Determination of Volatiles in Beer using Solid-Phase Microextraction in Combination with Gas Chromatography/Mass Spectrometry

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Abstract

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Headspace solid phase microextraction and purge and trap analysis were used for the determination of volatiles in beer. These methods were compared with the analysis of unconcentrated gas phase and liquid extraction. Solid phase microextraction proved to be the most useful and was investigated more closely. Volatiles were isolated by the means of different combinations of sorbents, sorption was performed at various temperatures and times. The addition of salt to the sample and stirring of the sample were examined to enhance the analyte concentration in the gas phase. Ultrasonic bath and filtration were tested to remove carbon dioxide. Not all methods improved the sorption of volatiles. Stirring was characterised by low repeatability and ultrasonic bath causes to the loss of volatile analytes. Distribution constants of volatiles depend on their boiling points and thus different sorption temperatures are suitable for different substances.

Keywords: GC; headspace technique; solid phase; microextraction; volatile compounds

Volatile compounds in beer are substances that contribute significantly to the organoleptic properties of beverages and thus affect product quality both in a positive and negative way. The Maillard reaction products are formed during kilning and roasting of malt. The temperature of 125–160°C is the most favourable for the formation of flavour. Intermediate chromophores are more sensory active than the final reaction products – the melanoidins (Coghe *et al*. 2004; Cramer *et al*. 2005). Essential oils, which are released to the wort during brewing, are another group of volatile compounds in hops. The most important phase in terms of volatile substances is a fermentation process when a lot of compounds with low boiling point are formed (Kovačevič & Kač 2001; Lermusieau *et al*. 2011).

The best technique for the determination of volatile compounds in beer is gas chromatography. Due to the complex matrix of beer and a large concentration range among the substances the analytes must be isolated and concentrated first. Liquid extraction, distillation, supercritical fluid extraction, solid phase extraction, stir bar sorptive extraction or headspace techniques are used for the sample preparation. The most popular is solid phase microextraction (SPME). During SPME the analytes are sorbed to the small amount of extraction phase on the surface of the fibre. The fibre is ejected directly into the sample or into the gas phase above the sample (headspace), which is preferable for the analysis of volatile compounds in beer (Kolb 1999; Štěrba *et al*. 2011; Pawliszyn 2000; Wardecki et al. 2003).

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MATERIAL AND METHODS

Materials. All chemicals were purchased from Sigma Aldrich (St. Louis, USA) and were of the highest purity available. Nitrogen (5.0) and helium (5.5) were purchased from SIAD (Braňany, Czech Republic). Bottled beer (Starobrno, Brno, Czech Republic) was used for the method optimisation. It is a pilsner type beer containing 4% (v/v) alcohol.

Liquid extraction. Hexane and dichloromethane were examined as suitable solvents for liquid extraction. Samples were prepared in 40 ml vials from 15 ml of beer and 15 ml of solvent. For optimisation of extraction conditions different amounts of salt (5, 10, and 15 g) were added. Samples were extracted in an ultrasonic bath (Kraintek, Hradec Králové, Czech Republic) for 10 minutes. Subsequently, extracts were spun in the centrifuge (MPW Med. Instruments, Warsaw, Poland) at 18 000 per min for 5 minutes.

Static headspace analysis (SHS). The sample for SHS was prepared from 5 ml of beer and 2 g of NaCl in a 15-ml vial, heated at 50°C for 30 minutes. Then 25 µl sample of the gaseous phase was taken with a gastight syringe (Hamilton, Reno, USA) and injected immediately.

Purge and trap (P&T) analysis. During the P&T analysis 10 ml of the sample was exposed to inert gas – nitrogen – bubbling through the liquid in a strip tube. Exposure time was 30 min, nitrogen flow rate was 10 ml/minutes. After completing the exposure time, the sorption tube was placed in a module for the fully automatic thermal desorption of the sample, Model TD-4 (Scientific Instrument Services, Ringoes, USA). The thermodesorption tube was filled with 100 mg of Tenax (Scientific Instrument Services). Thermal desorption lasted 3 min, initial temperature was 200°C and it was immediately raised at the rate of 20°C/min to 250°C.

