyl-1-butanethiol (2-MeBuSH) [1878-18-8], 3-methyl-1-butanethiol (3-MeBuSH) [541-31-3], 3-methyl-2-butene-1-thiol (3-MBT) [5287-45-6], 2-methyl-3-furanthiol (MeFuSH) [28588-74-1], methyl-3-(methylthio)propionate [13532-18-8], 1-methyl-1-propanethiol (1-MePrSH) [513-53-1), 2-methyl-2-propanethiol (2-MePrSH) [75-66-1], methyl thioacetate (MeSAc) [1534-08-3], 2-methylthiophene (2-MeThPh) [554-14-3], 3-methylthiophene (3-MeThPh) [616-44-4], 3-(methylthio)propionic acid [646-01-5], 3-(methylthio)propyl acetate (3-MeSPrAc) [16630-55-0], 1-pentanethiol (1-PeSH) [110-66-7], 1-propanethiol (1-PrSH) [107-03-9], 2-propanethiol (2-PrSH) [75-33-2]. Ethyl methyl sulphide (EMS) [624-89-5], 1-hexylmercaptan (HexSH) [111-31-9] and 1-propyl thioacetate (PrSAc) [2307-10-0] were used as internal standards. DMTriS, DMTetraS and 3-MeSPrAC were supplied by Oxford Chemicals (Hartlepool, UK). DEDS, ethyl-3-(methylthio)-propionate, EtSAc, HexSH, MeSAc, 3-(methylthio)-propionic acid and 1-PrSAc were supplied by Lancaster Synthesis (Mülheim am Main, Germany). 2-AcThPh, CS_2, DES, DMDS, DMS, EtSH and MeSH were supplied by Fluka (Buchs, Switzerland). The other sulphur compounds, with the exception of 3-MBT, were supplied by Aldrich (Steinheim, Germany). 3-MBT was synthesised by Newchem (Parkton, MD, USA). All compounds were obtained in the

highest grade purity available and were stored at 0°C to prevent decomposition.

Beer samples were obtained from three German breweries.

2.2. Equipment

2.2.1. SPME

The SPME fibres 7 μm polydimethylsiloxane (PDMS), 100 μm PDMS, 85 μm polyacrylate, 65 μm Carbowax–divinylbenzene (DVB) and 75 μm Carboxen–PDMS were purchased from Supelco (Bellefonte, PA, USA). Before use the fibres were conditioned by inserting them into a GC injector at the following temperatures: 7 μm PDMS, 320°C, 4 h; 100 μm PDMS, 250°C, 1 h; 85 μm polyacrylate, 300°C, 2 h; 65 μm Carbowax–DVB, 250°C, 30 min; 75 μm Carboxen–PDMS, 280°C, 30 min.

2.2.2. Chromatography

The analyses were carried out using a Varian 3800 gas chromatograph fitted with a Varian 8200 CX Autosampler with SPME III agitation modifications and heated carousel (Varian, Darmstadt, Germany). The chromatograph was equipped with a Varian 1079 split/splitless injector and a Varian pulsed flame photometric detector operated in the sulphur mode. A specially-designed 0.8 mm SPME injector liner (Supelco) was used to prevent peak broadening. The injections were carried out in the splitless mode at 250°C, the split being turned on after 0.8 min. The detector temperature was 210°C, the detector voltage 600 V, detector gate width 20 ms, the detector gate delay 6 ms and the detector trigger 200 mV. The gas flows to the detector were 10.3 ml/min of hydrogen, 16.9 ml/min of air1 and 9.8 ml/min of air2. The detector signals were evaluated using a Varian Star Workstation operated in the square root of peak height mode to compensate for the quadratic response of the pulsed flame photometric detector.

A number of columns were examined, VA-1 column [100% dimethylpolysiloxane (DMPS)] (60 m×0.25 mm, 0.5 μm) (Varian), DB-Wax column [polyethylene glycol (PEG)] (10 m×0.25 mm, 0.5 μm) (J&W Scientific, Folsom, CA, USA), OV-1701, 14% cyanopropyl-phenyl, 86% PDMS, 50 m×0.20 mm, 0.5 μm, (CS Chemie, Langerwehe, Germany); DB-Wax, PEG, 30 m×0.25 mm, 0.5 μm, (J&W Scientific); Optima δ-3, polysiloxane, 30 m×0.25 mm, 0.25 μm, (Macherey–Nagel, Düren, Germany).

2.3. Analytical method

2.3.1. Sample preparation of beer

The samples of cans or bottles of beer were cooled to 0°C to minimise the loss of very volatile compounds. The container was opened and 9 ml of beer sample was pipetted into a 15-ml glass vial. A 1-ml volume of water–ethanol (95:5) internal standard solution containing EMS and 1-PrSAc was added, giving final concentrations of 5.0 μg/l and 2.5 μg/l, respectively. The vials were tightly sealed with crimp caps and 20-mm Black Viton septa (Supelco).

2.3.2. Method of extraction and separation

During adsorption the beer samples were warmed to 45°C and the fibre agitated. The fibres were exposed to the headspace of the sample for 32 min and desorbed in the GC injector at a temperature of 250°C for 3 min. Separation of the volatile and non-volatile compounds was achieved using a combined polar/non-polar capillary column. A non-polar VA-1 column (100% DMPS) (60 m×0.25 mm, 0.5 μm) (Varian) was preceded by a short piece of DB-Wax column (PEG) (10 m×0.25 mm, 0.5 μm) (J&W Scientific). Hydrogen was used as the carrier gas and kept at a constant flow-rate of 2.7 ml/min. The oven program was as follows: 7 min at 32°C, increased to 110°C at 7°C/min, increased to 190°C at 11°C/min, increased to 235°C at 22°C/min and held for 6 min.

