

# Analysis of Selected Esters in Beer: Comparison of Solid-Phase Microextraction and Stir Bar Sorptive Extraction

Tomáš Horák\*, Jiří Čulík, Vladimír Kellner, Marie Jurková, Pavel Čejka, Danuša Hašková and Josef Dvořák

## ABSTRACT

*J. Inst. Brew.* 116(1), 81–85, 2010

Esters represent one of the most important flavour groups in beer. The aim of this work was focused on the comparison of two optimized, simple, rapid and low cost methods, the solid-phase microextraction technique and the stir bar sorptive extraction technique, for the determination of beer esters, in particular isoamyl acetate, ethyl caproate, ethyl caprylate, ethyl caprate, phenylethyl acetate, ethyl laurate, ethyl myristate, and ethyl palmitate. Subsequent gas chromatographic analyses with flame ionization detection were used for the determination of these compounds. Linearity, recovery, and repeatability of these methods were compared. Working parameters of both procedures were similar and characterized by high repeatability (2.1–7.3%) and good linearity (correlation coefficient ranging from 0.9991 to 0.9999). Results obtained by these two procedures were in good correlation.

**Key words:** beer, beer esters, solid-phase microextraction (SPME), solvent back extraction, stir bar sorptive extraction (SBSE)

## INTRODUCTION

From the point of view of composition, beer belongs to a very complex matrix. The amount of known constituents considerably exceeds more than 800 compounds, many of them contributing to beer flavour. The impact of chemical composition on beer flavour was studied by Meilgaard<sup>24</sup>. Beer flavour consists of a combination of odour and taste impressions and it is a significant factor in consumer acceptance.

Esters represent one of the most important flavour groups and they play a considerable role in the organoleptic characteristics of the beer. The production of esters is influenced first of all by wort composition, fermentation parameters and yeast strain during the brewing process<sup>4,25</sup>. Thus, esters are often the target analytes in authentication and quality control methods.

The presence of esters in beer has been routinely determined by static headspace methods, purge-and-trap pre-concentration techniques, distillation procedures, liquid-liquid extraction or solid phase extraction<sup>2,7,11,17,10,18,21,31,35</sup>. With the development of solid-phase microextraction (SPME), new methods for specific target compounds have been investigated.

SPME is a sampling technique that is rapid, simple, selective, and solvent free, allowing the preconcentration of volatile samples<sup>27</sup>. SPME has been successfully applied in the determination of variety of compounds in beer. For instance, Jélen et al. compared the determination of 12 alcohols and esters in beer by SPME and static headspace analysis<sup>19</sup>. SPME was also used for the quantitative determination of dimethylsulfide<sup>30,32</sup>, vicinal diketones<sup>12</sup>, medium-chain free fatty acids (caproic – lauric acid)<sup>15</sup>, furfural, hexanol, 5-hydroxymethylfurfural, and trans-2-nonenal<sup>33</sup> and for the investigation of beer flavour stability by monitoring 32 selected compounds<sup>29</sup>. Carbonyl compounds in beer have been determined after on-fibre derivatization using *o*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine<sup>28,34</sup>. Polar analytes such as alcohols and fatty acids were better extracted using tailor-made alumina-based hybrid organic-inorganic coating for SPME<sup>22</sup>. Combining silica-based adsorbents and SPME fibres in the extraction of the volatiles in beer was introduced by Biazon et al.<sup>5</sup>

Stir bar sorptive extraction (SBSE), like the SPME method, is a solventless enrichment technique. SBSE is based on the sorption of analytes onto a thick film of polydimethylsiloxane (PDMS) coated on a stir bar<sup>3</sup>. SBSE has been shown to have a much higher sensitivity than SPME due to the higher volume of the PDMS phase, in which the amount of analyte extracted is proportional to the coating thickness, increasing the limit of detection during sampling<sup>6</sup>.

After this extraction and enrichment step, the analytes are thermally desorbed from the stir bar and immediately transferred to a capillary column of a gas chromatograph. In brewing analytics, SBSE methods have been applied for the determination of sunstruck flavour (3-methyl-2-butene-1-thiol) and other sulphur compounds<sup>8</sup>, stale-flavour carbonyl compounds<sup>26</sup> or hop-derived terpenoids in beer<sup>20</sup>. In all these procedures, the analytes were thermally desorbed from the stir bar and were analyzed by gas chromatography. In the same way furfural, furfuryl ethyl ether, furyl hydroxymethyl ketone, 2,4-dodecadienal,  $\beta$ -dama-

Research Institute of Brewing and Malting PLC, Brewing Institute Prague, Lípová 15, CZ-120 44 Prague 2, Czech Republic.