SPME analysis. Unless stated otherwise, samples were prepared from 5 ml of beer in a 15-ml vial containing 2 g NaCl. All sample vials were equilibrated for 10 min at 50°C on a heating plate with an electronic contact thermometer (IKA-Werke GmbH, Staufen, Germany) followed by fibre exposure to the headspace for 20 minutes. SPME fibre holder (manual) and 100 µm polydimethylsiloxane coated fibre were used for the SPME method (Supelco, Bellefonte, USA).

Stirring with a magnetic stirrer was examined during the equilibration time. Samples were stirred during heating on the heating plate at a speed of 100 per minute. Ultrasonic bath degassing and filtration through filter paper of type 388 and 390 (Filtrak, Wiesenbad, Germany) were tested.

For the optimisation of SPME conditions different amounts of beer (2.5, 5, 7.5, and 10 ml) were prepared for SPME sorption. The amount of NaCl was another investigated sampling parameter; different amounts of NaCl (1, 2, 3, 4, 5 g) were added to the sample with 5 ml of beer. Samples were retained for 24 h in the refrigerator before analysis. Sorption time was also tested: 10, 20, 30, 40, 60, 90 and 120 min at 50°C. The sample was equilibrated for 10 min at 50°C before sorption.

To optimise the temperature used for the extraction of volatile substances, the samples were heated on a heating plate and the temperature of the samples was measured using an electronic contact thermometer. Temperature of the heating plate was set to 40, 50, 60, 70, 80, 90, 100, 110, and 130°C. Actual temperature of the sample was 32, 38, 45, 52, 57, 62, 68, 72, and 84°C.

To determine the most suitable volume of vials, standard vials with a capacity of 15 (20 mm o.d.), 20 and 40 ml (23 mm o.d.) were used. Beer samples (5 and 10 ml) were measured in vials with capacity of 15, 10, and 15 ml beer samples were tested in vials with capacity of 20 ml, 30 ml beer samples were prepared in vials with capacity of 40 ml. SPME sorption was carried out at 70, 80, and 100°C. The electronic contact thermometer measured the actual temperature of the sample and its changes due to changes in the sample volume.

Polydimethylsiloxane (PDMS), divinylbenzene (DVB), and carboxen (CAR) sorbents were used for SPME. The fibres tested for the extraction of volatile compounds were as follows: 100 µm PDMS, 65 µm PDMS/DVB, and 75 µm CAR/PDMS (Supelco, Bellefonte, USA). Each analysis was undertaken triplicate using different vials.

Gas chromatography. Gas chromatography with mass spectrometry (GC-MS) and gas chromatography with flame ionisation detector (GC-FID) were compared. Samples for testing were extracted by SPME and prepared in the same way as samples used for the optimisation of SPME conditions.

An HP-6890 gas chromatograph with an HP-5973 mass spectrometric detector was used for GC-MS. GC-FID was performed using HP-4890D. The HP-5973 detector was used in a classic electron impact mode. The electron ionizing energy was 70 eV. The temperature of the ion source was 230°C. Qualitative analysis was done by a comparison of the mass spectrum of volatile analytes (G1036A NIST Chem. Library) and