3. Results and discussion

The ‘‘new and improved analytical techniques’’ demanded by Peppard [22] for the analysis of sulphur compounds in beer require a detector more sensitive and less susceptible to quenching than the flame photometric detector. Our initial work examined the sulphur chemiluminescence detector, but found it to be very unstable, providing sensitive sulphur-specific detection for only one or two analy-

ses before a marked loss in sensitivity was seen. Mass spectrometric detectors are insufficiently sensitive to allow the analysis of many sulphur compounds at the very low levels in which they are found in beer. First impressions of atomic emission detection were favourable, but its high cost ruled it out. One alternative appeared to be the pulsed flame photometric detector.

3.1. Sulphur detection with PFPD

The pulsed flame photometric detector uses a flame source and gas rates that cannot sustain a continuous flame. The sample is combusted by a propagated ignited flame, a pulse of light is seen and the flame self-terminates. This cycle is repeated 2–4 times a second. Selectivity is provided by the appropriate filter and the added dimension of time. Hydrocarbon emission is faster than that of heteroatomic species, which allows separation in time of the sulphur and hydrocarbon emission signals. This not only increases selectivity but also provides higher sensitivity owing to the reduction of flame background signal. The sulphur response of the pulsed flame photometric detector is claimed to be purely quadratic and equimolar [32], i.e., the sulphur response is independent of the structure of the sulphur-containing molecule.

PFPD proved to be a very sensitive sulphur-specific detection method. Sulphur sensitivity was found to be optimal at a relatively low operating temperature of 210°C. The sensitivity of the optimised detector was experimentally determined at 0.7 pg sulphur/s. The claim that the detector response is equimolar was checked using a solution of four sulphur compounds (dimethyl sulphide, ethyl methyl sulphide, methyl thioacetate and ethyl thioacetate) in isooctane, the solution being so prepared that each of the four compounds contained exactly the same mass of sulphur. This experiment was repeated a total of 13 times, with relative standard deviations in the sulphur concentration between the four peaks lying between 1.99% and 5.12%. The average relative standard deviation over the 13 runs was 3.50%, confirming that the PFPD response can be considered to be equimolar.

3.2. SPME

Despite the sensitivity of the pulsed flame photometric detector, some form of sample enrichment was required to allow analysis of sulphur compounds at trace levels. SPME was assessed as a possible technique for this purpose. Headspace SPME sampling was employed to avoid heavier, non-volatile molecules being adsorbed and subsequently ''baked'' onto the fibre in the injector as that would decrease the lifetime of the fibre and possibly lead to artefact formation, which would falsify the results [34]. Another advantage of headspace SPME is that equilibrium is reached much more quickly than with direct sampling SPME. Theoretically headspace SPME can not be as sensitive as direct SPME owing to the presence of the sample headspace [24,35], but in practice high sensitivity with headspace SPME can be obtained by keeping the sample headspace volume to an absolute minimum. This was achieved by filling 10 ml of matrix (9 ml sample plus 1 ml internal standard) into a 15-ml glass vial, leaving just enough headspace for the fibre to be inserted.

The selectivity and yield from five different fibres were studied using identical conditions. After extraction and desorption the intensity of the signals of the sulphur compounds seen was compared. From the results it was seen that the most effective fibre for the extraction of sulphur compounds was the 75 μm Carboxen–PDMS fibre. This fibre has not been widely used in the analysis of flavours and aromas and only a few applications have been published [36,37]. However, two authors reported that although the 75 μm Carboxen–PDMS fibre provided efficient sample enrichment, it displayed poorer repeatability than other SPME fibres [37,38].

The following SPME parameters were optimised: time of extraction, extraction temperature, sample agitation, sample to headspace ratio.

The time of extraction using the Carboxen–PDMS fibre was optimised by exposure to six identical beer samples for differing periods of time (5, 10, 20, 30 and 40 min). The changes in the areas of the individual peaks were plotted against time. Using the extraction time profiles (Fig. 1), the optimum adsorption time was determined as being 32 min. The peaks were identified by their retention times.