\*Corresponding author. E-mail: horak@beerresearch.cz.

Publication no. G-2010-0325-1063

© 2010 The Institute of Brewing & Distilling

scenone and nicotinic acid ethyl ester were extracted, only GC-TOF-MS instrumentation was used for the final determination<sup>23</sup>.

Instead of thermal desorption, compounds can also be eluted from polydimethylsiloxane phase by a small volume of organic solvent and solvent back extraction. Some esters in beer<sup>16</sup>, free medium-chain fatty acids<sup>11,14</sup> or vicinal diketones<sup>13</sup> have been determined in this way. Capillary gas chromatography was used for the separation of these compounds followed by flame ionization detection (esters, fatty acids), respectively by electron capture detection (vicinal diketones). After solvent back extraction, SBSE can also be applied in conjugation with high pressure liquid chromatography as described in the determination of bitter acids in beer<sup>9</sup>.

The aim of this work was to compare different extraction methods for the determination of some flavour active esters in beer. The data obtained by headspace SPME technique and by SBSE with solvent back extraction were compared. The advantages and limitations of compared method are discussed.

## MATERIAL AND METHODS

### Beer samples

All analyzed beer samples were fresh commercial lagers of the Pilsner type, produced and bottled in the Czech Republic.

### Reagents

Standards of analyzed compounds (isoamyl acetate, ethyl caproate, ethyl caprylate, phenyl acetate, ethyl caprate, phenylethyl acetate, ethyl laurate, ethyl myristate, ethyl palmitate) were purchased from Sigma-Aldrich (USA) and were of >99% purity.

Analytical reagent grade ethanol (Lach-Ner, Czech Republic), methanol, hexane, dichloromethane (Merck, Germany), purified water (Milli-RO 5plus, Millipore, USA), helium 5.0 quality, hydrogen 5.0 quality, and synthetic air (Messer, Czech Republic) were used.

### SPME analysis

A manual SPME device and divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 65  $\mu\text{m}$  fibres were obtained from Supelco Co. (Bellefonte, PA).

Before use, the fibre was preconditioned in the GC injection port at 250°C for 0.5 h. Bottled beers were kept cool (4°C) until they were analyzed. Ethanol (5% v/v) was used for the evaluation of the method and for the calibration curves. Headspace SPME was applied for all analyses. The SPME fibre was exposed to the headspace above 10 mL of the sample in a 20 mL glass vial with an aluminium-coated septum. The vial was vigorously shaken for 10 sec prior to the commencement of headspace SPME. Others optimized conditions are discussed below.

### SBSE analysis

The stir bar was supplied from Gerstel GmbH (Mülheim a/d Ruhr, Germany). A 10 mm long and 3.2 mm o. d. stir bar with a 0.5 mm thickness of polydimethylsiloxane (PDMS) coating was used.

The stir bar was conditioned in a glass tube at 300°C for 60 min by passing helium through a tube (50 mL/min). Sample extraction was performed by placing 10 mL of sample in a 20 mL glass vial, adding a stir bar. The vial was crimped with a PTFE/silicone septum purchased from Chromacol (Herts, United Kingdom). The extraction procedure was based on conditions described by Horák et al.<sup>16</sup> i.e., stirring at 1,200 rpm for 60 min at ambient temperature. After extraction the stir bar was removed with forceps, rinsed briefly in distilled water and dried with a lint-free tissue. For solvent back extraction, the stir bar was placed into a 350  $\mu\text{L}$  glass insert containing 200  $\mu\text{L}$  of the solvent mixture dichloromethane:hexane, 50:50. This insert was immersed into a 2 mL vial, closed by a PTFE/silicone septum, and stirred at 1,200 rpm for 40 min.

For the determination of beer esters, 2  $\mu\text{L}$  of this extract was injected into the GC column under the chromatographic parameters mentioned below.