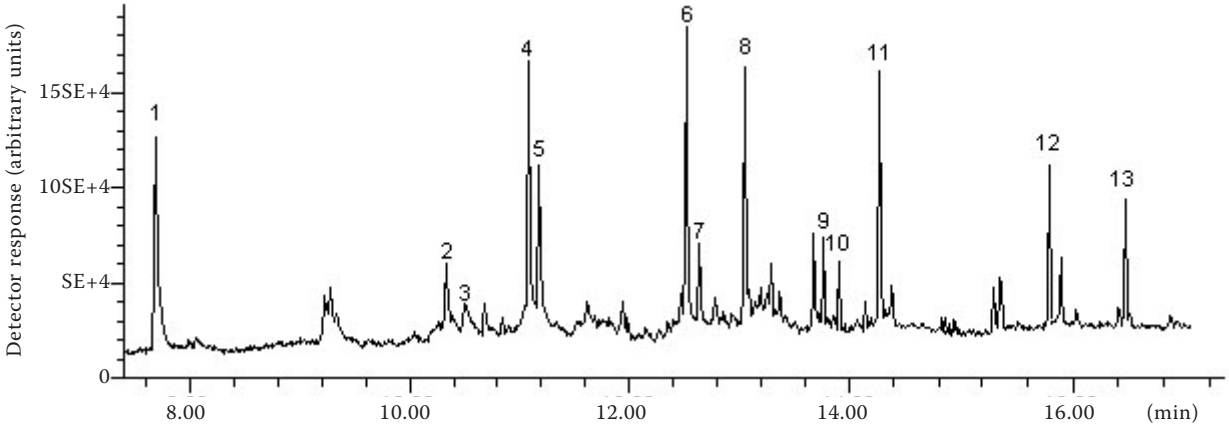

Figure 1. Chromatogram of volatile compounds in beer obtained using SHS: (1) 1-butanol, 3-methyl-, acetate; (2) nonanal; (3) phenylethyl alcohol; (4) octanoic acid, ethyl ester; (5) decanal; (6) decanoic acid, ethyl ester; (7) dodecanal; (8) 2-propenamide, 2-methyl-N-phenyl-; (9) dodecanoic acid, ethyl ester; (10) octadecanal; (11) cyclododecane; (12) dibutyl phthalate; (13) nonadecane

corresponding peaks in the beer sample. The mass spectrometric detector operated in a scan mode at *m*/*z* 35–200. The scan time was 0.2 s, peak areas were calculated from the total ion current. The gas chromatograph was controlled by ChemStation software (Version A.03.00; Agilent, Waldbronn, Germany).

Three types of chromatography columns were tested on GC-MS: HP-5MS (5% phenylmethylsiloxane, 30 m × 0.25 mm *i.d.* × 0.25 μm), DB-WAX (polyethylene glycol, 30 m × 0.25 mm *i.d.* × 0.25 μm) and HP-5MS of 60 m in length (Agilent, Waldbronn, Germany).

The basic temperature programme was as follows: T_1 = 40°C, t_1 = 5 min, 20°C/min to T_2 = 250°C, t_2 = 5 minutes. Two temperature programmes with gradual increase were tested: T_1 = 40°C, t_1 = 5 min, 10°C/min to T_2 = 270°C, t_2 = 5 min and T_1 = 40°C, t_1 = 5 min, 5°C/min to T_2 = 200°C, t_2 = 5 min, 15°C/min to T_3 = 250°C, t_3 = 1 minute.

Samples were injected in a splitless mode. The flow rate was 1 ml/min, injector temperature 240°C and temperature of the detector 250°C.

RESULTS AND DISCUSSION

Comparison of sample preparation methods

Liquid extraction. In comparison with SPME, liquid extraction was totally unsatisfactory. The

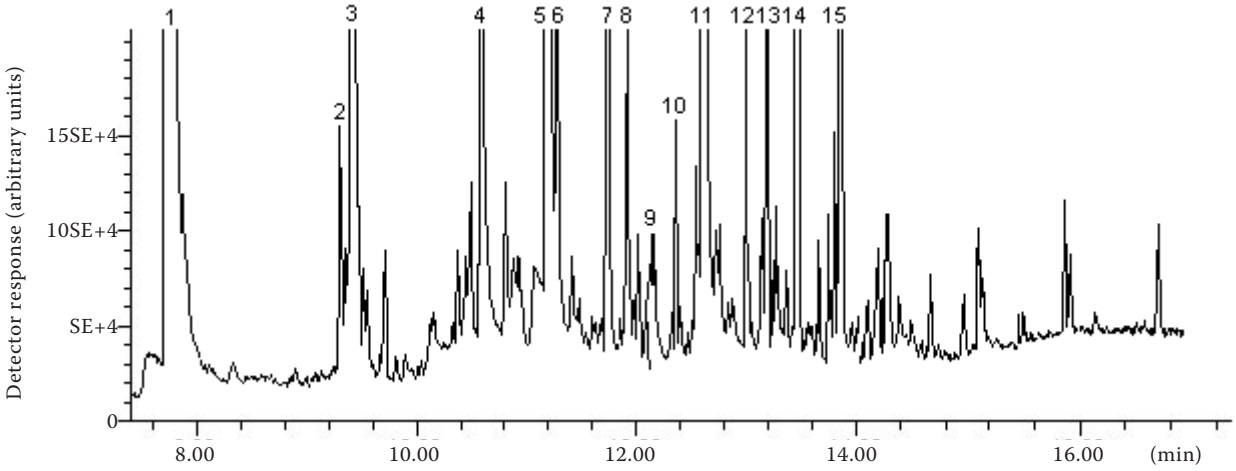

Figure 2. Chromatogram of volatile compounds in beer obtained using SPME: (1) 1-butanol, 3-methyl-, acetate; (2) myrcene; (3) hexanoic acid, ethyl ester; (4) phenylethyl alcohol; (5) octanoic acid, ethyl ester; (6) acetic acid, octyl ester; (7) acetic acid, 2-phenylethyl ester; (8) nonanoic acid, ethyl ester; (9) citronellyl acetate; (10) 9-decenoic acid, ethyl ester; (11) decanoic acid, ethyl ester; (12) octanoic acid, 3-methylbutyl ester; (13) humulene; (14) dibutylhydroxytoluene; (15) dodecanoic acid, ethyl ester