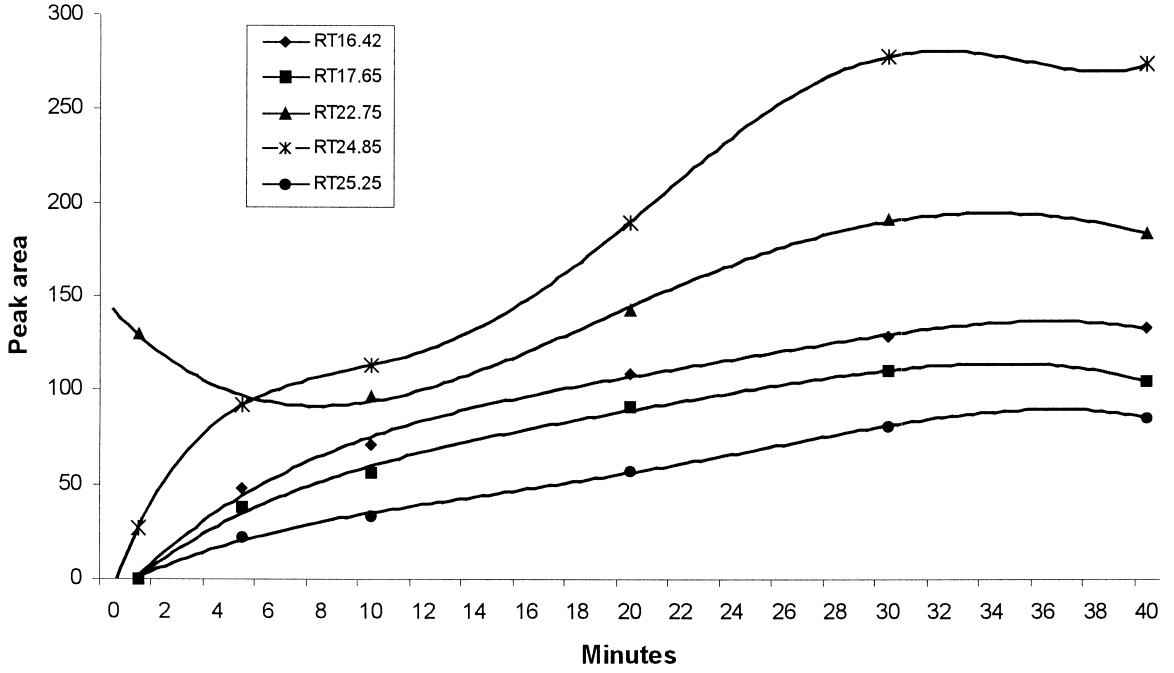

Fig. 1. SPME extraction profiles for several sulphur compounds (identified by retention time). SPME–GC–PFPD conditions as in the Experimental section.

The influence of temperature on the extraction of sulphur compounds was assessed by using a heatable sample carousel accessory. The extraction at 45°C was approximately twice as efficient as at ambient room temperature. The effects of higher temperatures were not studied: at temperatures in excess of 45°C the probability of artefacts being produced through the Maillard reaction is too high [39]. Therefore the beer samples were extracted at 45°C to provide better sample enrichment.

The autosampler is additionally capable of agitating the sample during extraction. Tests showed that the differences in sensitivity between agitated and non-agitated samples were very small, the extraction with agitation providing a very slight sensitivity advantage near the limit of detection. This is important, however, as even very minor sulphur compounds can have a significant impact on the sensory quality of the beer and are therefore diagnostically of interest. Therefore the SPME fibre was continually agitated during the extraction of beer samples.

Finally, matrix effects were examined. As SPME was originally developed for the analysis of organic compounds in purely aqueous samples, the problem of co-solvent matrix effects did not arise. The only mention in the early literature on SPME was in the 1992 publication by Aurthur et al. [40], where the effects of methanol in the aqueous sample were briefly discussed, with the conclusion that matrix effects with less than 1% of methanol were insignificant. Reports that 20% methanol in aqueous solution reduced the peak sizes of pesticides after SPME [41,42] led Urruty and Montury [43] to investigate the effects of ethanol on the same systems. They found that the variations in the ethanol concentration of the aqueous solutions had no influence on the equilibration time the system but had a great effect on the amount of analyte extracted. Various recent investigations on the application of SPME for the analysis of wine [28,37,44–46] have confirmed these findings. The reason for the decrease of organic analytes extracted on increasing ethanol concentration is not clear: Urruty and Montury conclude that ethanol acts as a co-solvent [43],

retaining the analytes in solution, whereas Mestres and co-workers [37,46] claim that competition for adsorption onto the fibre is responsible. Nedjma and Maujean [47] witnessed the same negative effect of ethanol on the amount of sulphur compounds extracted from brandy using static headspace, suggesting that ethanol indeed acts as a co-solvent.

The effect of the sample matrix on the extraction of sulphur compounds from beer was investigated by adding two standards – ethyl methyl sulphide (EMS) and propyl methyl thioacetate (PrSAc) – to the following matrices: water; water+5% ethanol (EtOH); non-alcoholic beer (NAB); NAB+5% EtOH; European Pilsener beer (5% EtOH). NAB is normal Pilsener beer which has been subjected to vacuum distillation to remove ethanol. During this process other volatile compounds are also removed from the beer; the non-volatile components remain unchanged. The final concentrations of the standards in the samples were 5 μg/l EMS and 2.5 μg/l PrSAc.

From the results in Table 1 it can be seen that the ethanol concentration has a great effect on the amount of sulphur compounds extracted by SPME. In addition, when the differences in the peak areas of the two standards between non-alcoholic beer with 5% ethanol added and European Pilsener beer, which contains 5% ethanol, are studied, it becomes clear that other matrix effects apart from the ethanol effect also play an important part in retaining sulphur compounds in the matrix. The extraction of PrSAc by SPME is influenced by the ethanol content to a much lesser extent than that of EMS. These effects can be compensated for by the use of internal standards. They should be taken into account during calibration.

Table 1
Peak areas of two sulphur compound standards in various matrices[a]

Matrix	Peak area (counts)/%			
	EMS		PrSAc	
	Peak area	%	Peak area	%
Water	5 758 400	100	1 867 863	100
Water+5% EtOH	3 649 140	63	1 028 349	55
NAB	5 094 138	88	1 222 000	65
NAB+5% EtOH	2 959 221	51	1 340 675	71
Beer (5% EtOH)	1 572 160	27	1 217 428	65

[a] SPME–GC–PFPD conditions as in the Experimental section.