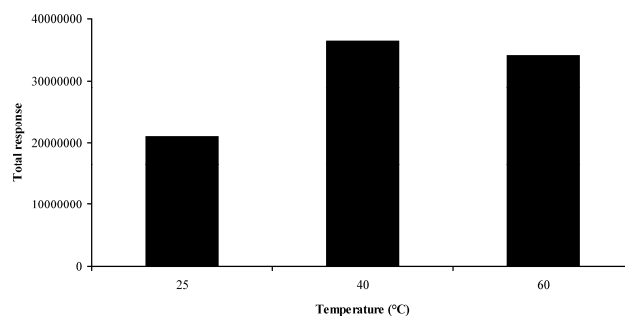
### GC analysis

The GC analysis was carried out using a Chrompack CP 9001 gas chromatograph equipped with autosampler Labio ASG 40. Analytes were separated on 30 m  $\times$  0.32 mm i.d. fused silica capillary column of Phenomenex ZB-WAX with 0.25  $\mu\text{m}$  film thickness. The GC column was maintained at 80°C for 1 min, ramped at a rate of 8°C/min to 240°C and then held at this temperature for 3 min. The split-splitless injector was used and the split vent was opened after 0.25 min. Temperatures of the injector and the flame ionisation detector were 250°C and 270°C respectively. The carrier gas was helium, 5.0 quality, with a column head pressure of 150 kPa at 80°C.

## RESULTS AND DISCUSSION

### Development of the SPME method

In the first experiment the effect of temperature was examined, as the temperature influences the vapour pressure of analytes and this was therefore a simple way of improving the sensitivity. The ambient temperature, and a temperature 40°C and 60°C were tested. Fig. 1 shows that the total response of esters of interest was the best at 40°C. At this temperature it attained 175% and 107% in comparison with ambient temperature and 60°C, respec-

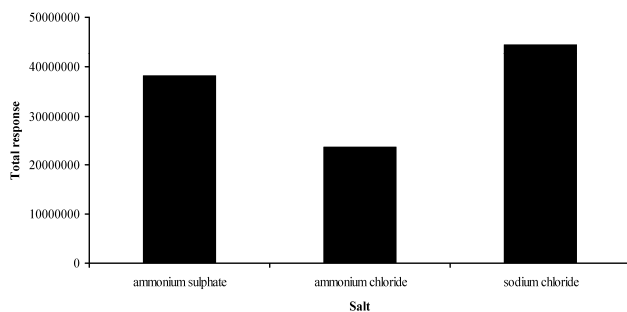


**Fig. 1.** Effect of the sampling temperature on the total response of selected esters (isoamyl acetate, ethyl caproate, ethyl caprylate, phenyl acetate, ethyl caprate, phenylethyl acetate, ethyl laurate, ethyl myristate, ethyl palmitate) extracted by SPME.

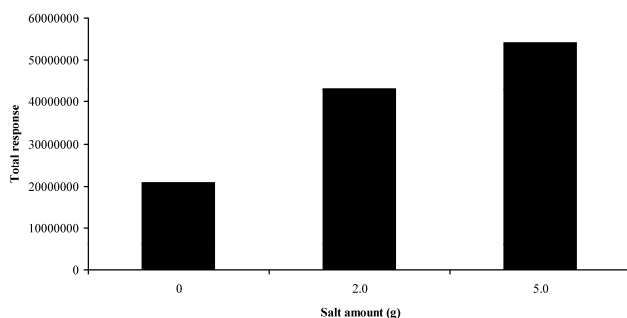
tively. Thus all other experiments were carried out at 40°C.

In the next experiment, the effect of the addition of different salts (ammonium sulphate, ammonium chloride and sodium chloride) on the extraction of some esters was studied. Fig. 2 demonstrates that the salting-out effect took place. The maximum responses were obtained by the use of sodium chloride (189% and 116% in comparison with ammonium chloride and ammonium sulphate, respectively). Addition of sodium chloride to analyzed beer lowers the detection limits of the SPME method and therefore could be helpful in running samples in which esters, occurring in trace quantities, are the main point of interest. Thus, the influence of varying concentrations of sodium chloride (0–5.0 g) in 10 mL of sample was tested. The total responses obtained with 5.0 g salt addition reached 260% of total responses found without salt addition (Fig. 3). Based on these results, a salt amount of 5.0 g was chosen for future experimentation.