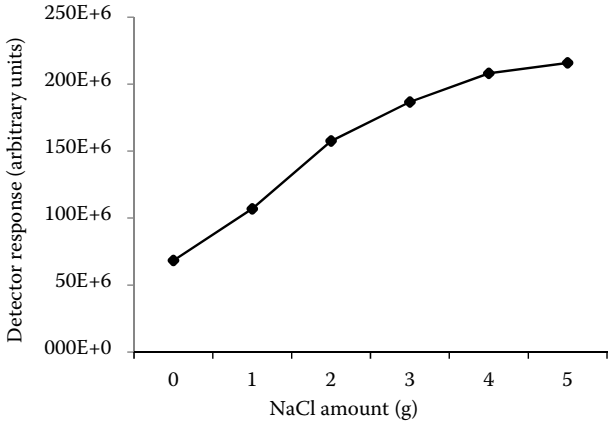

Figure 3. The effect of ionic strength increase on the extraction efficiency of solid phase microextraction (SPME). Sodium chloride was added to 5 ml of beer in a 15-ml vial (y-axis: total volatile compounds expressed as peak area arbitrary)

Table 1. Comparison of the repeatability of solid phase microextraction (SPME) without stirring and SPME with stirring during 20 minutes of extraction

Compound	SPME without stirring RSD[a] (%)	SPME with stirring during equilibration RSD (%)
1-Butanol, 3-methyl-, acetate	3.75	21.43
Hexanoic acid, ethyl ester	3.29	34.09
Phenylethyl alcohol	8.41	14.91
Octanoic acid, ethyl ester	7.34	16.55
Acetic acid, 2-phenethyl ester	5.66	53.67
9-Decenoic acid, ethyl ester	5.97	23.81
Decanoic acid, ethyl ester	3.11	23.27
Dibutylhydroxytoluene	5.10	7.34
Dodecanoic acid, ethyl ester	4.48	16.88

[a]relative standard deviation (estimated for peak areas, $n = 5$)

dichloromethane extracted 8 volatiles and hexane extracted 10 volatiles, which is less than one third of volatile substances extracted by SPME. However, it can be used to reduce the costs of analysis in cases where the analytes of interest are among the compounds that are extracted well. Salting out increased the sensitivity of the method. The obtained relative areas for the sample were 73, 96, 100, and 99% for dichloromethane and 34, 71, 90, and 100% for hexane thanks to the increasing amount of salt.

SHS. SPME is used for the preconcentration of analytes, so it can be assumed that SPME is more suitable for the analysis of volatile compounds than SHS. On the other hand, it is not possible to ensure the transfer of all the substances by SPME because it is dependent on the affinity of the analytes to the sorbent. Analyses showed that none of the required analytes was missing when using SPME. Figures 1 and 2 show the volatile analytes detected after direct sampling and volatile substances detected from the PDMS SPME fibre. The source of contaminating compounds 8, 11, and 12 from Figure 1 is urban air pollution.

P&T. SPME is preferable for the isolation of hop essential oils and products of fermentation. P&T is a better method for the isolation of Maillard reaction products such as maltol, furfural and 2-furanmethanol.