3.3. GC separation of the sulphur analytes

SPME is capable of extracting sulphur compounds across a wide range of boiling points and polarities. As a result, the demands on a chromatographic column for the analysis of sulphur compounds in beer are high: the separation of highly volatile compounds must be possible whilst at the same time, the retention capacity should not be too high to prevent the elution of semi-volatile compounds within reasonable analysis times. Thick film columns provide good separation of volatile compounds but retain the less volatile compounds very strongly. Although elution can be forced by intensive heating of the column, this leads to increased column bleeding, a phenomenon to which thick film columns are particularly susceptible.

Several different columns were tested. A non-polar VA-1 column (60 m×0.25 mm, 0.5 μm) provided good separation of volatile components and allowed acceptable retention times for heavier compounds. However, a seemingly polar group of semi-volatile sulphur compounds displayed poor peak form and could not be separated on the column. Consequently, a DB-Wax column (30 m×0.25 mm, 0.5 μm) was tested: the polar group was resolved but the separation of the highly volatile compounds was incomplete. Two further columns, a middle-polarity OV-1701 (50 m×0.20 mm, 0.5 μm) and an Optima δ-3 (30 m×0.25 mm, 0.25 μm) also failed to meet the high chromatographic demands. The solution was to use a VA-1 column (60 m×0.25 mm, 0.5 μm) preceded by a shorter length of polar DB-Wax column (10 m×0.25 mm, 0.5 μm). This combined column allows both resolution of the polar group of compounds and separation of highly volatile compounds. It is important that the shorter piece of polar column precedes the main non-polar column: the placing of the polar column behind the non-polar column gave a poorer separation.

3.4. Identification of sulphur compounds in beer

In comparison to previously-used methods for the analysis of compounds in beer, such as static [10,18]

or dynamic headspace with FPD or SCD, many additional sulphur compounds were seen, especially less volatile sulphur compounds. Previously at room temperature the least volatile compound analysed using static headspace was methyl thioacetate (b.p. 98°C) [10]; with dynamic headspace the least volatile sulphur compound extracted was dimethyl trisulphuide (b.p. 239°C) [11,12,48].

The identification of the individual sulphur compounds in samples of beer using the optimised SPME–GC–PFPD method was difficult, owing to the low concentrations present and the relatively high concentrations of other, non-sulphur compounds. Several different GC–MS systems connected to a variety of sample enrichment techniques were used, but none supplied the required selectivity and sensitivity to identify the sulphur compounds in beer. The only remaining possible method of identification was by retention time. Reference standards of the highest available purity were obtained and analysed using the SPME–GC–PFPD method. The main sulphur compounds in a European Pilsener beer sample were identified in this way (Fig. 2) although some components remain unidentified.

The sensitivity of the SPME–GC–PFPD system allowed two sulphur compounds which had previously been unreported in beer to be detected and identified by comparison with standards. 3-Methylthiophene, which has previously been found in hops [49–51], and 2-methyl-1-butanethiol were both determined in European Pilsener beers in ng/l levels.

A sulphur compound of great interest to the brewing industry is 3-methyl-2-butene-1-thiol (3-