Fig. 4 shows the influence of different sampling times on extraction. As expected, the responses increased with sampling time. While the total response was increased about 55% after a 60 min sampling time in comparison with a 30 min sampling, the total response was greater only by 19% after a 90 min sampling in comparison with a 60 min sampling. SPME is an equilibrium technique. In practice, full equilibration is not necessary for an accurate



**Fig. 2.** Effect of the addition of 2 g of the different salts on the total response of selected esters (isoamyl acetate, ethyl caproate, ethyl caprylate, phenyl acetate, ethyl caprate, phenylethyl acetate, ethyl laurate, ethyl myristate, ethyl palmitate) extracted by SPME.



**Fig. 3.** Effect of the amount of sodium chloride on the total response of selected esters (isoamyl acetate, ethyl caproate, ethyl caprylate, phenyl acetate, ethyl caprate, phenylethyl acetate, ethyl laurate, ethyl myristate, ethyl palmitate) extracted by SPME.

determination. However, a relatively short sampling time will not only result in a loss of sensitivity, but also of precision. Therefore a 60 min sampling time was selected for time saving and for precision.

The sample was stirred during extraction at 800 rpm. Each analysis was carried out three times.

After exposure, the fibre was thermally desorbed into a GC and left in the injection port at 250°C for 5 min. The injector was equipped with a 0.75 mm i.d. inlet liner.

### Comparison of SPME and SBSE method

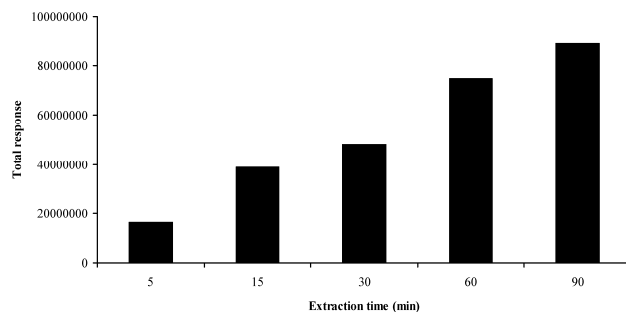
For method comparison, the following parameters have been determined: linearity, recovery and repeatability. Results of this comparison are shown in Table I.

For all methods calibration curves were measured throughout a range of the esters, covering the concentration usually found in beers of the Pilsner type (concentration from 0.015 mg/L to 30 mg/L for each compound). The correlation coefficients to straight line for all determined esters were the best for the SPME method at >0.9995. The coefficients for SBSE procedure were >0.9991.

All methods were characterized by high linearity in the examined concentration ranges.

The accuracy of the methods was investigated by conducting recovery tests. The tests were performed by measuring the natural concentration of the esters in five different beers. Then the same beers were spiked with a known amount of each ester (7.5 mg/L) and concentrations of the esters were determined again. The results showed that the recoveries of the SPME and SBSE methods for esters were similar and in the range 85–102% and 78–107%, respectively.

The repeatability of the methods was examined by repeating all procedures five times during the same day using the same beer sample. The results demonstrated that there were not significant differences between the methods. The procedures had good repeatability for the determination of esters in beer. The relative standard deviations (RSD) extended from 2.6 to 6.3% for SPME, and from 2.1 to 7.3% for SBSE. Although SBSE includes a two step extraction and solvent back extraction, the averages of the RSD values were very similar (for the SPME method they reached 4.9% and for the SBSE procedure 5.4%).



**Fig. 4.** Effect of the sampling time on the total response of selected esters (isoamyl acetate, ethyl caproate, ethyl caprylate, phenyl acetate, ethyl caprate, phenylethyl acetate, ethyl laurate, ethyl myristate, ethyl palmitate) extracted by SPME.

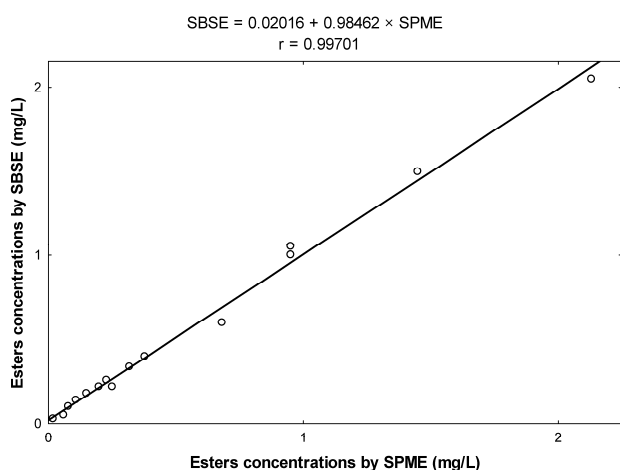
**Table I.** Comparison of linearity, recovery and repeatability of SPME and SBSE methods used for ester determination in beer.