SPME. SPME was determined to be the most effective method and became a subject of further optimisation. At first the effect of ionic strength increase on the extraction efficiency of SPME was evaluated. According to Jelen (1998) peak areas of all analysed compounds increased with increasing

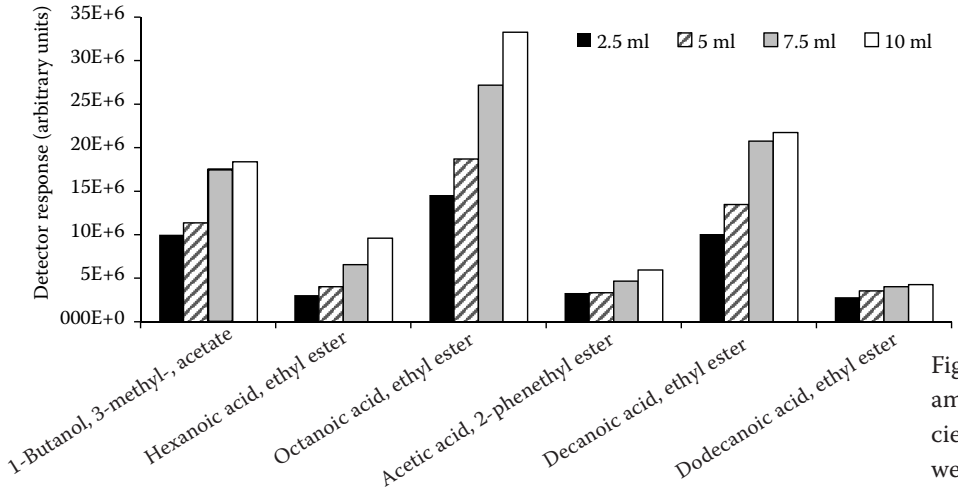

Figure 4. The effect of sample amount on the extraction efficiency of SPME. Beer samples were processed in a 15-ml vial

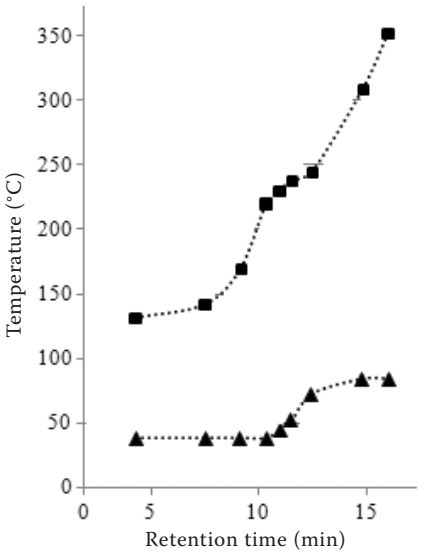

Figure 5. Optimal extraction temperature for volatiles according to retention time (t_R)

Volatile compounds	Rf (min)	Optimal extraction temperature (°C)	Boiling point
1-Butanol, 3-methyl	4.32	38	132
1-Butanol, 3-methyl-, acetate	7.54	38	142
Hexanoic acid, ethyl ester	9.17	38	170
Phenylethyl alcohol	10.39	38	219
Octanoic acid, ethyl ester	11.02	45	230
Acetic acid, 2-phenethyl ester	11.53	52	238
Decenoic acid, ethyl ester	12.47	72	245
Tetradecanoic acid, ethyl ester	14.83	84	309
Hexadecanoic acid, ethyl ester	16.03	84	352

salt concentration. Figure 3 shows the increase of detector response after sodium chloride addition to analysed beer. Addition of sodium chloride to samples lowers detection limits of the SPME method. It may be helpful for the analysis of compounds contained in trace quantities that are the main point of interest.

Figure 4 shows the effect of sample amount on the extraction. SPME adsorption increased with increasing sample amount. However, the volume of the vial is more important for extraction efficiency than the sample volume. Extraction efficiency of 20 ml beer in a 40-ml vial was worse than that of 10 ml beer in a 15-ml vial. It depends on phase rations. The headspace volume should be smaller than the volume of the sample. Analytes are more concentrated in the headspace of small volume and the efficiency of SPME is higher. A comparison of the analysis of 10 ml beer in a 15-ml vial and 30 ml beer in a 40-ml vial proved that a smaller volume of the sample was preferred.

The vial diameter also influences SPME adsorption. 10 ml of beer was processed to 15- and 20-ml vials of larger diameter. Some compounds showed higher recovery using the 20-ml vial although there was a larger volume of the headspace. On the other hand, there is a larger surface area that facilitates the transfer of volatile substances in the gas phase in the given time. Thus, thanks to the larger active surface the equilibrium is reached sooner and the extraction time can be adequately shorter. It enables to use more favourable extraction times for substances that work well with the higher headspace volume, which is very useful in the case of large monitoring studies of many samples.