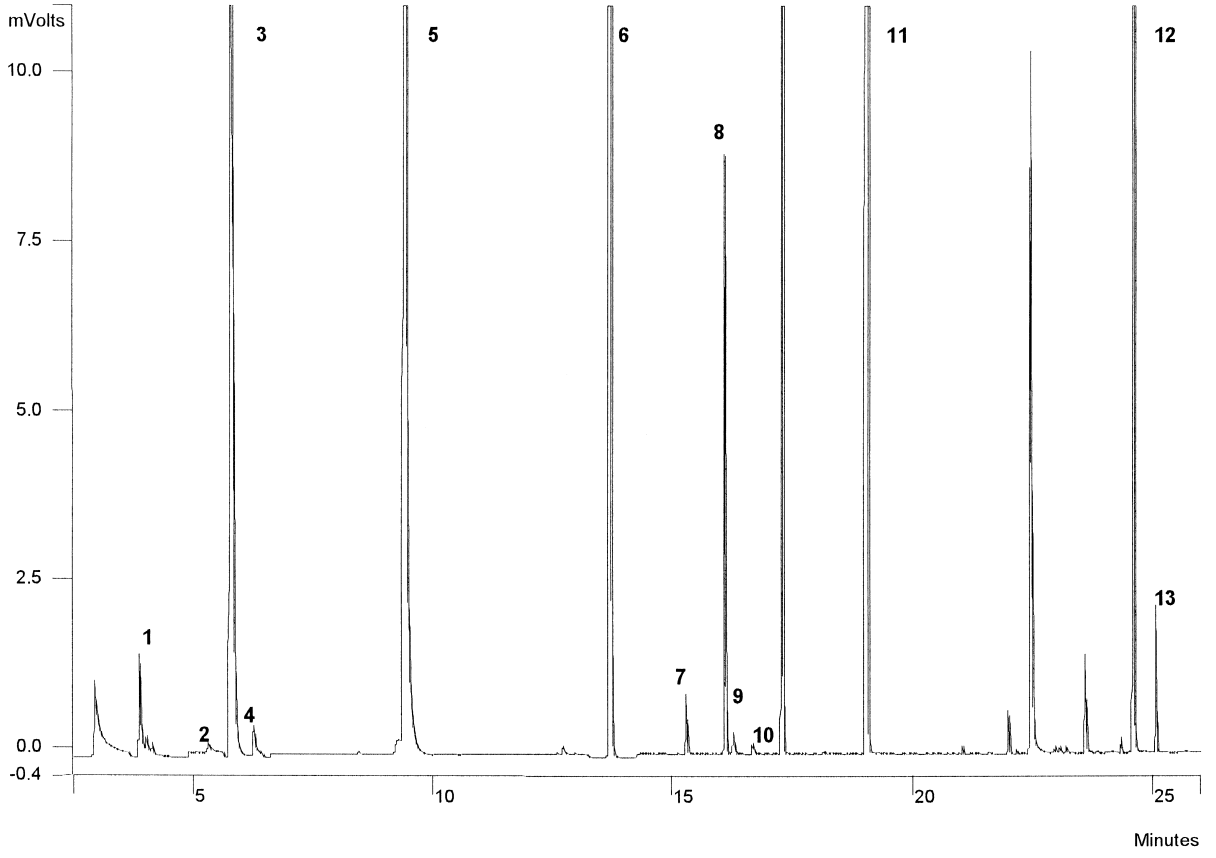

Fig. 2. Beer sample analysed using the SPME–GC–PFPD method described in Section 2. 1=MeSH, 2=EtSH, 3=DMS, 4=CS_2, 5=EMS (internal standard), 6=MeSAc, 7=DMDS, 8=EtSAc, 9=2-MeBuSH, 10=3-MeThPh, 11=PrSAc (internal standard), 12=methionol, 13=3-MeSPrAc. SPME–GC–PFPD conditions as in the Experimental section.

MBT), the so-called ''light mercaptan'' [52]. When beer is subjected to illumination, photolysis of iso-α-acids from the hops and of sulphur-containing amino acids leads to a free radical reaction which results in the formation of 3-MBT [53–55]. Lightstruck beers are often described as ''skunky'' in taste and smell [52]. This is in fact a misnomer, 3-MBT not being found in the anal secretions of the three main species of American skunk [56–59]. The sensory threshold for 3-MBT is considered to lie between 7 ng/l [60] and 1 μg/l [61]. The peak identified as 3-MBT using the SPME–GC–PFPD system could only be determined in illuminated beers.

3.5. Calibration

Following identification of the sulphur compounds the system was calibrated using the reference standards. Two internal standards, EMS (5 μg/l) and PrSAc (2.5 μg/l) were used. They were allotted to the various sulphur compounds according to volatility and functionality. For example, propyl thioacetate was used as the internal standard for methyl thioacetate, ethyl thioacetate and 3-methylthiopropyl acetate on the grounds of similar ester functionality, and for the less volatile sulphur compounds on the grounds of similar volatility. EMS was used as the internal standard for the sulphides and other highly volatile compounds. The equimolar sulphur response of the PFPD could not be used to calibrate all sulphur compounds in the beer samples on the basis of one calibrated compound because of the discriminatory nature of the extraction step by SPME, i.e., although the detector response is dependent solely on the absolute mass of sulphur in the compound and independent of the structure of the sulphur-containing molecule, the efficiency of SPME is structure and volatility dependent.

The standard addition method was employed, the compounds being calibrated using three different concentrations across a range of one-order of magnitude, the expected concentration of each individual compound in beer being used as the basis for the median concentrations of the standard solutions. The calibration was carried out in non-alcoholic beer with 5% added ethanol to compensate for matrix effects – and for volatility and SPME trapping differences. A similar approach to calibration using a ''deodorised'' matrix has already been employed for the SPME–GC analysis of flavour compounds in orange juice [62]. The square root of the height of the peaks was used as the basis for the calculations, to compensate for the quadratic response of the detector. Fig. 3 shows a calibration curve for a sulphur compound (methyl thioacetate) obtained using peak height. Fig. 4 shows a linear calibration curve obtained using the

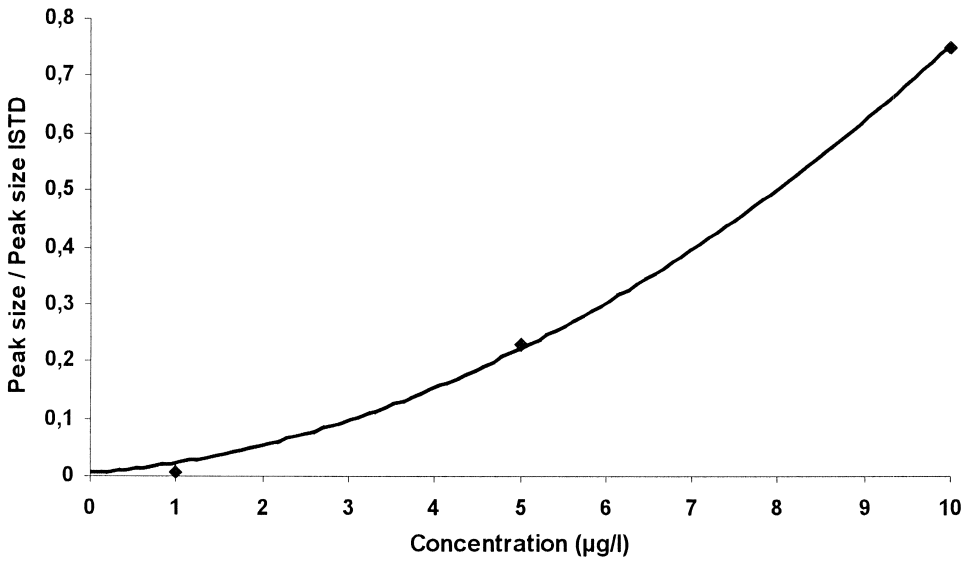

Fig. 3. Calibration curve for a sulphur compound (MeSAc) obtained using peak heights. SPME–GC–PFPD conditions as in the Experimental section.