Compound	Correlation coefficient		Recovery (%)		Repeatability RSD (%)	
	SPME	SBSE	SPME	SBSE	SPME	SBSE
Isoamyl acetate	0.9996	0.9992	95	107	4.8	5.2
Ethyl caproate	0.9999	0.9988	102	104	4.3	3.1
Ethyl caprylate	0.9999	0.9994	96	85	2.6	2.1
Ethyl caprate	0.9999	0.9996	94	78	5.6	7.3
Phenylethyl acetate	0.9999	0.9991	101	94	5.4	7.3
Ethyl laurate	0.9995	0.9988	91	81	4.8	5.8
Ethyl myristate	0.9998	0.9998	85	83	5.7	6.2
Ethyl palmitate	0.9995	0.9995	89	84	6.3	6.1

**Table II.** Concentration of esters as an average of three replicates in three beer samples determined by using SPME and SBSE and their differences after the analysis of dispersion test<sup>a</sup>.

Compound	Sample A (mg/L)		Sample B (mg/L)		Sample C (mg/L)	
	SPME	SBSE	SPME	SBSE	SPME	SBSE
Isoamyl acetate	<b>0.95</b>	<b>1.05</b>	<b>2.13</b>	<b>2.05</b>	<b>1.45</b>	<b>1.5</b>
Ethyl caproate	<b>0.25</b>	<b>0.22</b>	<b>0.15</b>	<b>0.18</b>	<b>0.32</b>	<b>0.34</b>
Ethyl caprylate	<b>0.38</b>	<b>0.4</b>	<b>0.11</b>	<b>0.14</b>	<b>0.20</b>	<b>0.22</b>
Ethyl caprate	<b>0.08</b>	<b>0.1</b>	<b>0.06</b>	<b>0.05</b>	<b>0.02</b>	<b>0.03</b>
Phenylethyl acetate	<b>0.95</b>	<b>1</b>	<b>0.68</b>	<b>0.6</b>	<b>0.23</b>	<b>0.26</b>
Ethyl laurate	0.02	0.03	0.02	0.02	0.01	0.02
Ethyl myristate	0.01	0.01	0.01	0.01	<0.01	<0.01
Ethyl palmitate	0.03	0.02	<0.01	<0.01	0.01	0.01

<sup>a</sup> Bold characters indicate results without statistically significant differences.

**Fig. 5.** Linear regression for esters in beer samples determined by SPME and SBSE methods.

These results indicated that both of the methods provided comparable working parameters.

Both methods have been applied for the analysis of three different Pilsner type beer samples. Each analysis was repeated three times. The concentrations, as averages of the triplets of the analyzed compounds, are shown in Table II.

An analysis of variation (ANOVA) has been applied to compare the statistical differences between the results obtained by the sample preparation procedures (Table II). The results without statistically significant differences are marked by bold characters. The determined concentrations of esters at levels >0.10 mg/L (isoamyl acetate, ethyl caproate, ethyl caprylate, ethyl caprate and phenylethyl acetate) obtained by using of both methods can be regarded as the same. Compounds at low concentrations (<0.05 mg/L) were not statistically tested.

The correlation of content of esters achieved by SPME and SBSE methods is presented in Fig. 5. The correlation coefficient reached a satisfactory value of 0.9970.

## CONCLUSIONS

The advantages of the SPME method in comparison with the SBSE technique are better simplicity, a lower time requirement (a further 40 min for solvent back extraction is necessary in the SBSE procedure) and due to a proper fibre phase, the possibility to determine more polar compounds, e.g. alcohols. On the other hand, the stir bar used in the SBSE method is much more robust than the fragile SPME fibre. The working parameters of both methods were similar. The obtained concentrations for all determined compounds were in good correlation.

## ACKNOWLEDGEMENTS

The authors thank members of the Czech Beer and Malt Association for financial support.