The optimal temperature for SPME sorption of individual volatiles in beer can be seen in Figure 5. It depends on boiling points of analytes. The sorption of volatiles is proportional to their concentration in the headspace. The concentration of volatiles with lower boiling point in the headspace is higher using a lower temperature because their vapour pressure is higher. The retention time of all analysed compounds increased with increasing boiling point because partition and diffusion coefficients of analytes depend on temperature. Therefore a lower temperature of SPME sorption is more suitable for compounds with low retention time and conversely. The lowest tested temperature (32°C) is not suitable for any analysed substance. 38°C is the most suitable for phenylethyl alcohol and compounds with lower boiling points. Higher temperatures of SPME sorption are preferable for volatiles with higher boiling points. The highest temperature suitable for SPME is 84°C.

The extent of SPME adsorption increased rapidly with increasing the fibre exposure time from 10 to 40 minutes. The efficiency of SPME adsorption was highest after 60 minutes. It remained constant with longer exposure time. The dependence of analyte adsorption on the exposure time is shown in Figure 6. Analysed compounds are given in order according to the boiling point (upper compounds have the lower boiling points). Differences in the exposure time are largest for volatiles with higher boiling points. The use of longer exposure time is more important for SPME adsorption of volatiles with higher boiling points. Other groups of researchers came to similar results (Jelen *et al.* 1998; Pinho *et al.* 2006).

According to the literature an ultrasonic bath is usually used for removing carbon dioxide (Silva *et al.* 2008; Rodrigues *et al.* 2011). However, volatiles were detected in lower concentrations after the ultrasonic

Table 2. GC column and solid phase microextraction (SPME) fibre used for volatile identification

Compound	Retention time on GC column (min)			Relative areas achieved using different SPME fibre (%)		
	DB-WAX	5MS(30)	5MS(60)	D/C/P	P/D	C/P
Ethanol	2.23	3.25	3.97	96	37	100
Ethyl acetate	–	4.11	4.81	100	24	76
1-Butanol, 3-methyl-	4.80	5.89	6.80	100	42	44
1-Butanol, 2-methyl-	–	5.95	6.87	100	70	72
Acetic acid, butyl ester	–	6.66	7.54	100	47	–
1-Pentanol	–	–	6.67	–	–	–
Butanoic acid, ethyl ester	–	7.32	8.06	100	35	61
1-Butanol, 3-methyl-, acetate	6.74	9.53	10.22	100	51	43
1-Butanol, 2-methyl-, acetate	–	9.57	10.29	100	43	59
Myrcene	–	13.14	13.82	100	–	51
Hexanoic acid, ethyl ester	7.31	13.41	14.05	100	39	39
Octanoic acid, ethyl ester	12.35	19.65	20.25	100	65	43
Acetic acid, octyl ester	13.31	–	20.59	–	100	53
Acetic acid, hexyl ester	–	13.83	14.48	100	–	–
2,3-Butanediol	14.98	–	–	–	–	–
1-Octanol	–	15.74	16.35	100	48	–
Heptanoic acid, ethyl ester	–	16.56	17.18	100	50	–
Linalool	15.13	16.70	17.35	100	35	59
Acetic acid, heptyl ester	–	16.99	17.62	100	62	47
Decanoic acid, ethyl ester	17.22	25.17	25.75	92	100	35
Humulene	17.69	27.12	27.77	100	–	44
9-decenoic acid, ethyl ester	18.35	–	–	–	–	–
1-Decanol	20.03	–	–	–	–	–
Acetic acid, 2-phenylethyl ester	20.96	21.42	22.05	100	60	43
Nonanoic acid, ethyl ester	–	–	23.02	–	–	–
Dodecanoic acid, ethyl ester	21.66	30.14	30.64	33	100	–
Phenylethyl alcohol	22.99	17.21	17.94	100	47	56
1-Undecanol	24.24	–	–	–	–	–
Octanoic acid	25.95	18.93	19.70	100	–	19
Decanoic acid	29.85	–	25.09	–	–	–
Acetic acid, decyl ester	–	–	26.07	–	–	–
Octanoic acid, 3-methylbutyl ester	–	–	27.05	–	–	–
Dodecanoic acid, 1-methylethyl ester	–	–	31.35	–	–	–

(30) – 30 m long; (60) – 60 m long; P – polydimethylsiloxane; D – divinylbenzene; C – carboxen

bath, as the ultrasonic bath also removes volatiles. Filtration is more suitable for carbon dioxide removal. Filter paper of type 388 is the most suitable for volatiles with lower molecular weight. It is generally suitable for compounds with low boiling point. Type 390 is preferable for volatiles with higher boiling point. In the first group of volatiles, octanoic acid ethyl ester is a compound with the highest boiling point.