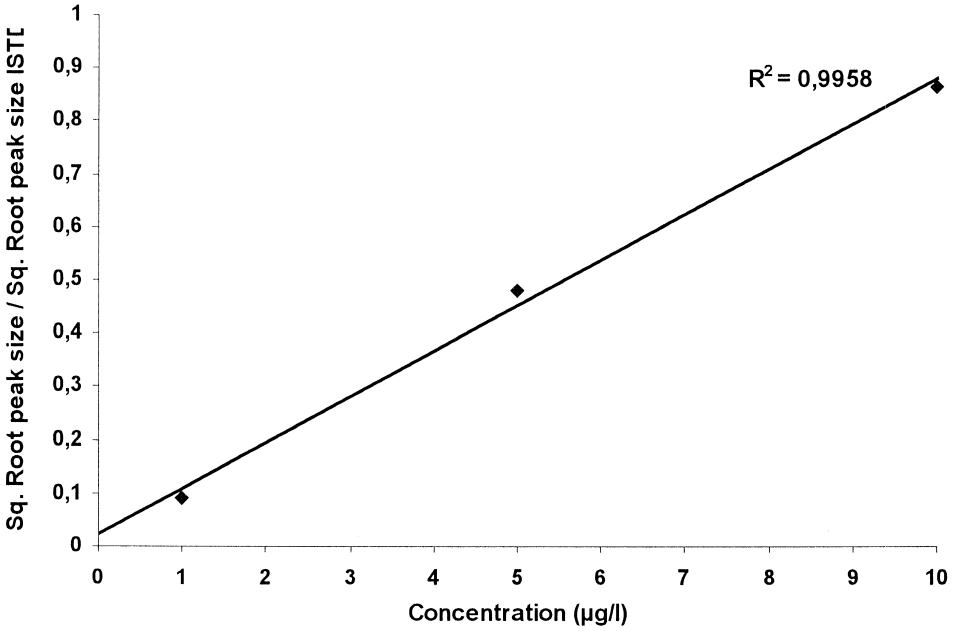

Fig. 4. Calibration curve for a sulphur compound (MeSAc) obtained using square roots of the peak heights. SPME–GC–PFPD conditions as in the Experimental section.

square root of the peak height to compensate for the quadratic response of the detector.

The linearity of the SPME–GC–PFPD system was good. The correlation coefficients for the calibration curves of all compounds were all over 0.9930, with the exception of methyl- and ethylmercaptan, which displayed correlation coefficients of 0.9869 and 0.9838, respectively. The correlation coefficients and calibration concentrations for each sulphur compound calibrated can be seen in Table 2. The repeatability of the method was also good. The relative standard deviations for most sulphur compounds were under 10%. Methionol was the exception with a high relative standard deviation of 18.3%. The limits of detection (LODs) of the SPME–GC–PFPD method were difficult to determine owing to the effects of the sample matrix. The SPME–GC–PFPD system is most sensitive when 100% aqueous samples are analysed, the sensitivity decreasing with increasing ethanol concentration. Therefore, the LODs vary from sample to sample. As a result, the determination of LODs in aqueous standard does not give any indication of the LODs in real samples. Additionally, because of the discriminatory nature of SPME, the limits of detection vary from compound to compound. The LODs for certain compounds in a European Pilsener beer (approx. 5% ethanol) could, however, be approximated by studying selected compounds. The system appeared to be most sensitive for 3-methylthiophene with a LOD of 1–5 ng/l

Table 2
Calibration concentrations and correlation coefficients for all sulphur compounds calibrated using the SPME–GC–PFPD method[a]

Analyte	Calibration concentrations (µg/l)	Correlation coefficient
MeSH	1.00; 5.00; 10.00	0.9869
EtSH	0.25; 1.245; 2.50	0.9838
DMS	6.00; 30.0; 60.0	0.9972
CS_2	0.05; 0.25; 0.50	0.9953
MeSAc	1.00; 5.00; 10.00	0.9958
DMDS	0.10; 0.50; 1.00	0.9997
EtSAc	0.20; 1.00; 2.00	0.9984
2-MeBuSH	0.05; 0.25; 0.50	0.9993
3-MeThPh	0.01; 0.05; 0.10	0.9993
3-MBT	0.80; 4.00; 8.00	0.9960
DEDS	0.05; 0.25; 0.50	0.9996
DMTriS	0.25; 1.25; 2.50	0.9938
Methionol	50.0; 250.0; 500.0	0.9999
3-MeSPrAc	4.00; 20.0; 40.0	0.9998

[a] SPME–GC–PFPD conditions as in the Experimental section.