This work is a part of the Research Plan of the RIBM No. MSM 6019369701

## REFERENCES

- Alvarez, P., Malcorps, P., Sa Almeida, A., Ferreira, A., Meyer, A. M. and Dufour, J. P., Analysis of free fatty acids, fused alcohols and esters in beer: an alternative to CS<sub>2</sub> extraction. *J. Am. Soc. Brew. Chem.*, 1994, **52**, 127-134.
- Baker, C. D., Determination of lower boiling point volatile compounds in beer by headspace gas chromatography, Collaborative trial. *J. Inst. Brew.*, 1989, **95**, 267-270.
- Baltussen, E., Sandra, P., David, F. and Cramers, C., Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: Theory and principles. *J. Microcolumn Separation*, 1999, **11**, 737-747.

4. Berry, D. R., Products of primary metabolic pathways. In: Physiology of Industrial Fungi. D. R. Berry, Ed., Blackwell Scientific Publications: Oxford, U. K., 1988.
5. Biazon, C. L., Brambilla, R., Rigacci, A., Pizzolato, T. M. and dos Santos, J. H., Combining silica-based adsorbents and SPME fibers in the extraction of the volatiles of beer: an exploratory study. *Anal. Bioanal. Chem.*, 2009, **394**, 549-556.
6. Bicchi, C., Iori, C., Rubiolo, P. and Sandra, P., Headspace sorptive extraction (HSSE), stir bar sorptive extraction (SBSE) and solid phase microextraction (SPME) applied to the analysis of roasted Arabica coffee and coffee brew. *J. Agr. Food Chem.*, 2002, **50**, 449-459.
7. Buckee, G. K., Determination of the volatile components of beer. *J. Inst. Brew.*, 1992, **98**, 78-79.
8. David, F., Sandra P., Hoffmann, A., Harms, D. and Nietzsche F., Elucidation of the hoppy aroma in beers by stir bar and headspace sorptive extraction followed by thermal desorption-CGC-MS/PFPD, AppNote 4/2001, Gerstel, 2001, 1-7.
9. Harms, D., Nietzsche, F., Hoffmann, A., David, F. and Sandra, P., The analysis of the bitter and other flavour compounds in beer and wort by stir bar sorptive extraction (SBSE) followed by HPLC, AppNote 5/2001, Gerstel, 2001, 1-6.
10. Hawthorne, D. B., Kavanagh, T. E. and Clarke, P. J., Determination of low molecular weight organic compounds in beer using capillary gas chromatography. *J. Am. Soc. Brew. Chem.*, 1987, **45**, 23-27.
11. Horák, T., Čulík, J., Čejka, P., Jurková M., Kellner, V., Dvořák, J., Hašková, D., Analysis of free fatty acids in beer: comparison of solid-phase extraction, solid-phase microextraction, and stir bar sorptive extraction. *J. Agr. Food Chem.*, 2009, **57**, 11081-11085.
12. Horák, T., Čulík, J., Čejka, P., Jurková, M. and Kellner, V., Determination of vicinal diketones in beer by SPME method. *Kvasny Prum.*, **47**, 2001, 316-321.
13. Horák, T., Čulík, J., Jurková, M., Čejka, P. and Kellner, V., Application of some modern sample preparation procedures for quantitative determination of vicinal diketones in beer. *Kvasny Prum.*, 2009, **55**, 66-72.
14. Horák, T., Čulík, J., Jurková, M., Čejka, P. and Kellner, V., Determination of free medium-chain fatty acids in beer by stir bar sorptive extraction. *J. Chromatogr. A*, 2008, **1196-1197**, 96-99.
15. Horák, T., Čulík, J., Jurková, M., Čejka, P. and Kellner, V., Determination of the fatty acids in beer by SPME. *Kvasny Prum.*, 2005, **51**, 374-377.
16. Horák, T., Kellner, V., Čulík, J., Jurková, M. and Čejka, P., Determination of some beer flavours by stir bar sorptive extraction and solvent back extraction. *J. Inst. Brew.*, 2007, **113**, 154-158.
17. Chen, E. C. H., Analysis of volatile beer flavor compounds by a dynamic headspace entrainment technique. *J. Am. Soc. Brew. Chem.*, 1983, **41**, 28-31.
18. Irwin, A. J. and Thompson, D. J., A rapid method for the extraction and analysis of beer flavour components. *J. Inst. Brew.*, 1987, **93**, 113-115.