Reportedly, a good way to speed up the extraction process is to use stirring (Silva *et al*. 2008). However, stirring is not suitable for short-time extractions because the volatiles in the sample are not equilibrated with the gas phase above the sample. It causes too low repeatability (Table 1).

Optimisation of gas chromatography conditions

Choice of detector. The optimisation started by selection of the best available detector for the given type of analytes: MS and FID were compared. Although a flame ionisation detector used by Tian (2010)

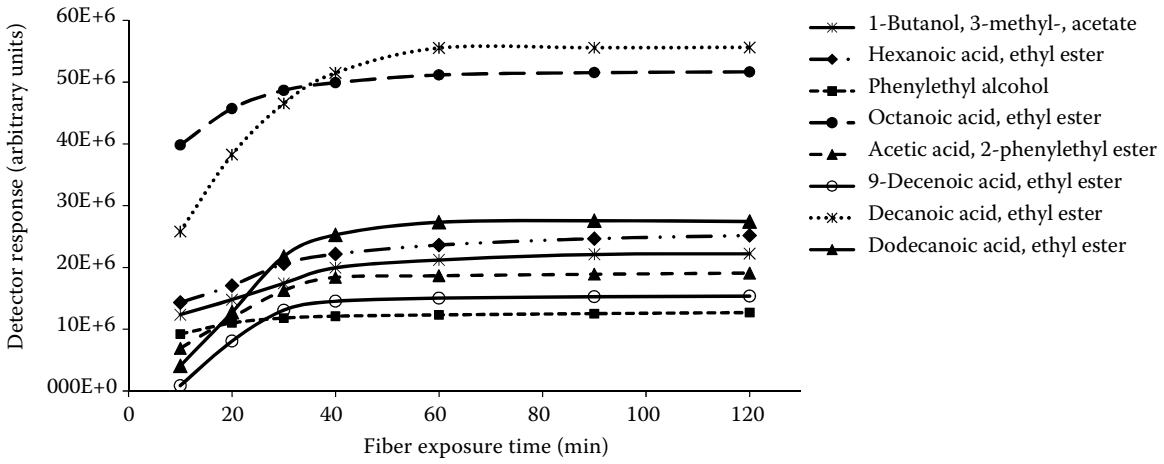

Figure 6. The effect of adsorption time on the extraction efficiency of PDMS fibre

ensures symmetrical peaks, a mass spectrometric detector was more suitable, because 43 volatile compounds were determined by GC-MS above the limit of quantification. The flame ionisation detector was able to quantify only 26 analytes. Using the mass spectra for the identification of analytes is another advantage of GC-MS.

Choice of temperature programme. The temperature programme with the slowest temperature increase proved to be the most suitable for the determination of volatile compounds in beer. On the other hand, sufficient separation of basic volatile components was ensured also with the fastest increase of temperature at a much shorter time of analysis.

Choice of chromatographic column. Several stationary phase sorbents were tested during the research. Table 2 shows that DB5 column of 60 m in length and SPME fibre with combination of sorbents are the most suitable, because the highest amount of substances of interest was identified using these sorbents. The longest temperature programme was used for this research. These results are in good accordance with data given in other papers (Cajka *et al*. 2010).

CONCLUSION

SPME is the most efficient sample preparation method for determination of volatiles in beer. The extraction efficiency of SPME is increased by salt addition, sample volume increase and proper temperature selection. The use of sonication for CO_2 removal decreases the recovery of volatile analytes. The use of filter paper is more favourable. The use of a stirrer causes worse reproducibility. GC analysis was performed with MS detector and DB5 column to obtain maximum sensitivity for the highest number of compounds.

Volatiles in beer have different boiling points and molecular weights. Thus, different conditions according to individual analyte properties are preferable to improve the determination of these analytes, especially if contained in trace quantities. This way the detection limits can be shifted to levels that enable identification and quantitative determination of volatiles which are contained in trace quantities in the sample, but due to the low threshold of sensory perception are important for the quality of the product and are the main point of interest.

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