Table 3
Concentrations of sulphur compounds in various European beers[a]

Sample	Sulphur compounds in beer (μg/l)											
	MeSH	EtSH	DMS	CS_2	MeSAc	DMDS	EtSAc	2-MeBuSH	3-MeThPh	3-MBT	Methionol	3-MeSPrAc
Pilsener beer												
Brewery A	3.074	0.560	70.52	0.167	11.93	0.306	0.688	0.049	0.026	0.000	356.0	4.286
Lager beer												
Brewery B	3.019	0.581	59.18	0.398	11.88	0.247	1.085	0.042	0.021	0.000	454.8	9.427
Alcohol-free beer												
Brewery A	1.633	0.099	5.112	0.068	0.055	0.081	0.000	0.000	0.000	0.000	206.2	1.163
Bock beer												
Brewery B	3.537	0.634	64.49	0.187	11.10	0.176	1.402	0.043	0.011	0.000	576.7	18.51
Wheat beer												
Brewery C	3.589	0.184	47.78	0.267	4.288	0.170	0.289	0.066	0.031	0.000	2621.0	136.9

[a] SPME–GC–PFPD conditions as in the Experimental section.

in beer. For several other compounds, such as 3-MBT, DMDS, EtSAc and CS_2, the LODs lay between 10–60 ng/l.

The calibrated SPME–GC–PFPD method was used to determine the concentrations of sulphur compounds in a range of different beer varieties (Table 3). It can be seen that there are significant differences in the concentrations of sulphur compounds in the different beer varieties. Pilsener, lager and bock beers are all bottom-fermenting beers and a similarity in the sulphur compound levels between the three varieties are seen. Wheat beer is a top-fermented beer and contains significantly higher levels of methionol and 3-methyl thiopropyl acetate. The non-alcoholic beer was a normal Pilsener beer which had been subjected to vacuum distillation to remove ethanol. The results show that volatile sulphur compounds are also removed in the vacuum distillation process.

4. Conclusions

SPME–GC–PFPD is a simple, fast and sensitive method for the routine analysis of volatile and semi-volatile sulphur compounds in beer. Headspace SPME using a 75 μm Carboxen–PDMS fibre provided effective sample enrichment. The SPME adsorption and desorption parameters were optimised and the effect of the sample matrix, in particular the influence of ethanol, on the extraction of trace amounts of sulphur compounds was investigated.

Separation of sulphur compounds across a wide range of boiling points and polarities was possible using a combined column consisting of a 10 m length of polar wax column connected to a 60 m non-polar PDMS column. Stable and sensitive sulphur-specific detection was provided by a pulsed flame photometric detector.

The SPME–GC–PFPD system displayed good linearity and reproducibility and excellent sensitivity. The sensitivity of the system allowed two compounds which had previously been unreported in beer – 3-methylthiophene and 2-methyl-1-butanethiol – to be identified. Using the SPME–GC–PFPD method described, 3-methyl-2-butene-1-thiol, the so-called ''light mercaptan'', can be determined routinely. Further applications of the method under investigation are the formation of sulphur compounds caused by the influence of light on beer and the determination of the concentrations of sulphur compounds at different stages of beer production.

References

[1] C.J. Mussinan, M.E. Keelan (Eds.), Sulfur Compounds in Foods, ACS Symposium Series, No. 564, American Chemical Society, Washington, DC, 1993, p. 1, Chapter 1.
[2] M.H. Boelens, L.J. van Gemert, Perfume Flavour 18 (1993) 29.