19. Jélen, H. H., Wlazly, K., Wasowicz, E. and Kaminski, E., Solid-phase microextraction for the analysis of some alcohols and esters in beer: Comparison with static headspace method. *J. Agr. Food Chem.*, 1998, **46**, 1469-1473.
20. Kishimoto, T., Wanikawa, A., Kagami, N. and Kawatsura, K., Analysis of hop-derived terpenoids in beer and evaluation of their behavior using the stir bar-sorptive extraction method with GC-MS. *J. Agr. Food Chem.*, 2005, **53**, 4701-4707.
21. Lindsay, R. C., Withycombe, D. A. and Micketts, R. J., Comparison of gas chromatographic methods for analysis of beer flavors. *Proc. Am. Soc. Brew. Chem.*, 1972, 4-7.
22. Liu, M., Liu, Y., Zeng, Z. and Peng, T., Preparation and characteristics of high pH-resistant sol-gel alumina-based hybrid organic-inorganic coating for solid-phase microextraction of polar compounds. *J. Chromatogr. A*, 2006, **1108**, 149-157.
23. Marsili, R. T., Laskonis, L. C. and Kanaan, C., Evaluation of PDMS-based extraction techniques and GC-TOFMS for the analysis of off-flavor chemicals in beer. *J. Am. Soc. Brew. Chem.*, 2007, **65**, 129-137.
24. Meilgaard, M. C., Prediction of flavor differences between beers from their chemical composition. *J. Agr. Food Chem.*, 1982, **30**, 1009-1017.
25. Nykänen, L. and Suomalainen, H., Formation of aroma compounds by yeast. In: Aroma of Beer, Wine and Distilled Alcoholic Beverages, Akademie-Verlag: Berlin, 1983.
26. Ochiai, N., Sasamoto, K., Daishima, S., Heiden, A. C. and Hoffmann, A., Determination of stale-flavor carbonyl compounds in beer by stir bar sorptive extraction with in-situ derivatization and thermal desorption-gas chromatography-mass spectrometry. *J. Chromatogr. A*, 2003, **986**, 101-110.
27. Pawliszyn, J., Solid phase microextraction theory and practise. Wiley: New York, 1997.
28. Saison, D., De Schutter, D. P., Delvaux, F. and Delvaux, F. R., Determination of carbonyl compounds in beer by derivatisation and headspace solid-phase microextraction in combination with gas chromatography and mass spectrometry. *J. Chromatogr. A*, 2009, **1216**, 5061-5068.
29. Saison, D., De Schutter, D. P., Delvaux, F. and Delvaux, F. R., Optimisation of a complete method for the analysis of volatiles involved in the flavour stability of beer by solid-phase microextraction in combination with gas chromatography and mass spectrometry. *J. Chromatogr. A*, 2008, **1190**, 324-349.
30. Scarlata, C. J. and Ebeler, S. E., Headspace solid-phase microextraction for the analysis of dimethyl sulfide in beer. *J. Agr. Food Chem.*, 1999, **47**, 2505-2508.
31. Stenroos, L. E., Grabowski, J., Spearman, J. and Siebert, K. J., The semi-routine use of capillary gas chromatography for analysis of aroma volatiles in beer. *J. Am. Soc. Brew. Chem.*, 1985, **43**, 203-208.
32. Šulák, M., Šmogrovičová, D. and Leitner, E., Comparison of the content of volatile sulphur-containing compounds in Slovak beers by the SPME method. *Kvasny Prum.*, 2008, **54**, 70-74.
33. Techakriengkrai, I., Paterson, A. and Taidi, B., Relationship of sensory staleness in two lagers to headspace concentrations of trans-2-nonenal and three staling aldehydes. *J. Inst. Brew.*, 2006, **112**, 36-40.
34. Veselý, P., Lusk, L., Basařová, G., Seabrooks, J. and Ryder, D., Analysis of aldehydes in beer using solid-phase microextraction with on-fibre derivatization and gas chromatography/mass spectrometry. *J. Agr. Food Chem.*, 2003, **51**, 6941-6944.
35. Wampler, T. P. and Washall, J. W., Matheson, M. J., Applications of purge-and-trap to the analysis of beers. *Am. Lab.*, 1996, **28**, 18T-18V.

(Manuscript accepted for publication March 2010)