[3] H.-W. Chin, R.C. Lindsay, in: C.J. Mussinan, M.E. Keelan (Eds.), Sulfur Compounds in Foods, ACS Symposium Series, No. 564, American Chemical Society, Washington, DC, 1993, p. 90, Chapter 8.
[4] L.C. Maillard, Compt. Rend. Sci., Paris 66 (1912) 154.
[5] C. Eriksson (Ed.), Maillard Reactions in Food, Permagon Press, Oxford, 1981.
[6] L. Nykänen, H. Suomalinen, in: Aroma of Beer, Wine and Distilled Alcoholic Beverages, Akademie Verlag, Berlin, 1983, p. 233, Chapter 16.
[7] B.J. Anness, European Brewing Convention Monograph VII, Flavour Symposium, Copenhagen, 1981, p. 135.
[8] P. Schreier, F. Drawert, A. Junker, Brauwissenschaft 28 (1975) 73.
[9] L. Narziß, H. Miedaner, U. Kattein, Monatssch. Brauwiss. 39 (1986) 360.
[10] M.S. Burmeister, C.J. Drummond, E.A. Pfisterer, D.W. Hysert, Y.O. Sin, K.J. Sime, D.B. Hawthorne, J. Am. Soc. Brew. Chem. 50 (1992) 53.
[11] M.D. Walker, J. Inst. Brew. 98 (1992) 283.
[12] A. Dercksen, J. Laurens, P. Torline, B.C. Axcell, E. Rohwer, J. Am. Soc. Brew. Chem. 54 (1996) 228.
[13] F. Schur, Brauerei Rundsch. 99 (1988) 165.
[14] L. Narziß, H. Miedaner, P. Zinsberger, Monatssch. Brauwiss. 41 (1988) 244.
[15] T. Wainwright, in: Proceedings European Brewing Congress, Estoril, 1971, p. 437.
[16] M.D. Walker, W.J. Simpson, Lett. Appl. Microbiol. 16 (1993) 40.
[17] J.R.M. Hammond, in: A.H. Rose, J.S. Harrison (Eds.), The Yeasts, Vol. 5, Academic Press, London, 1993, Chapter 2, p. 7.
[18] H.J. Niefind, G. Späth, in: Proceedings European Brewing Congress, Estoril, 1971, p. 459.
[19] R.J. Anderson, G.A. Howard, J.S. Hough, in: Proceedings European Brewing Congress, Estoril, 1971, p. 253.
[20] R.J. Anderson, G.A. Howard, J. Inst. Brew. 80 (1974) 357.
[21] H. Garza-Ulloa, Brew. Digest 55 (1980) 20.
[22] T. Peppard, in: H.F. Listens, J.F. Jackson (Eds.), Modern Methods of Plant Analysis, Beer Analysis, Vol. 7, Springer Verlag, Berlin, 1988, p. 241.
[23] A.W. Dercksen, I. Meijering, B. Axcell, J. Am. Soc. Brew. Chem. 50 (1992) 93.
[24] J. Pawliszyn, Solid Phase Microextraction – Theory and Practice, Wiley–VCH, New York, 1997.
[25] X. Yang, T. Peppard, J. Agric. Food Chem. 42 (1994) 1925.
[26] J.A. Field, G. Nickerson, D.D. James, C. Heider, J. Agric. Food Chem. 44 (1996) 1768.
[27] A. Steffen, J. Pawliszyn, J. Agric. Food Chem. 44 (1996) 2187.
[28] D. De la Calle Garcia, M. Reichenbächer, K. Danzer, C. Hurlbeck, C. Bartzsch, K.-H. Feller, J. High Resolut. Chromatogr. 20 (1997) 665.
[29] A.J. Matich, in: J. Pawliszyn (Ed.), Applications of Solid Phase Microextraction, Royal Society of Chemistry, Cambridge, 1999, p. 349, Chapter 26.
[30] T.J. Braggins, C.C. Grimm, F.R. Visser, in: J. Pawliszyn (Ed.), Applications of Solid Phase Microextraction, Royal Society of Chemistry, Cambridge, 1999, p. 407, Chapter 30.
[31] E. Atar, S. Cheskis, A. Amirav, Anal. Chem. 63 (1991) 2064.
[32] S. Cheskis, E. Atar, A. Amirav, Anal. Chem. 65 (1993) 539.
[33] A. Amirav, H. Jing, Anal. Chem. 67 (1995) 3305.
[34] H. Verhoeven, T. Beuerle, W. Schwab, Chromatographia 46 (1997) 63.
[35] Z. Zhang, J. Pawliszyn, Anal. Chem. 65 (1993) 1843.
[36] M.E. Miller, J.D. Stuart, Anal. Chem. 71 (1999) 23.
[37] M. Mestres, C. Sala, M.P. Marti, O. Busto, J. Guasch, J. Chromatogr. A 835 (1999) 137.
[38] P. Popp, A. Paschke, Chromatographia 46 (1997) 419.
[39] G.A. Reineccius, in: P.A. Finot, H.U. Aeschbacher, R.F. Hurrell, R. Liardon (Eds.), The Maillard Reaction in Food Processing, Human Nutrition and Physiology, Birkhäuser, Basel, 1990, p. 157.
[40] C.L. Arthur, L.M. Killiam, K.D. Buchholz, J. Pawliszyn, J.R. Berg, Anal. Chem. 64 (1992) 1960.
[41] R. Eisert, K. Levsen, Fresenius J. Anal. Chem. 351 (1995) 555.
[42] R. Eisert, K. Levsen, J. Am. Soc. Mass Spectrom. 6 (1995) 1119.
[43] L. Urruty, M. Montury, J. Agric. Food Chem. 44 (1996) 3871.
[44] D. De la Calle Garcia, S. Magnaghi, M. Reichenbächer, K. Danzer, J. High Resolut. Chromatogr. 19 (1996) 257.
[45] C. Fischer, U. Fischer, J. Agric. Food Chem. 45 (1997) 1995.
[46] M. Mestres, O. Busto, J. Guasch, J. Chromatogr. A 808 (1998) 211.
[47] M. Nedjma, A. Maujean, J. Chromatogr. A 704 (1995) 495.
[48] L. Narziß, H. Miedaner, P. Zinsberger, Monatssch. Brauwiss. 4 (1988) 72.
[49] A. Suggett, M. Moir, J.C. Seaton, in: Proceedings European Brewing Congress, Berlin (West), 1979, p. 78.
[50] F.R. Sharpe, D.R.J. Laws, J. Inst. Brew. 87 (1981) 96.
[51] T.L. Peppard, J. Inst. Brew. 87 (1981) 376.
[52] J. Templar, K. Arrigan, W.J. Simpson, Brew. Digest 70 (1995) 18.
[53] Y. Kuroiwa, N. Hashimoto, in: Proceedings of the American Society of Brewing Chemists, 1961, p. 28.
[54] F. Gunst, M. Verzele, J. Inst. Brew. 84 (1978) 291.
[55] S. Sakuma, Y. Rikimaru, K. Kobayashi, M. Kowaka, J. Am. Soc. Brew. Chem. 49 (1991) 162.
[56] W.F. Wood, J. Chem. Ecol. 16 (1990) 2057.
[57] W.F. Wood, C.G. Morgan, A. Miller, J. Chem. Ecol. 17 (1991) 1415.
[58] W.F. Wood, C.O. Fisher, G.A. Graham, J. Chem. Ecol. 19 (1993) 837.
[59] W.F. Wood, personal communication.
[60] A.J. Irwin, L. Bordeleau, R.L. Barker, J. Am. Soc. Brew. Chem. 51 (1993) 1.
[61] M.C. Meilgaard, Brygmesteren 39 (1982) 159.
[62] M. Jia, H. Zhang, D.B. Min, J. Agric. Food Chem. 46 (1998) 2744.